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Low dose irradiation of plum peel anthocyanins: Impact on stability and antioxidant activity

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ABSTRACT

Consumer demand for both natural food colorants and non-thermal methods of preservation has increased overwhelmingly during the last few decades. Preventing loss of appeal and color through non-thermal methods without compromising on quality will provide an alternative to conventional thermal methods. In the present study, irradiation of plum peel anthocyanins after extraction and purification was done in a range of 0.1-1.0 kGy. Results revealed that degradation was significantly ($p \leq 0.05$) dependent on both time and dose of irradiation. The percentage degradation observed in the first 3 h of treatment was $9.0-21.0 \pm 1.2\%$. The maximum anthocyanin degradation recorded during 8 h of irradiation was 45.3%. Anthocyanin degradation was apparently less at 0.1-0.5 kGy compared to 0.6-1.0 kGy. The retention of anthocyanins was up to the extent of 88.8-62.3% in samples irradiated at 0.1-0.5 kGy. Comparison of the individual anthocyanin indicated that diglucoside anthocyanins were stable towards irradiation than monoglucoside anthocyanins. For monoglucoside anthocyanins, a 50% decrease was observed as the irradiation treatment exceeded 0.6 kGy. A close comparison of the data revealed that at irradiation dose of 1.0 kGy, the decrease in L^* and a^* values was almost four and three times higher than that observed in samples irradiated at 0.5 kGy and below; while an increase in b^* value was almost double. In spite of marginal degradation in color, low dose irradiation (up to 0.5 kGy) significantly ($p \leq 0.05$) increased the antioxidant activity of the plum peel anthocyanins.

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1.Introduction

Anthocyanins are a group of naturally occurring glycosidic compounds responsible for innumerable attractive shades and colours of fruits, vegetables and flowers present in nature.

Chemically anthocyanins are polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylum cation. Anthocyanidins are the basic structures of anthocyanins, hence anthocyanidins in their glycosidic form (bonded to a sugar moiety) are called as anthocyanins.

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The large structural diversity of anthocyanin pigments is attributed to the glycosylation and acylation of anthocyanidin (aglycone) moiety by different sugars and acids at different positions (1,2). These anthocyanin pigments are found in roots, leaves, flowers and fruits of plants and have received tremendous attention during the past few decades owing to their wide range of biological, pharmacological, chemotherapeutic, immune modulating and potential antioxidant activities (3).

It is reported that anthocyanins besides imparting colour to food product also play important role in maintaining good health (4,5). Earlier reports reveal that anthocyanins help in reducing the risk of coronary heart diseases, have antidiabetic properties, protect against Alzheimer's disease and prevent several other chronic diseases due to their free radical scavenging effect (6-8). In spite of the vast potential health benefits of the natural anthocyanins extracted from plant based products, their use is limited in food industry because of their low stability under processing and storage conditions compared to food grade artificial dyes. The stability of the anthocyanins is markedly influenced by combination of chemical and environmental factors such as pH, temperature, anthocyanin concentration and structure, light, oxygen and other accompanying factors (9-11). Among the various factors which influence the anthocyanin stability, conventional heat treatment is one of the most widely studied factor as it is largely used method of preservation and shelf-life extension of foods including juices. Reports reveal that magnitude and duration of heating has a strong influence on anthocyanin stability and degradation rate (12). Several studies reported a logarithmic

degradation of anthocyanin with an increase in temperature (13,14). The high temperatures in combination with pasteurisation involved in various processing operations could result in about 50% loss in total monomeric anthocyanins compared to original levels found in fresh fruits and vegetables (15). Degradation of anthocyanins due to heat treatments for many fresh fruits and vegetables such as red cabbage (16), raspberry (17), pomegranate (18), blackberry (19), blueberry (20) and black carrot (21) has been studied extensively. The results obtained from these earlier studies indicate that heat labile factors can accelerate anthocyanin degradation and strongly supports the hypothesis that endogenous enzymes in fruits causes pigment destruction during processing.

Increased consumer demand for nutritious foods, which are minimally processed, without using artificial preservative, has led to an interest and search for non-thermal technologies, which can ensure product safety yet maintain the desired nutritional and sensory characteristics (22). Non thermal technologies are preservation treatments that are effective at ambient or sub-lethal temperatures, thereby minimising negative thermal effects on food nutritional and quality parameters. Among the various non thermal technologies currently in use for preservation purposes, food irradiation is one such method. Irradiation (also known as cold pasteurisation) of food is done by exposing the product to a source of ionizing energy. It is a physical means of food processing that involves exposing pre-packaged or bulk foodstuffs to gamma rays (Cobalt-60), X-rays, or accelerated electrons. Irradiation induces negligible or subtle losses of nutrients and sensory qualities in food compared to

thermal processing as it does not substantially raise the temperature of food during processing (23). Literature survey based on very few studies conducted till date reveals that induced effects of radiation treatment on anthocyanins depend on the type of anthocyanin and amount of dose applied. Alighourchi et al. (24) reported a significant reduction in the total and individual anthocyanin content in pomegranate juice after irradiation at higher doses. In a more recent study, Najafabadi et al. (25) reported that decrease in monoglycoside anthocyanins of jujube fruit was significant at doses above 1 kGy. However; to our best knowledge, there hardly seems to be any information available till date regarding the effect of low dose irradiation on degradation of plum peel anthocyanins and their antioxidant activity. Further the use of non-thermal methods in achieving the thermal pasteurization effects in liquid foods is increasing day by day; hence, the objective of current present study is to evaluate the effect of low dose irradiation on degradation of monomeric anthocyanins and changes in visual colour. The present study will prove beneficial in providing first hand information pertaining to effect of radiation treatments on anthocyanin stability and exploring the possible use of technology in food industry.

2. Materials and Methods

2.1. Raw materials and chemicals

Mature and fully ripe plum fruit of Santarozza variety was procured from plum orchards of Sheri Kashmir University of Agricultural Sciences and Technology, Shalimar, Kashmir during the month of July 2019 and 2020. The procurement of fruits was done from the pre-

selected plum trees for both the years of study. Fruit after harvest and subsequent package in cardboard boxes of size $0.5 \times 0.3 \times 0.3 \text{ m}^3$ was kept at $2 \pm 1^\circ\text{C}$ till further use. All the chemicals used in the present study were of high-purity analytical grade. Ethanol, HCl, Amberlite XAD 7N resins, HPLC-grade standards, i.e., cyanidin-3-glucoside, peonidin-3-glucoside, cyaniding-3-rutinosid, peonidin-3-rutinoside and cyaniding-3, 5-diglucoside, formic acid, methanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and other routine based chemicals were purchased from Sigma Aldrich and Hi-Media, Mumbai, India.

2.2. Extraction and concentration of anthocyanins

The extraction of plum peel anthocyanins was done as per the previously optimized procedures with slight modifications (26). Briefly 50 g of homogenized plum peel was extracted with ethanol: 1.5 N HCl (85:15, v/v) using sample to solution ration of 1:3. For efficient and complete extraction, the samples were kept overnight under dark. After extraction, extracts were combined and concentrated at 40°C under reduced pressure using rotary vacuum evaporator.

2.3. Purification and lyophilization of anthocyanins

Anthocyanins were purified using column chromatography. The concentrated samples after diluted with distilled water were loaded onto Amberlite XAD 7N macropore absorptive resins which was washed with 100 mL of distilled water. The absorbed anthocyanins were recovered with about 1000 mL of acidified ethanol. To remove the solvent, the ethanol elute was concentrated and stored at 2°C until use. Following concentration, anthocyanin extract was

freeze dried and stored in amber glass bottles under dark conditions.

2.4. Gamma irradiation treatment

Known weight of lyophilized anthocyanin extract was taken in different test tubes, re-dissolved in acidified water (100 mL) and irradiated at 0.1-1.0 kGy using Panoramic Batch Irradiator (BRIT, BARC, Mumbai, India). Following irradiation, samples were monitored immediately for changes in anthocyanins and activity. Triplicate samples were used for each irradiation treatment. The control samples did not receive and dose.

2.5. Determination of total anthocyanin content

Total anthocyanins were determined according to the pH- differential method (27). Clear extract (1 mL) of both irradiated and un-irradiated sample was taken in volumetric flask (25 mL) and volume made up with pH 1.0 buffer (1.49 g of KCl/100 mL water and 0.2 N HCl, with a ratio of 25:67). Another 1 mL of sample taken in another 25 mL volumetric flask, and volume made up with pH 4.5 buffer (1.64 g of sodium acetate/100 ml of water, adjusted to pH 4.5 with 0.2 N HCl). Absorbance was calculated as $\Delta A = (A_{510nm} - A_{700nm})_{pH 1.0} - (A_{510nm} - A_{700nm})_{pH 4.5}$. Total anthocyanins as mg of cyanidin -3- glucoside equivalents per 100 g of sample were calculated as $\text{Total anthocyanins (mg/100 g)} = (\Delta A / \epsilon L) \times MW \times D \times (V/G)$ (equation 1).

where ΔA is absorbance, ϵ the cyanidin3-glucoside molar extinction coefficient (26,900), L the cell path length (1cm), MW the molecular weight of anthocyanin (449.2), D a dilution factor, V the final volume (ml) and G the sample weight (g).

2.6. HPLC analysis of individual anthocyanins

The identification and quantification of individual plum peel anthocyanins was done by HPLC (JASCO, Japan) method. Prior to analysis, the extracted anthocyanin samples were centrifuged in a low temperature centrifuge at 14000 rpm for about 15 min. Aliquot of 20 μ L centrifuged sample was injected for estimation purposes in a C-18 column which was prewashed thoroughly with methanol followed by the mobile phase for 1 h. The analysis of the anthocyanins was carried at a detector (UV-visible) wavelength of 520 nm. The mobile phase used was 100% methanol (A) and 10% formic acid (B). Elution program employed was 0–20 min, 9–35% (A); 20–30 min, 35% (A); 30–40 min, 35–50% (A); and 40–55 min, 50–9% (A). The mobile phase was set at a flow rate of 1 mL/min. Anthocyanins were identified by comparison of the retention times (Rt) with standard samples. The quantification was performed with the help of standard curve.

2.7. Color measurement

The colour change during radiation treatment was monitored by a Ultra Scan VIS (HunterLab, USA) according to the previously used method (28). The color was expressed in L^* , a^* , b^* , where the L^* represents lightness ($L^* = 0$ yields black and $L^* = 100$ denotes white), a^* expresses red (+) or green (-), and b^* indicates yellow (+) or blue (-). L^* , a^* and b^* parameters were measured against a white calibration plate and were directly obtained from the apparatus. In the present study, 2 mL sample was diluted with 20 mL water for color measurement. ΔE was calculated by the following equation

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \text{ (equation 2)}$$

Here, ΔL^* , Δa^* , and Δb^* , represent the change in the color coordinates compared to sample before irradiation treatment

2.8. Determination of polymeric colour index

Bleaching method (27) was used to measure polymeric colour index after diluting the samples in a citrate-phosphate buffer at a pH of 2.2 (1:20 v/v). Four mL of the diluted samples were added to 1 mL of metabisulfite (1 M) or citrate-phosphate buffer for measuring the polymeric color and color density respectively after equilibrating for 40 min. Polymeric color index was determined as

Polymeric color index = Polymeric colour / Color density (equation 3)

Polymeric color = $[(A_{420} - A_{700}) + (A_{525} - A_{700}) \times$ dilution factor (equation 4)

Where A is the absorbance measured in the sample mixed with metabisulfite.

Color density = $[(A_{420} - A_{700}) + (A_{525} - A_{700}) \times$ dilution factor (equation 5)

Where A is the absorbance measured in the sample mixed with citrate-phosphate buffer.

2.9. Determination of antioxidant activity

Known weight of the lyophilized anthocyanin extract was dissolved in acidified water so as to get a working concentration of 0.1 mg/mL. Different volumes of working solution were taken in different test tubes and irradiated at 0.1–1.0 kGy. Following irradiation, antioxidant activity of both irradiated and un-irradiated anthocyanin extracts was determined by using DPPH radical scavenging and ferric reducing ability power (FRAP) method. DPPH radical scavenging activity and ferric reducing ability power

was measured according to the previously described methods with slight modifications (29,30).

2.10. Statistical analysis

The data were analyzed statistically using completely randomized design experiment (31). using MINITAB statistical analysis software package (Minitab, version 11.12, 32 bit, Minitab, Inc., State College, PA, USA).

3. Results

3.1. Effect of irradiation time and dose on anthocyanin degradation

Effect of irradiation time and dose on anthocyanin degradation is shown in Figure 1. It is evident that percentage degradation observed in first 3 h of treatment was in the range of 9.0–21.0±1.2%. Between 5-8 h of irradiation, the anthocyanins contents differed non-significantly ($p \geq 0.05$).

The maximum degradation recorded during 8 h of irradiation was of the order of 45.3%. The data pertaining to irradiation dose indicated that anthocyanin degradation was apparently less between 0.1–0.5 kGy compared to 0.6–1.0 kGy range. The retention in anthocyanin content of plum peel irradiated between 0.1–0.5 kGy was 88.8–62.3%. Data further indicated that maximum degradation of anthocyanins observed at 1.0 kGy was 60.9%.

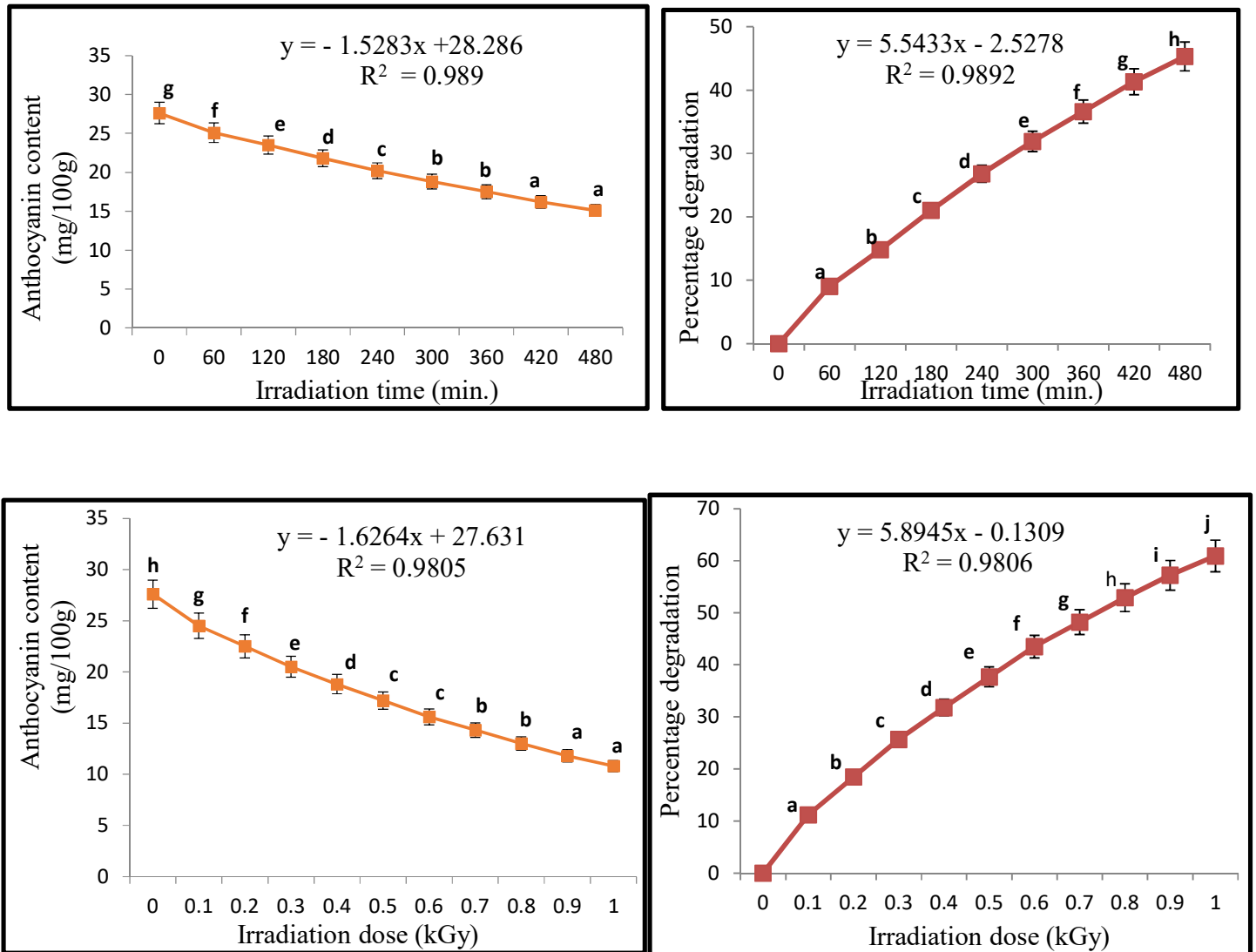


Figure 1. Effect of irradiation time and dose on anthocyanin content and degradation percentage

3.2. Effect of radiation treatment on stability of individual anthocyanins

Effect of radiation treatment on stability of individual anthocyanins of plum peel is depicted in Table 1. Presented data revealed the presence of six main anthocyanins in plum peel (Figure 2) as analysed by HPLC. Out of the six anthocyanin pigments; four were monoglucoside anthocyanins (cyaniding-3-glucoside, cyaniding-3-rutinoside, peonidin-3-glucoside and peonidin-3-rutinoside) and two were diglucoside anthocyanins (cyaniding-3, 5-diglucoside and pelargonidin-3, 5-diglucoside).

Among the monoglucoside anthocyanins, cyaniding-3-rutinoside was the dominant pigment present at a concentration of 9.5 ± 0.61 mg/100 g followed by peonidin-3-rutinoside (6.7 ± 0.42 mg/100 g) and cyanidin-3-glucoside (4.2 ± 0.51 mg/100 g). The two diglucoside anthocyanins were present at a concentration of 1.9 ± 0.12 mg/100 g and 1.1 ± 0.11 mg/100 g respectively. The present study revealed that diglucoside anthocyanins were relatively stable to irradiation compared to monoglucoside anthocyanins which is in conformity to the earlier findings. In the present study, the average radiation induced decrease in diglucoside anthocyanins was 35.6% for pelargonidin-3, 5-diglucoside and 36.2% for cyaniding-3, 5-diglucoside. However, in case of monoglucoside anthocyanins, decrease was higher in cyaniding-3-glucoside and peonidin-3-glucoside; while as cyanidin-3-rutinoside exhibited an average decrease of 39.9% following irradiation.

3.3. Colour changes and polymeric colour index

The colour change during anthocyanin irradiation was monitored visually and characterized by the CIELAB color-space.

Colour changes during radiation treatment of plum peel anthocyanins are shown in Table 2. After irradiation, the color of the anthocyanins changed in a dose dependent manner and progressed significantly ($p \leq 0.05$) from red to pale red at doses above 0.5 kGy and finally to brown colour (Figure 3). The data presented indicated that before irradiation, color coordinate CIE L^* , a^* and b^* values of anthocyanins were 45.3 ± 0.01 , 71.3 ± 0.01 and 17.5 ± 0.06 , respectively. After irradiation, lightness (L^*) and redness (a^*) values decreased from 44.6 ± 0.02 to 30.7 ± 0.04 and 69.9 ± 0.04 to 53.4 ± 0.12 respectively and decrease was significant beyond 0.5 kGy. In contrast, the yellowness (b^*) value increased from 18.4 ± 0.16 to 38.8 ± 0.07 and increase was non-significant up to 0.3 kGy. The ΔE values were relatively lower in samples irradiated up to 0.5 kGy and increased significantly ($p \leq 0.05$) beyond dose of 0.5 kGy.

Table 1. Effect of irradiation treatments on individual anthocyanins (mg/100g) of plum peel.

Dose (kGy)	C3G	C3R	P3G	P3R	C 3, 5 dG	Pg 3,5 dG
0	4.2±0.51 ^e	9.5±0.61 ^f	3.2±0.32 ^c	6.7±0.42 ^e	1.9±0.12 ^d	1.1±0.11
0.1	3.8±0.31 ^e (9.5)	8.4±0.52 ^e (11.6)	2.8±0.31 ^c (12.5)	5.9±0.43 ^d (11.9)	1.7±0.13 ^c (10.5)	0.99±0.04 ^c (10.0)
0.2	3.4±0.46 ^d (19.0)	7.7±0.56 ^e (18.9)	2.6±0.30 ^c (18.7)	5.4±0.37 ^d (19.4)	1.6±0.10 ^c (15.8)	0.93±0.07 ^c (15.4)
0.3	3.1±0.47 ^d (26.2)	7.0±0.57 ^d (27.2)	2.3±0.28 ^b (28.1)	4.9±0.35 ^c (26.9)	1.5±0.11 ^c (21.0)	0.86±0.06 ^b (21.8)
0.4	2.8±0.44 ^c (33.4)	6.3±0.54 ^d (33.7)	2.1±0.25 ^b (34.4)	4.4±0.32 ^c (34.3)	1.3±0.10 ^b (31.6)	0.76±0.05 ^b (30.9)
0.5	2.6±0.41 ^c (38.1)	5.8±0.47 ^c (38.9)	1.9±0.22 ^b (40.6)	4.1±0.29 ^b (38.8)	1.2±0.08 ^b (36.8)	0.71±0.04 ^b (35.4)
0.6	2.2±0.35 ^b (47.6)	5.2±0.41 ^c (45.3)	1.7±0.18 ^b (46.9)	3.7±0.25 ^b (44.8)	1.1±0.07 ^b (42.1)	0.66±0.04 ^a (40.0)
0.7	1.9±0.21 ^b (54.8)	4.9±0.31 ^b (48.4)	1.6±0.14 ^a (50.0)	3.3±0.22 ^b (50.7)	0.98±0.05 ^a (48.4)	0.59±0.03 ^a (43.4)
0.8	1.8±0.18 ^a (57.1)	4.4±0.21 ^b (53.7)	1.4±0.12 ^a (56.2)	3.0±0.16 ^a (55.2)	0.95±0.05 ^a (50.0)	0.55±0.03 ^a (50.0)
0.9	1.6±0.15 ^a (61.9)	3.9±0.18 ^a (58.9)	1.2±0.11 ^a (62.5)	2.8±0.14 ^a (58.2)	0.93±0.03 ^a (51.0)	0.52±0.01 ^a (52.7)
1.0	1.4±0.13 ^a (66.7)	3.5±0.15 ^a (63.1)	1.0±0.10 ^a (65.6)	2.4±0.12 ^a (64.2)	0.86±0.02 ^a (54.7)	0.48±0.01 ^a (56.4)
LSD	0.4	0.7	0.6	0.8	0.2	0.2
Avd. (%)	41.4	39.9	41.5	40.4	36.2	35.6

Values are mean±SD (n=3); Avd. = average degradation; LSD = least significant difference (p ≤ 0.05)

Values in parenthesis is the percentage degradation in particular anthocyanin during irradiation

C3D = cyanidin-3-glucoside; C3R = cyanidin-3-rutinoside; P3G = peonidin-3-glucoside; P3R = peonidin-3-rutinoside; C 3, 5 dG = cyanidin-3,5-diglucoside; Pg 3,5 dG = pelargonidin-3,5-diglucoside

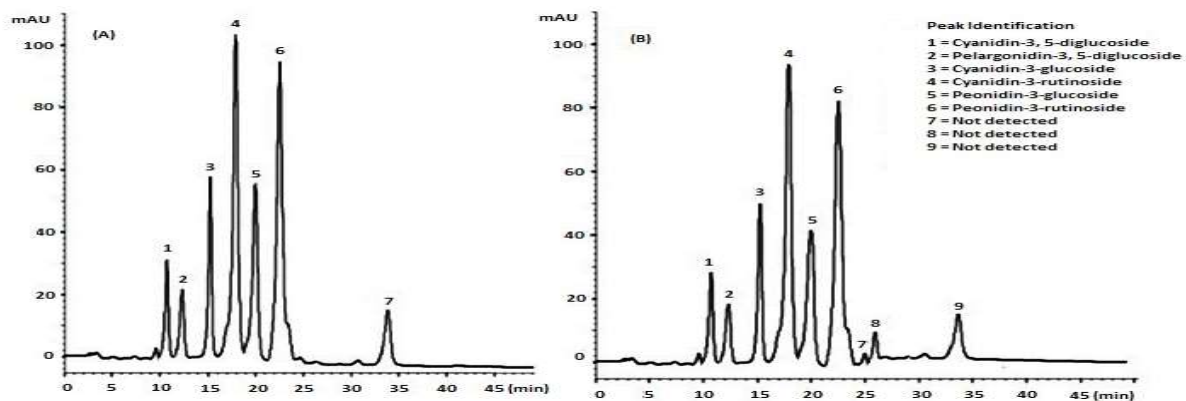


Figure 2. HPLC chromatograms of un-irradiated (A) and irradiated 1.0 kGy (B) plum peel anthocyanins

Table 2. Effect of low dose irradiation treatments on colour values and polymeric color index of plum peel anthocyanins

Dose (kGy)	L	a	b	ΔE	PCI
0	45.3±0.01 ^d	71.3±0.01 ^d	17.5±0.06 ^a	-	-
0.1	44.6±0.02 ^c	69.9±0.04 ^d	18.4±0.16 ^a	1.8±0.16 ^a	0.124±0.001 ^a
0.2	43.4±0.03 ^c	69.2±0.11 ^d	19.5±0.06 ^a	3.5±0.05 ^a	0.162±0.003 ^a
0.3	42.8±0.02 ^c	68.4±0.01 ^c	20.8±0.02 ^a	5.1±0.03 ^a	0.196±0.002 ^a
0.4	42.1±0.01 ^c	66.1±0.01 ^c	22.2±0.00 ^b	7.7±0.02 ^b	0.202±0.003 ^a
0.5	41.4±0.04 ^c	64.9±0.04 ^c	23.6±0.08 ^b	9.7±0.04 ^b	0.222±0.011 ^a
0.6	39.4±0.12 ^b	59.7±0.13 ^b	27.4±0.34 ^c	16.4±0.23 ^c	0.281±0.013 ^b
0.7	38.3±0.12 ^b	58.5±0.11 ^b	29.3±0.53 ^c	18.7±0.45 ^c	0.332±0.021 ^b
0.8	36.2±0.44 ^b	56.7±0.19 ^a	32.4±0.76 ^d	22.7±0.63 ^d	0.373±0.031 ^b
0.9	33.8±0.04 ^a	55.2±0.12 ^a	35.3±0.07 ^d	26.6±0.78 ^e	0.421±0.033 ^c
1.0	30.7±0.04 ^a	53.4±0.12 ^a	38.8±0.07 ^e	31.4±0.92 ^f	0.453±0.031 ^c
LSD	3.2	3.7	3.8	3.7	0.1

Values are mean±SD (n=3); LSD = least significant difference ($p \leq 0.05$); PCI = polymeric color index, ΔE = color difference
 Values within treatments in a column with different superscript lowercase letters differ significantly ($p \leq 0.05$).

The effect of irradiation treatment on the formation of polymeric colour index in plum peel anthocyanins is depicted in Table 2. It was observed that polymeric colour index ranged from 0.124–0.453 and was significantly higher in samples irradiated at doses above 0.5 kGy.

Positive correlation ($r = 0.86$) existed between irradiation treatment and polymeric colour index, thereby indicating that polymeric colour formation increases with irradiation dose.

The significant increase in polymeric colour index in samples irradiated at doses above 0.5 kGy may be attributed to formation of chalcone upon anthocyanin degradation and further degradation of chalcone to brown products.

A more reasonable explanation for the enhancement of polymeric colour index during irradiation treatment is the reaction among anthocyanins. Further, the comparison of HPLC chromatograms of unirradiated and irradiated (1.0 kGy) anthocyanin samples indicated the presence of some new peaks in irradiated chromatogram, which could indicate the degradation of anthocyanins and formation of polymeric compounds.

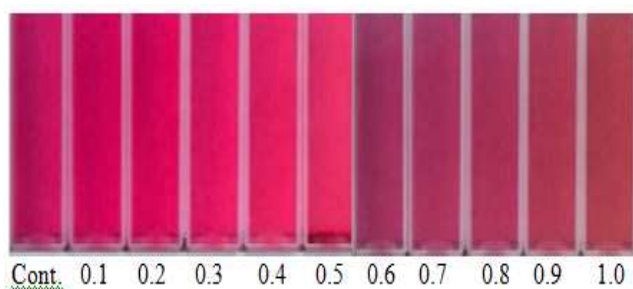


Figure 3. Comparison of visual color quality of un-irradiated and irradiated plum peel anthocyanins

3.4. Antioxidant activity

3.4.1. DPPH radical scavenging activity and ferric reducing ability power (FRAP)

Among the various antioxidant assays currently being used, DPPH and FRAP are most widely used methods to evaluate the antioxidant potential due to their reproducibility, accuracy, reliability, high sensitivity and good correlation with bioactive compounds with regression factor $R > 0.8$. Further, the demand of performing DPPH test is due to the fact that radical scavenging time is 30 min. which allows DPPH to react efficiently even with weak antioxidants; while as FRAP can screen a wide spectrum of biological samples including plasma, blood samples, saliva, organic extracts of drugs etc. in addition to foods and plants.

The effect of gamma irradiation treatment on DPPH radical scavenging activity and ferric reducing ability power of plum peel anthocyanins is plotted in Fig. 4 (A, B). It is clear that DPPH radical scavenging activity as well as ferric reducing ability power of plum peel anthocyanins increased with increase in irradiation dose compared to un-irradiated control and the increase was significant ($p \leq 0.05$) up to 0.5 kGy dose. Beyond 0.5 kGy dose, DPPH radical scavenging activity as well as ferric reducing ability power showed a decreasing trend and decrease was marginal ($p \geq 0.05$). DPPH radical scavenging activity of control sample was 58.2% compared to 62.3 ± 0.27 – $79.1 \pm 0.35\%$ in irradiated samples. The ferric reducing ability power of control (reported as OD at 700 nm) was of the order of 0.42 ± 0.02 compared to 0.48 ± 0.03 – 0.78 ± 0.04 in case of irradiated samples. Highest DPPH radical scavenging activity ($79.1 \pm 0.35\%$) and ferric reducing ability (0.78 ± 0.04) was observed in samples irradiated at 0.5

kGy, thereby resulting in an increase of 20.9% in DPPH radical scavenging activity and 85.7% in ferric reducing ability power over control. The corresponding decrease in DPPH radical scavenging activity and FRAP from their maximum values obtained at 0.5 kGy dose was of the order of 5.4% and 7.7% at 0.6 kGy dose; 6.3% and 12.8% at 0.7 kGy dose; 7.2% and 20.5% at 0.8 kGy dose; 8.3% and 24.3% at 0.9 kGy dose and 11.4% and 30.7% at 1.0 kGy dose respectively. From the data, it is clear that radiation induced decrease at doses beyond 0.5 kGy was higher in FRAP in comparison to DPPH radical scavenging activity.

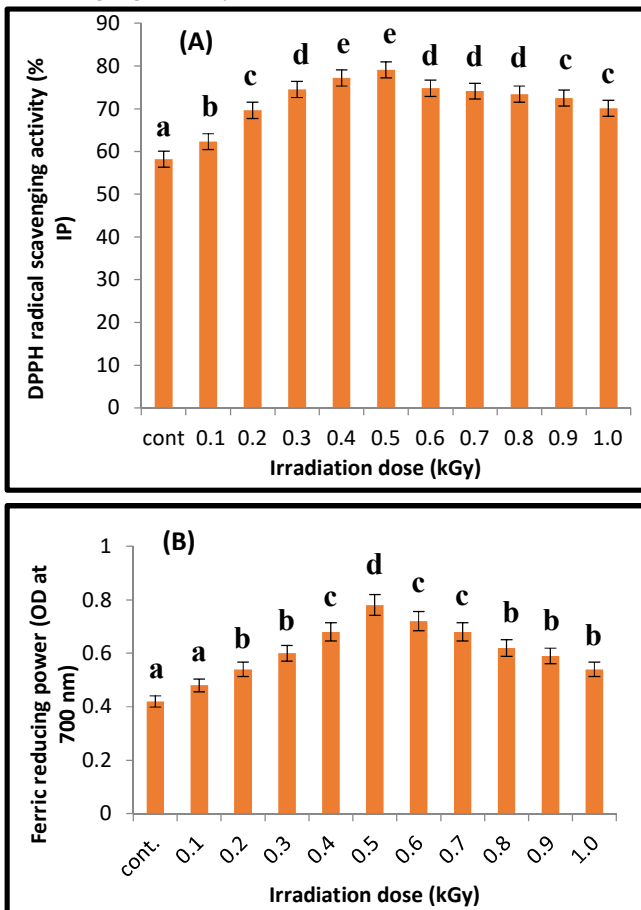


Figure 4. Effect of irradiation dose on DPPH radical scavenging activity (A) and ferric reducing ability power (B) of plum peel anthocyanins

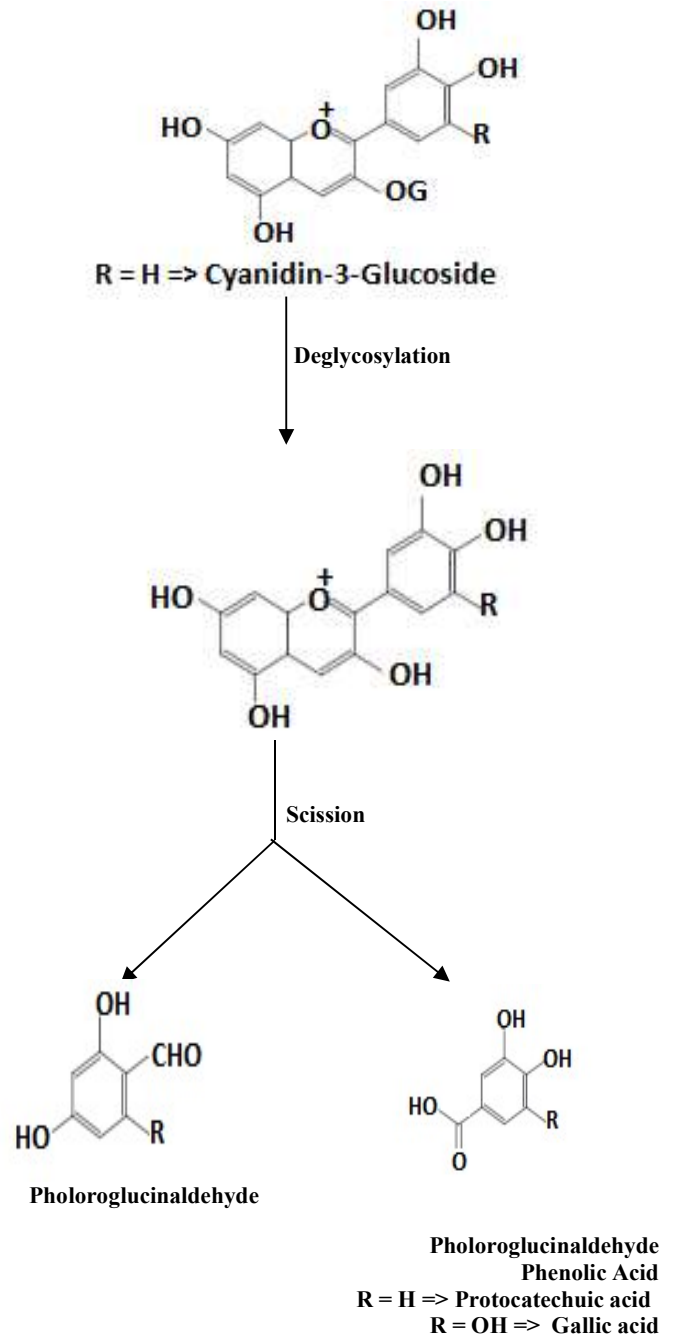


Figure 5. Mechanism of degradation of anthocyanins and formation of phloroglucinaldehyde and phenolic acids

4. Discussion

Anthocyanin pigments have received tremendous attention during the past few decades owing to their wide range of biological, pharmacological, chemotherapeutic, immune modulating and potential antioxidant activities (3). It is reported that anthocyanins besides imparting colour to food product also play important role in maintaining good health (4, 5). Earlier reports reveal that anthocyanins help in reducing the risk of coronary heart diseases, have antidiabetic properties, protect against Alzheimer's disease and prevent several other chronic diseases due to their free radical scavenging effect (6-8). The stability of the anthocyanins is markedly influenced by combination of chemical and environmental factors such as pH, temperature, anthocyanin concentration and structure, light, oxygen and other accompanying factors (9-11). Among the various factors which influence the anthocyanin stability, conventional heat treatment is one of the most widely studied factors. Reports reveal that magnitude and duration of heating has a strong influence on anthocyanin stability and degradation rate (12). Increased consumer demand for nutritious foods, which are minimally processed, without using artificial preservative, has led to an interest and search for non-thermal technologies, which can ensure product safety yet maintain the desired nutritional and sensory characteristics (22). Results based on very few studies conducted on anthocyanins till date reveals that irradiation induced changes depend on the type of anthocyanin and amount of dose applied (24).

The results of the present study also do indicate that anthocyanin degradation was highly dependent on both time and dose of irradiation. The degradation of anthocyanins increased with time as well as dose, but the degradation was faster during the first three h of irradiation. Positive correlations existed between both anthocyanin degradation and irradiation time ($r = 0.92$) and dose ($r = 0.94$). The faster degradation of plum peel anthocyanins in the initial h of treatment is attributed to the fact that during initial h of irradiation, the anthocyanin concentration being higher; hence likelihood of interactions between anthocyanins and radiolytically generated products are higher which causes degradation of anthocyanins. However, towards late h of irradiation the interaction chances between anthocyanins and radiolytic products become lower due to exhaustion of anthocyanin contents (32). The degradation of anthocyanins during irradiation has also been reported in Jujuba fruit (25). Alighourchi et al. (24) reported that average loss of total anthocyanins in juices of three pomegranate varieties after irradiation (0 - 10 kGy) were of the order of 22.0% and 90.0%. The degradation of anthocyanins during irradiation is associated with the free radical induced cleavage of glycosidic bonds of anthocyanins and eventually the formation of colourless products (33). Further an increase in hydroxylation due to the loss of glucosylation or free radical substitution increases the instability of anthocyanins under different conditions (24). Changes in the content of individual anthocyanins following irradiation demonstrated that anthocyanin pigments recorded a decreasing trend with increase of irradiation dose; however decrease was dependent upon anthocyanin composition and structure.

It is reported that sugar substitution increases the stability of anthocyanin molecule. During radiation processing under different conditions, increase in hydroxylation favours the instability of anthocyanins whereas an increase in glycosylation confers the greater stability to the anthocyanins (34). Further during irradiation, stability of the individual anthocyanins is also influenced by irradiation dose applied besides other factors such as water content and structure of material (24). Alighouechi et al. (24) also reported that diglucoside anthocyanins were stable to low dose irradiation compared to monoglucoside anthocyanins. Higher stability of diglucoside anthocyanins compared to monoglucoside anthocyanins to irradiation under different conditions has also been reported by other researchers (35,36). Further; our study indicated that for monoglucoside anthocyanins, 50% decrease in anthocyanins was observed as the irradiation treatment exceeded beyond 0.6 kGy.

Color of anthocyanins that is strongly affects the quality and appeal. The decrease in a^* values indicates that the color has considerably changed upon irradiation at doses above 0.5 kGy. Close comparison of the data revealed that at irradiation dose of 1.0 kGy, the decrease in L^* and a^* values was almost four and three times higher than that observed in samples irradiated at 0.5 kGy and below; while as increase in b^* value was almost double. Our results are in agreement with earlier reports available in literature. Najafabadi et al. (25) reported that lightness (L^*) value of Jujube juice anthocyanins decreased significantly by increasing the irradiation dose above 0.6 kGy while as the a^* and b^* values changed slightly until the dose of 5 kGy. On the other hand Naresh et al. (37) reported a significant

decrease in L^* value and increase in a^* and b^* values of mango juice irradiated at doses of 0 – 3 kGy. In contrast, Lee et al. (35) observed a significant reduction in both a^* and b^* values in fresh and stored ready to use tamarind juice after gamma irradiation at 0–5 kGy. Several previous reports have shown that loss of anthocyanins was accompanied with increase in polymeric colour index (4,5). Our results also show a strong negative correlation ($r = -0.83$) between anthocyanin content and polymeric colour index; hence indicating that degradation of anthocyanins is accompanied by formation of polymer colour.

In view of the growing interest in screening and quantifying antioxidants from natural products with the objective to combat free radical related pathological complications; antioxidant assays play a crucial and important role in high-throughput and cost-effective assessment of antioxidant capacities of natural products such as food samples and other medicinal products.

The DPPH and FRAP assays used for measuring the antioxidant capacity are both electron transfer based assays. DPPH assay measures the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of the colour change is correlated with the samples antioxidant activity. The FRAP method is based on the reduction of Fe^{3+} to Fe^{2+} forming an intense blue coloured ferrous complex under acidic conditions. The increase in antioxidant activity of plum peel anthocyanins treated with gamma irradiation reported in the present study may be due to degradation of anthocyanins in to the products that have higher antioxidant capability (38, 39). When anthocyanins are irradiated, the hydroxyl

radicals generated precede the cleavage of glycosidic bonds of anthocyanins resulting in the formation of aglycone anthocyanidins. Following deglycosylation, scission reaction take place and result in the degradation products of phenolic acids such as protocatechuic acid, gallic acid and phloroglucinaldehyde (5). These degradation products have antioxidant activity comparable to the commercial antioxidant such as butylated hydroxytoluene (38). The increase in antioxidant activity of anthocyanins in spite of their degradation has also been reported earlier and has been attributed to the formation of phloroglucinaldehyde and phenolic acids (34,40). These earlier findings support the increased antioxidant activity of irradiated plum peel anthocyanins observed in the present study. Variyar et al. (41) also demonstrated breaking the glycosidic bonds of polyphenols and releasing of soluble phenols during irradiation; leading to an increase of antioxidant rich phenolics responsible for higher antioxidant activities. A more recent study on black soybean extracts also demonstrated that increase in activity occurred in a dose-dependent manner (42). Sarver et al. (43) also reported similar effects on antioxidant activity of dried apricot and quince irradiated by gamma and electron beam in a dose dependent manner.

5. Conclusion

The present investigation revealed that anthocyanin degradation was dependent both on time as well as dose of irradiation; however, degradation was faster up to first 3 h of irradiation. Comparison of individual anthocyanins indicated that stability of diglucoside anthocyanins towards irradiation was higher than

monoglucoside anthocyanins. In spite of anthocyanin degradation, low dose irradiation (up to 0.5 kGy) increased the antioxidant activity of the plum peel anthocyanins. Finally, it is recommended that plum peel anthocyanins can be irradiated without any appreciable color change with doses not exceeding 0.5 kGy. However, the studies demand further investigation to ensure microbiological safety and retention of other quality attributes in juices and beverages prepared from anthocyanin rich fruits at the above optimized dose range.

Conflict of interest

The authors of the present study declare no conflict of interest.

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