

Effect of Gamma Irradiation on Total Phenolic Content and *in Vitro* Antioxidant Activity of Pomegranate (*Punica Granatum* L.) Peels

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ABSTRACT

Pomegranate peels were studied for the effect of gamma irradiation on microbial decontamination along with its effect on total phenolic content and *in vitro* antioxidant activity. Gamma irradiation was applied at various dose levels (5.0, 10.0, 15.0 and 25.0 kGy) on pomegranate peel powder. Both the values of total phenolic content and *in vitro* antioxidant activity were positively correlated and showed a significant increase ($p < 0.05$) for 10.0 kGy irradiated dose level immediately after irradiation and 60 days of post irradiation storage. At 5.0 kGy and above dose level, gamma irradiation has reduced microbial count of pomegranate peel powder to nil. Post irradiation storage studies also showed that, the irradiated peel powder was microbiologically safe even after 90 days of storage period.

Keywords: Pomegranate Peels, Food Irradiation, Total Phenolic Content, *In Vitro* Antioxidant Activity, Microbial Load

1. Introduction

Pomegranate (*Punica granatum* L.) is native to the Mediterranean region and has been used extensively in the folk medicine of Indian subcontinent and many other countries. India is second largest producer of pomegranate fruit after Iran [1]. The fruit can be divided into three parts: the seeds (3% w/w), the juice (30% w/w) and peels including the interior network of membranes (67% w/w) [2]. *P. granatum* is rich in tannins that have remarkable antimicrobial activity [3,4]. Interestingly, the peel fraction of pomegranate has higher total phenolic content and antioxidant activity than the pulp fraction. It contains 249.4 mg/g total phenolics as compared to just 24.4 mg/g total phenolics present in its pulp [5]. It has substantial amount of polyphenols such as ellagic acid, ellagitannins and gallic acids [6]. Yasoubi *et al.* [7] showed that, acetone with sonication produced the maximum amount of phenolic compounds from pomegranate peel extracts (PPE). Pomegranate peel extracts have been shown to possess significant *in vitro* and *in vivo* antioxidant activity [8,9]. In addition to antioxidant activity, the peel extract also has antimicrobial, antibacterial, antiviral, antifungal and antimutagenic properties

as well as beneficial effects on the oral and cardiovascular diseases [10]. It has both antioxidant and antimutagenic properties which can be exploited as bio-preservatives in various food applications and nutraceuticals [11]. The antimicrobial activity against some food-borne pathogens by various extracts from pomegranate fruit peels was evaluated using both *in vitro* (agar diffusion) and *in situ* (food) methods [12]. The pomegranate peel extract improves the process intensity due to acceleration of deposit precipitation of the haze-forming substance of fruit juices [13]. After processing of fruits, their peels generally have been discarded as waste. This waste is responsible for causing a severe problem of environmental pollution as they gradually ferment and released off odour due to microbial contamination. Thus being valuable source of natural antioxidants, these peels cannot be used utilized in food products or any other applications.

Food irradiation (controlled application of ionizing radiation such as x-rays, gamma rays, electron beam, etc.) refers to improve hygiene, safety and to reduce microbial load in order to extend the shelf life of perishable food products. Gamma irradiation is considered

as effective method of food processing to reduce microbial load and to extend the shelf life of product without any detrimental effect on food quality. Gamma irradiation (10 kGy) has also showed increased phenolic acid content in cinnamon and clove while phenolic content in nutmeg remained unaltered [14]. There are several reports on effect of irradiation processing on total phenolic content and antioxidant activity from several plant and food products. Although, some studies report that, gamma irradiation does maintain or enhance the antioxidant properties; there are a few examples wherein the antioxidant properties of the plant material were decreased [15].

The main objectives of this study were thus, to investigate the effects of various gamma irradiation dose levels on 1) total phenolic content 2) *in vitro* antioxidant activity as ABTS⁺ free radical scavenging activity 3) microbial decontamination from pomegranate peel powder.

2. Material and Methods

2.1. Materials

Pomegranate peels (Ganesh var.) were procured from Surekha Fruit Extract Pvt. Ltd. Baramati, Maharashtra, India. Peels were cleaned, hot air dried at 60°C for 7 h. Initial moisture content of pomegranate peels was found to be 61.0% that was reduced to 3.36% on drying. The said ground material was passed through a 30-mesh sieve, that was packed in High Density Polyethylene (HDPE) self sealed pouches (thickness 0.1 mm) until they are used for further analysis. All samples were stored under ambient conditions (25°C) during the study.

2.2. Reagents and Solutions

[2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) were purchased from Sigma Chemicals (St. Louis, MO). Folin-Ciocalteu reagent, gallic acid, methanol, ethanol, ethyl acetate, sodium carbonate used were of analytical grade and obtained from AMI Chemicals, Mumbai, India.

2.3. Irradiation Treatment and Storage

Three pouches containing each 25 g of dried pomegranate peel powder (<5% M.O.) were irradiated at the various doses 5.0, 10.0, 15.0 and 25.0 kilogray (kGy) using gamma chamber-900 housing with ⁶⁰Co gamma source at dose rate 1.93 kGy/h at Board of Radiation and Isotope Technology (BRIT), Vashi, Navi Mumbai, India. Fricke's Dosimeter was used for the measurement of the applied irradiation dose. The temperature during irradiation was 40°C ± 2°C. The irradiated samples were then

stored in polyethylene bags at laboratory conditions (25°C, relative humidity 40%) along with the control (0 kGy) for 90 days.

2.4. Extraction Studies

Solvent extraction studies were carried out using modified method as described by Chirinos *et al.* [16] for extraction of total phenolic content and to determine *in vitro* antioxidant activity of pomegranate peel powder. Both control and irradiated peel powder samples were extracted with methanol concentration (60%) (v/v), sample to solvent ratio (1/30) (w/v), kept on rotary shaker (Associated Entrepreneurs, Bombay, India) at a speed of 180 rpm for 1 h at 37°C ± 2°C. Further various antioxidant rich extracts were collected by centrifugation (Remi Compufuge CPR 30, 6000 g, 15 mins) and filtered through Whatman filter no. 1. The clear filtrates were stored in an amber coloured bottle at 4°C for the period of one week and later at -20°C freezer. These filtrates were then subsequently used for the determination of total phenolic content and *in vitro* antioxidant activity.

2.5. Total Phenolic Content

The Total Phenolic Content (TPC) was measured using spectrophotometer (Spectronic Genesys-5, Thermo Electron, USA) by Folin-Ciocalteu colorimetric method [7]. Sample (0.2 ml) was mixed with 1 ml of 10-fold-diluted Folin-Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate solution. After the mixture has been allowed to stand for 30 minutes at room temperature, the absorbance was measured at 765 nm. All the measurements were taken in triplicates and mean values were calculated. The concentration of total phenolics in both the extracts (from control and irradiated) was determined and results were expressed as gram of gallic acid equivalents per 100 gram of dry weight (g GE/100 g DW).

2.6. *In Vitro* Antioxidant Activity

In vitro Antioxidant activity was measured using the improved ABTS method [17,18]. The ABTS radical cation (ABTS⁺) solution was prepared by the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23°C in the dark for 16 h. The ABTS⁺ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. ABTS⁺ solution (3.9 ml; absorbance of 0.700 ± 0.005) was added to 0.1 ml of the test sample and mixed thoroughly. The reaction mixture was allowed to stand at 23°C for 6 min and the absorbance at 734 nm using spectrophotometer (Spectronic Genesys-5, Thermo Electron, USA) was immediately recorded. The samples were diluted with 80% ethanol so as to give 20% - 80% reduction of the blank absorbance

with 0.1 ml of sample. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 15 μ M) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard. Trolox Equivalent Antioxidant Capacity (TEAC) was expressed in millimoles of Trolox equivalents (TE) per 100 gram of dry weight (mM TE/100 g DW).

2.7. Microbial Analysis

Total plate counts and Total fungal (yeasts and moulds) counts were carried out by the method described by Alighourchi *et al.* [19]. Each value represents the mean of three samples and results were expressed as log of colony forming units only per gram of the sample (CFU/g).

2.8. Stastical Analysis

All determinations were obtained from triplicate measurements and results were expressed as mean \pm standard deviation. The Statistical Package for Social Sciences (SPSS) for Windows version (16.0) was used to analyse the data (SPSS Inc., Chicago, IL). Statistical significance was declared at $p < 0.05$ or mentioned otherwise.

3. Results and Discussion

Pomegranate peel powder samples were exposed to gamma radiation at various dose levels of 5.0 10.0, 15.0 and 25.0 kGy. The effect of irradiation on total phenolic content and *in vitro* antioxidant activity along with microbial decontamination was studied for all the irradiated and control (0 kGy) samples. To check post irradiation storage effect, all the samples were stored for 25°C with relative humidity 40% along with the control (0 kGy) for 90 days.

Table 1 shows, the effect of different irradiation doses on TPC and *in vitro* antioxidant activity immediately (0 day) after the irradiation. The total phenolic content and average *in vitro* antioxidant activity of control (0 kGy) and irradiated (at 10.0 kGy) peel powder were increased by 4% and 12% respectively. It was determined to be 16.10 ± 0.21 g GE/100 g DW in the control (0 kGy), that

was gradually increased up to 16.80 ± 0.15 g GE/100 g DW for 10.0 kGy dose level. This increase in total phenolic content could be attributed to the degradation of tannins present in pomegranate peel powder having higher molecular weight into the release of simple phenolic compounds like gallic acid, tannic acid, etc. Irradiation may break this complex to facilitate release of active ingredients, which were contributed to increase the total phenolic content [20]. The enhanced antioxidant capacity/activity of a plant after irradiation is mainly attributed either to increased enzyme activity (e.g. phenylalanine ammonia-lyase and peroxidase activity) or to the increased extractability from the tissues extractability by depolymerization and dissolution of cell wall polysaccharides by irradiation [15]. Bhatt *et al.* [21] observed that, except for 2.5 kGy, rest of the doses showed a significant dose-dependent increase in total phenolics to higher extractability by depolymerization and dissolution of cell wall polysaccharides by irradiation, which was known to increase the activity of phenylalanine ammonia-lyase, responsible for the synthesis of phenolic compounds. ABTS⁺ free radical scavenging activity, expressed as (mM TE/100 g DW) was used for the evaluation of *in vitro* antioxidant activity of peel powder samples (both irradiated and control). **Table 1** also shows a significant increase in *in vitro* antioxidant activity values from control (0 kGy) to 10.0 kGy (12.12 ± 0.43 mM TE/100 g DW and 13.70 ± 0.29 mM TE/100 g DW respectively). This increase could be attributed to enhanced free phenolics of the irradiated samples. The antioxidant activity is due to the presence of polyphenols or gallic acid. As mentioned earlier, this significant increase in total phenolic content was thus suggestive of their enhanced antioxidant properties. Kumari *et al.* [20] also showed similar results with Triphala, wherein they have found out a significant increase in gallic acid concentration and total phenolics in the water extract due to irradiation that leads to increase in antioxidant property.

Figure 1 shows, post irradiation storage effect (30, 60 and 90 days) on total phenolic content of control and irradiated peel powder samples. After 30 days of post irradiation storage period, total phenolic content was significantly increased for 10.0 kGy of irradiated dose level ($p < 0.05$). The same increase was also observed for 10.0 kGy as compared to control (0 kGy) even after 60 and 90 days of post irradiation storage. This increase in total phenolic content may be associated with changes in the molecular conformation as a result of irradiation treatment [20]. The differences in the effect of irradiation on total phenolic content (increase or decrease) may be due to plant type, geographical and environmental conditions, state of the sample (solid or dry), phenolic content

Table 1. Effect of gamma irradiation on total phenolic content and *in vitro* antioxidant activity of pomegranate peel powder.

Irradiation dose (kGy)	Total phenolic content (g GE/100 g DW)*	<i>In vitro</i> antioxidant activity (g TE/100 g DW)*
0	16.10 ± 0.21^a	12.12 ± 0.43^a
5.0	16.14 ± 0.52^a	13.43 ± 0.45^a
10.0	16.80 ± 0.15^b	13.70 ± 0.29^b
15.0	15.38 ± 0.56^a	12.97 ± 0.26^a
25.0	16.66 ± 0.35^a	12.57 ± 0.25^a

*Means in the same column with different alphabetical letters are significantly different ($p < 0.05$).

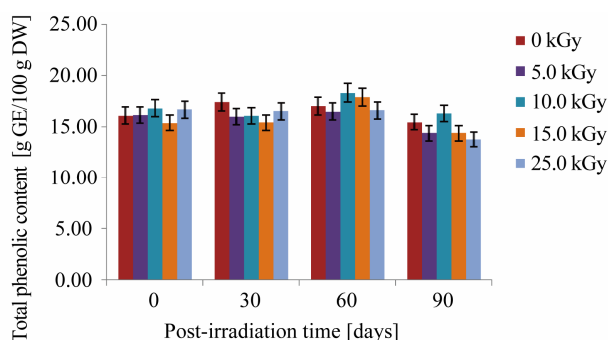


Figure 1. Effect of gamma irradiation on total phenolic content of pomegranate peel powder during 90 days of storage at 25°C.

composition, extraction solvent, extraction procedures, temperature, dose of gamma irradiation, etc. [22]. Our studies showed that, there is a non significant increase in TPC value for control (0 kGy), which was decreased after 90 days of storage. However for 10.0 kGy of irradiated dose level, there was a significant gradual increase after 30 and 60 days of storage period as compared to 0 day. At this dose level, TPC value was also found to be decreased after 90 days of storage period. Thus, gamma irradiation of 10.0 kGy dose level significantly affects the total phenolic content of pomegranate peel powder over 60 days of post irradiation storage period.

Post irradiation storage studies after 30, 60 and 90 days showed variable *in vitro* antioxidant activities (**Figure 2**). After 30 days of storage period, there was an increase in *in vitro* antioxidant activity for all the irradiated dose levels as compared to control (0 kGy). However, at 10.0 kGy there was a significant increase ($p < 0.05$) in TEAC value of peel extracts. Further storage studies for 60 and 90 days, also showed significant increase in TEAC values of irradiated peel powder extracts for 10.0 kGy irradiated dose level. Statistical analysis also showed that, there was a decrease after 60 days of storage period

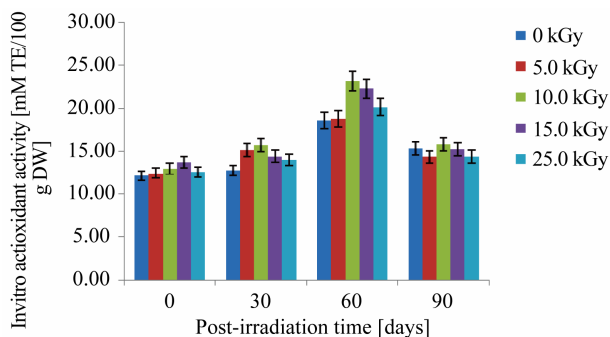


Figure 2. Effect of gamma irradiation on *in vitro* antioxidant activity of pomegranate peel powder during 90 days of storage at 25°C.

as compared to 0 day for all the irradiated dose level. The antioxidant concentration in a plant produce depends upon the time of evaluation, like analyzing immediately after the irradiation treatment or after certain period of time duration: Song *et al.* [23] reported that the total phenols analyzed in irradiated kale juice immediately after the irradiation, was significantly lower than the control. Irradiation also did not influence the antioxidant activities of cinnamon to a great extent, although a small decrease was observed in the range of 20 - 25 kGy [24]. Murcia *et al.* [25] carried out quantitative evaluation of antioxidant capacity based on Trolox Equivalent Antioxidant Capacity (TEAC) that can be used to provide a ranking order of antioxidants. They have also observed that, the irradiated samples did not show differences from non-irradiated ones in TEAC values. Our studies showed that, there was a positive correlation between total phenolic content and *in vitro* antioxidant activity of pomegranate peel powder extracts (control and irradiated). Post irradiation storage studies showed, a decrease in TEAC values after 90 days of storage period. This could be due to, relative decrease in total phenolic content as showed in **Figure 1**. The increase in total phenolic content was thus suggestive of enhanced anti-oxidant properties as shown earlier (**Table 1**). Therefore, for 10.0 kGy there was an increase in both TPC and TEAC values as compared to control (0 kGy) even after 60 days of post irradiation storage period.

Microbial Analysis

Table 2 shows, microbial counts (total plate counts and total fungal counts) measured by pour plate method in control and irradiated pomegranate peel powder. The initial mean populations of the total plate and total fungal counts for pomegranate peel powder were 3.2×10^3 CFU/g and 1.8×10^3 CFU/g respectively. Samples that were irradiated at 5.0 kGy and above dose levels did not show any bacterial and fungal counts. The immediate effects of radiation on food-borne microorganisms can be described by the different types of interactions (photon or electron interactions with particular atoms) [26]. The most important target of ionizing radiation in a microorganism is the DNA molecule. Any alteration or destruction of the DNA molecule can cause a cell to lose its ability to survive or reproduce as explained by Moreira *et al.* [27]. The results are in accordance with the reports of Alighourchi *et al.* [19] on pomegranate juice. In other studies, Thomas *et al.* [28] reported that irradiation dose of 7 kGy is effective to control microbial growth in black tea and in extending their shelf life without any significant deterioration of quality constituents. **Table 2** also showed that, after 90 days of post irradiation storage

Table 2. Effect of post irradiation on microbial load (CFU/g) of pomegranate peel powder during 90 days of storage at 25°C.

Irradiation dose (kGy)	Total Plate Count(CFU/g)*				Total Fungal Count (CFU/g)*			
	0 Day	30 Days	60 Days	90 Days	0 Day	30 Days	60 Days	90 Days
0	3200 ± 120	3300 ± 100	3400 ± 120	3550 ± 100	1800 ± 70	1850 ± 70	1950 ± 70	1900 ± 90
5.0 - 25.0	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

*Each value is expressed as mean ± standard deviation (n = 3).

period, total bacterial and total fungal count was nil for samples that were irradiated at dose levels of 5.0 kGy and above; indicating that those samples were microbiologically safe. Thus, gamma irradiation (does levels of 5.0 kGy and above) resulted in a complete removal of microbes and extended shelf life of pomegranate peel powder.

4. Conclusions

Pomegranate peel contains high amount of polyphenols and tannins but are often discarded as waste. These discarded peels then further results into microbial contamination. Studies at various dose level of gamma irradiation were carried out for sterilization of these peels. All the irradiated samples along with control (0 kGy) were then stored for 90 days, to check its effect on total phenolic content and *in vitro* antioxidant activity. Immediately, after the irradiation (0 day), there was a significant increase in total phenolic content and *in vitro* antioxidant activity at 10.0 kGy as compared to control (16.80 ± 0.15 g GE/100 g DW and 16.10 ± 0.21 g GE/100 g DW respectively). For control as well as for all the irradiated dose levels, post irradiation storage of 60 days, showed a significant increase ($p < 0.05$) in TPC and TEAC values of peel powder extracts that shows a good positive correlation between the two. After 90 days of storage, both the values were found to be decreased. There was also a complete removal of microbial load at 5.0 kGy and above dose levels. Thus, pomegranate peel powder, that has been irradiated at (5.0 - 10.0 kGy) could be used in various food products as a potential source of natural antioxidants. This irradiated peel powder can be stored for 60 days with stable nutrient activities.

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