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# The Effect of Gamma Irradiation on Phytochemical Content and Antioxidant Activity of Stored and None Stored Almond (*Amygdalus communis* L.) Hull

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**ABSTRACT**

***Background:*** Gamma radiation has been widely used as a post-harvest food preservation process for many years. Irradiation can affect the content of phytochemicals. During processing of almonds, large amounts of by-products such as hull and shell are produced. This study evaluates the effect of gamma radiation on phytochemical content and antioxidant activity of none stored (H1) and stored (H2) almond hull. ***Methods:*** Both almond hull samples were irradiated with 0, 2, 6 and 10 kGy gamma rays. Total phenolic content (TPC), total flavonoid content (TFC) and bioactivity of the treated samples extracts were investigated by various *In vitro* colorimetric methods. ***Results:*** Irradiation dose of 10 kGy slightly decreased the TPC and TFC values but maintained FRAP value in H1 extracts. The TPC of H2 was increased ($p<0.05$) at the dose of 10 kGy, while the TFC and FRAP values were constant. 2 kGy dose of gamma irradiation slightly increased the antiradical activity of H1 and H2, but the other doses significantly reduced antiradical activity of extracts. ***Conclusion:*** Results showed that gamma irradiation can change the antioxidant content and activity of almond hull.

## Introduction

High amounts of almond hull were removed from almonds after harvesting which are used as supplemental livestock feed, is estimated to exceed 6 million tons annually.[1] In the recent years, almond hulls are used as a natural source for sweetener concentrate, dietary fiber and natural antioxidants.[2] Almond hulls contain triterpenoids, betulinic, urosolic and oleanolic acids [3], as well as flavonol glycosides and phenolic acids.[4] Due to the presence of these valuable compounds, almond hulls possess potent antioxidant capacities.[5-9] Recently, reports have shown that natural antioxidants play a major health protective role.[10] These antioxidants have a wide range of biological activities that act against possible harmful effects of free radical-induced damage in the body.[11] For example, polyphenolic compounds like flavonols, flavones or the flavonoids, have gained considerable interest as they are proved to be very effective protecting against cardiovascular diseases by reducing the oxidation of LDL as well as preventing other degenerative diseases.[12,13]

Some studies underlined different factors such as genotype, cultivation techniques, climatic conditions that occur during the pre-harvest period, post-harvest storage conditions and processing which may affect the chemical composition of plant foods and they may have an important role in determining the phenolic composition and the bioactivity of these compounds.[14,15] Radiation processing is well established as a physical, non-thermal method to preserve various food products that involves the exposure of food products (raw or processed) to ionizing or non-ionizing radiation.[16] Irradiation of food products causes a minimal modification in the flavor, color, nutrients, taste, and other quality attributes of food.[17] However, the levels of these modifications might vary depending on the basic raw material used, irradiation dose delivered, and on the type of radiation source employed (gamma-ray, X-ray, UV, electron beam).[11,18,19] Irradiation can also influence the levels of antioxidants/phytochemicals in plant products.[20] It has been reported that under certain favorable conditions, the concentration of plant phytochemicals might be increased. These conditions contain exposure to irradiation sources, wounding, storage at low temperatures, and/or exposure to extreme temperatures.[21]

Gamma radiation, more energetic than X-rays, is used from sources of radioactive isotopes, cesium-137 or cobalt-60, and it is identified by the World Health

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Organization as a food preservation technique that improves food safety without altering the toxicological, biological or nutritional quality of the food.[17,22-24] Gamma irradiation treatment has been widely studied on vegetables and fruits for many years, and the results of this sterilization method are very interesting. It has been reported that gamma irradiation could increase phenolic content in almond skin[25], cinnamon and clove while phenolic content in nutmeg remained unaltered [26,27]. Irradiation reduced the tannin content and activity of antioxidants of pistachio hull extracts but increased the total phenolic content[20]. Whereas many reports have shown that gamma irradiation decreased phenolics[28]. Collectively, this prompted us to investigate the effects of gamma irradiation treatment on both stored and none stored almond hulls total phenolics, flavonoid and antioxidant activities.

## Materials and methods

### *Chemicals and reagents*

Quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (tripyridyl-S-triazine), gallic acid were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Folin-Ciocalteu Reagent was prepared from Merck Chemical Company (Darmstadt, Germany). All other reagents were of analytical reagent grade.

### *Sample preparation*

Almond (*Amygdalus communis* L.) hull was collected from different locations of West and East Azarbaijan provinces of Iran and was air dried and reduced to fine powder.[8] This powder was stored at room temperature for five years (H2). For fresh samples (H1) we collected almond hull from the same locations in August-September 2012.

### *Gamma irradiation*

Almond hulls (H1: none stored and H2: stored) were irradiated by $^{60}Co$ radiation in a gamma cell (Issledo Vatel Gamma Cell Facility, Model PX-30) at a dose rate of 0.8 kGy at a absorbed dose of 2, 6 and 10 kGy in polyethylene bags (10×10 $cm^2$) under the same conditions of temperature and humidity. Samples were treated uniformly by packing them in small sizes.

### *Preparation of extracts*

For extraction of the antioxidant compounds, 1 g of each irradiated powder sample was mixed with 20 ml of pure methanol and then stirred for 30 min using a magnetic stirrer [29]. The yield extracts were filtered through filter paper and stored at 4 °C until use.

### *Determination of Total phenolic content*

Total phenolic content was determined with Folin-Ciocalteu Reagent (FCR) according to the method of Singleton and Rossi [30] with some modifications. Briefly, 0.5 ml of each phenolic extract was mixed with 2 ml of 7.5% sodium carbonate, and then the mixture was allowed to stand at room temperature for 2 min. After addition of 2.5 ml ten-fold Folin-Ciocalteu reagent, the mixture was incubated in the dark room for 30 min. The absorbance was measured at 720 nm by using a spectrophotometer (T60, PG Instruments Ltd., Leicestershire, UK). The concentration of phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per gram of extract.

### *Total flavonoid content assay*

Total flavonoid content of the extracts were assayed by the colorimetric method described by Zhishen et al. [31] and Jahanban Esfahlan and Jamei [32] , with minor modifications. Almond hull extract (250 µl) was mixed with 1.25 ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After five minutes, 150 µl of a 10% $AlCl_3$. $H_2O$ solution, 500 µl of 1 M NaOH and 275µl of distilled water were added to the mixture. The absorbance of the mixture was measured at 507 nm. The content of flavonoids was expressed as mg quercetin per gram of extract.

### *FRAP assay*

The ferric reducing antioxidant power (FRAP) assay was done according to Benzie and Strain [33] method with some modifications. The stock solutions were included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3.6H_2O$ solution. Almond hull extract (50 µl) were allowed to react with 950 µl of the FRAP solution for 20 min in the dark condition. Reading of the colored product (ferrous tripyridyltriazine complex) was then taken at 593 nm. FRAP values for all samples were achieved by standard calibration curve obtained by using different concentrations of $FeSO_4.7H_2O$. Results were expressed as mg $FeSO_4$ / g extract.

### *DPPH free radical scavenging activity*

The DPPH radical scavenging activity was determined as described Brand-Williams et al. [34] with some modifications. Various volumes of extracts (30, 50, 70 and 100 mL) were added to 1mL of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 mM in methanol) and the reaction mixture shaken vigorously. After incubation at room temperature for 10 min, the absorbance of this solution was determined at 517 nm, by using a spectrophotometer. Radical scavenging activity was expressed as IC50 values and percentage of DPPH radical scavenging obtained from the following equation:

$$RSA\% = (A_{blank} - A_{sample}) / A_{blank} \times 100 \quad \text{Eq.(1)}$$

### *Statistical analysis*

All of the assays were obtained from triplicate measurements and results are expressed as means ±

standard deviations. The Statistical Package for Social Sciences (SPSS, version 20 for windows) was used to analyze the data. The means were compared using Duncan's multiple range (DMRT) test at $p<0.05$ following analysis of variance (ANOVA).

## Results and discussion

### *Total phenolic content*

The effect of gamma irradiation on total phenolic contents is shown in Table 1. Gamma irradiation decreased ($p<0.05$) the TPC of the H1 extracts compared with the control. H2 extract showed an increase in phenolic content by about 21% at an irradiation dose of 10 kGy compared to the control, but absorbed dose of 2 and 6 kGy slightly decreased the TPC of the H2 extracts. This shows that high levels of gamma irradiation may enhance or preserved the phenolic compounds in almond hull. In some of the studies using ionizing irradiation, it was shown an increase in the level of TPC.[20,25,35,36] While, some studies did not exhibit increase in phenolic content with irradiation.[27] In contrast, many investigations established that gamma irradiation decreased the content of phenolic compounds in plant products.[18] De Toledo et al. [28] found that the effects of ionizing irradiation on tannin and phenolic compounds are dose dependent. Universally, gamma radiation studies have shown different effects that were attributed to the different phenolic compounds present in the various plant materials. Some of the materials have appreciable amounts of hydrolysable compounds, which may be more susceptible to gamma-irradiation compared to the condensed compounds present in other products. In the present study the differences in the effect of radiation on H1 and H2 extracts might be due to the storage of almond hull that can influence the antioxidant content and its activities. Accordingly, it has been shown that during post-harvest storage of agricultural crops, significant changes in antioxidant status can occur.[37]

**Table 1.** Effect of gamma radiation on total phenolic content (mg GAE /g extract) of almond hull.

| | Irradiation dose (kGy) | | | |
|---|---|---|---|---|
| | 0 | 2 | 6 | 10 |
| H1 | 398±2.45$^{a}$ | 275±1.66$^{c}$ | 378±4.32$^{b}$ | 390±3.12$^{ab}$ |
| H2 | 316±2.42$^{b}$ | 289±6.07$^{c}$ | 295±5.68$^{c}$ | 400±2.37$^{a}$ |

Different letters within a row are significant ($p< 0.05$, n = 3).

### *Total flavonoid content*

Total flavonoid content of treated and none treated almond hull extracts (H1 and H2) by irradiation is shown in Table 2. The TFC value of H1 was decreased approximately 41% at the dose of 2 kGy, but irradiation doses of 6 and 10 kGy decreased the TFC of H1 by about 16% compared to the control. In agreement with these results, Carocho et al.[38] showed that ionizing radiation decreased flavonoids content in chestnut, revealing that these compounds may be sensitive to ionizing radiation. For the H2 extracts, irradiation decreased the TFC by about 35% at the doses of 2 and 6 kGy while, at 10 kGy there was no significant effect on TFC value.

**Table 2.** Effect of gamma radiation on total flavonoid content (mg Q) of almond hull.

| | Irradiation dose (kGy) | | | |
|---|---|---|---|---|
| | 0 | 2 | 6 | 10 |
| H1 | 73.9±3.25$^{a}$ | 43.7±0.76$^{c}$ | 62.6±4.32$^{b}$ | 61.8±1.69$^{b}$ |
| H2 | 51.1±4.02$^{a}$ | 32.5±2.05$^{b}$ | 34.8±3.08$^{b}$ | 52.6±4.63$^{a}$ |

Different letters within a row are significant ($p$ 0.05, n = 3).

### *FRAP assay*

The antioxidant potential of the control and irradiated samples were estimated from their ability to reduce the TPTZ-Fe(III) complex to the TPTZ-Fe(II) complex as shown in Table 3. The FRAP value of the H1 extracts was slightly decreased at 2 kGy and remained stable at 6 and 10 kGy. There was a high correlation ($R^2$= 0.912) between the phenolic contents and the FRAP values of H1 extracts. Irradiation at the doses of 2 and 6 kGy caused a decrease ($p<0.05$) in FRAP values of H2 extracts compared to the control. But there was no difference in FRAP value of irradiated H2 at 10 kGy compared to the control. This shows that higher irradiation maintained the antioxidant activity of almond hull.

**Table 3.** Effect of gamma radiation on FRAP values (mg FeSO4) of almond hull.

| | Irradiation dose (kGy) | | | |
|---|---|---|---|---|
| | 0 | 2 | 6 | 10 |
| H1 | 16.3±0.25$^{a}$ | 14.9±0.25$^{b}$ | 16.6±0.08$^{a}$ | 16.3±0.02$^{a}$ |
| H2 | 16.4±0.06$^{a}$ | 10.5±1.07$^{b}$ | 8.16±1.14$^{c}$ | 16.2±0.23$^{a}$ |

Different letters within a row are significant ($p< 0.05$, n = 3).

### *DPPH radical scavenging activity*

The antioxidant activity of the samples was also measured in terms of the radical scavenging power, according to the DPPH method (Table 4).

**Table 4.** Effect of gamma radiation on DPPH radical scavenging activity (IC50 values, mg/mL) of almond hull

| | Irradiation dose (kGy) | | | |
|---|---|---|---|---|
| | 0 | 2 | 6 | 10 |
| H1 | 74.8 | 72.4 | 77.5 | 113.3 |
| H2 | 62.4 | 59.8 | 128.4 | 132.5 |

It was found that irradiation at 2 kGy slightly increased radical scavenging activity of H1 and H2 extracts. The lowest values of scavenging activity (highest $IC_{50}$) were observed for H1 and H2 extracts at 10 kGy. Behgar et al. [20] observed higher activity in control

pistachio hull than those of irradiated ones (10–60 kGy). The decrease of radical scavenging activity with gamma irradiation in this study is consistent with the reduction in tannin content. Gamma irradiation (5–30 kGy) significantly decreased the DPPH radical-scavenging activity and reducing power of ground black pepper extracts.[39]

In contrast to the present study, Harrison and Were [25] reported an increase in phenolic content and antioxidant activity in almond skin extracts irradiated at doses greater than 4 or 12.7 kGy. In the study of Carocho et al. [38], electron beam and gamma irradiation (1 and 3 kGy) were increased phenolic compounds and radical scavenging activity of chestnuts. The differences in the effect of ionizing radiation on antioxidant content and activity (increase or decrease) may be due to the variation in plant type, geographical and environmental conditions, state of the sample (solid or dry), phenolic content composition, extraction solvent, extraction procedures, temperature, dose of irradiation, etc.[40]

We observed no correlation between TPC and TFC content and DPPH radical scavenging capacity of almond hull extracts. An explanation of this result might be due to the effects of irradiation on other chemicals present in almond hull such as tannins and lipids. Akbari et al. [41] observed low correlation coefficient between phenol content and radical scavenging activity in kernel extract (R2 = 0.1) . The carboxyl group is an electron-withdrawing group, which does not benefit the radical scavenging activity of the compound [42], inversely the presence of higher numbers of hydroxyl groups in phenolic compounds most likely associated with the increased DPPH radical scavenging activity.[43]

## Conclusions

Almond hull contains high amount of polyphenols and possess potent antioxidant capacity. In general, gamma irradiation changed the phytochemical content and antioxidant activity of almond hull. 10 kGy was the effective dose to maintain antioxidant content and activity for both almond hull (H1 and H2) extracts. Gamma irradiation decreased radical scavenging capacity of almond hull extracts compared to the control. More investigations are needed to evaluate the effect of gamma radiation on individual phenolic compounds, using HPLC and chromatographic techniques.

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