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Influence of Gamma Irradiation Treatment on Storage Quality, Antioxidant Composition and Nutraceutical Potential of Onion Sprouts

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Abstract: Ten days old onion sprouts after harvest were gamma irradiated in the dose range of 0.5–1.5 kGy followed by storage under refrigerated conditions (3 ± 1°C, RH 85%) for 6 days. Samples were evaluated at intervals of 1 day for storage quality, bioactive contents, antioxidant and hypoglycemic activities. Results of the study revealed that gamma irradiation treatment (1.5 kGy) besides maintaining the external appearance, texture and appeal; significantly ($p \leq 0.05$) increased the content of bioactive compounds and enhanced the nutraceutical potential of irradiated sprouts. For both control and irradiated sprouts, content of the bioactive compounds especially total phenolics increased during storage and reached its maximum values at 3 days with concomitant increase in phenylalanine ammonialyase (PAL) enzyme activity. The results of the antioxidant activity showed a significant ($p \leq 0.05$) decrease in EC_{50} values and a corresponding increase in antioxidant content and activity due to irradiation. Data analysis indicated strong positive correlation of total phenols for reducing power ($r = 0.89$), beta-carotene bleaching assay ($r = 0.95$), hydroxyl radical scavenging ($r = 0.92$) and ferrous ion chelating effect ($r = 0.93$). Comparison of the hypoglycemic activity revealed that irradiation treatment resulted in significant ($p \leq 0.05$) inhibition of alpha-glucosidase activity compared to alpha-amylase activity. All the extracts inhibited α-amylase and α-glucosidase activities in concentration-dependent manner. The present investigation suggested that radiation processing of onion sprouts besides extending the storage life has a potential to enhance their antioxidant content and nutraceutical potential.

Keywords: Onion Sprouts, Gamma Irradiation, Antioxidant Composition, Nutraceutical Potential

1. Introduction

Sprouts are the germinated seeds of legumes, grains and bulb crops, which are full of rejuvenating and health promoting qualities. Unlike other sprouts, onion sprouts are the micro-shoots of the onion plant and take longer time to grow than other sprouts. Sprouts are believed to be rich in health promoting phytochemicals compared to their mature counterparts. Sprouting has been suggested as an inexpensive and effective way to improve the quality of food by increasing the levels of health promoting antioxidants and decreasing of some anti-nutritional factors [1 – 3]. Sprouts are also rich in fiber, proteins, minerals, vitamins (A, C, E) and enzymes. Being rich in dietary fiber, sprouts promote healthy bowel movements and intestinal functions. Sprouts assist in activating the immune system and cleansing of the body. Those high in chlorophyll are especially effective at removing toxins from the cells and lymphatic system [4, 5]. As the seed or bulb is nutritionally activated through the process of sprouting, multitude of chemical changes occur to mobilize the stored carbohydrate and protein reserves into the growing sprout resulting in breakdown of starches into simpler carbohydrates, fats into fatty acids and proteins into amino acids [6, 7]. This, in effect, makes these nutritional properties more bio-available and improves the nutritional value of the sprout significantly [8].

Initially the consumption of sprouts was limited to countries like China, Korea and Japan but more recently their consumption has spread to other countries, such as Europe, Australia and the United States [9], where different types of

seeds such as adzuki beans, alfalfa, buckwheat, watercress, cabbage, mustard, radish, red cabbage and soybeans are appreciated by the consumers. The reason that consumption of ready-to-eat sprouts has increased during the past few years is attributed to their high content of nutrients, notably amino acids, peptides, vitamin and minerals [10 – 12] and their lower content of non-nutrients such as trypsin inhibitors, galactosides, tannins and lectins [13, 14] compared to non-germinated analogs. Previous studies also revealed that sprouts are good sources of antioxidants and possess high antioxidant activity. Laus et al. [15] reported that an increase up to three times in antioxidant capacity of sprouts compared to seeds as measured by trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) methods.

Fresh onion sprouts are crisp with white hypocotyls and green cotyledons and grow usually within 10 – 15 days from start to finish. Due to high moisture content and high metabolic activity, onion sprouts like other high moisture foods are highly perishable and vulnerable to rapid desiccation and other quality defects. At normal room temperatures, onion sprouts can last just for 2 days compared to 3-6 days at refrigeration temperatures [16]. Darkening of the cotyledons, development of dark streaks on the hypocotyls, sliminess, fungal decay and musty odor are the main deteriorative impediments in the storage and subsequent marketing of the onion sprouts. Overcoming the quality loss with concomitant retention of nutritional quality, improvement in nutraceutical potential and extension in shelf-life of onion sprouts demands for the search of innovative preservation methods. Judicious use of conventional chemicals as anti-ripening, anti-senescence and microbial fumigants has been phased out and restricted throughout the world as these chemicals pose serious health hazards and environmental effects [17].

Gamma irradiation has become an effective means of processing and preserving food products. The process is gaining much importance as it can be performed at room temperature, and due to its cold nature and high efficiency, for inactivation of food borne pathogens and parasites [18]. Irradiation has been recognized as an alternative to chemicals for treating fresh and dried agricultural products to overcome quarantine barriers in international trade, as a mode of decontamination, disinfestations, delaying the ripening and senescence of fruits and vegetables and for improving nutritional attributes and shelf-life [19]. Gamma irradiation can extend shelf-life of treated foods without including the formation of any radionuclide in food products. Food can be treated with radiations in a pre-packed form thereby limiting the chances of cross contamination. Review of literature revealed that few studies have been conducted to preserve and maintain the quality of fresh sprouts. Studies conducted on the effectiveness of hot water immersion in elimination of microorganisms in alfalfa and mung bean sprouts revealed suppressed respiration, transpiration and chlorophyll breakdown, which resulted in prolonged shelf-life [20]. DeEll and Vigneault [21] reported that perforated film packaging helped to maintain the quality of fresh sprouts by reducing water loss. A recent study reported that ethanol vapour treatment significantly improved the nutritional quality of mung bean sprouts, both at room as well as refrigerated temperatures [4]. D'ambrosioa *et al*. [22] more recently studied the effect of modified atmosphere packaging (MAP) on the quality of quinoa sprouts and reported that sprouts stored in active MAP (5% O_2 + 20% CO_2) showed a better texture and a minor production of off-flavor, compared to samples stored in passive MAP; thus active packaging may be a potential solution for allowing the distribution of quinoa sprouts. However, to our knowledge; there is no information available in the literature till this date on the radiation preservation of sprouts, in particular the onion sprouts. Therefore, the present study was conducted with the objectives to investigate the effect of radiation processing on maintaining the storage quality and enhancement in nutraceutical potential of onion sprouts.

2. Materials and Methods

2.1. Onion Sprouts and Gamma Irradiation

Matured Red onions were procured from the Department of Agriculture, Lalmandi, Srinagar, Jammu and Kashmir. The onions were stored at 15 – 20°C, RH 85-90% and allowed to sprout under normal conditions. Ten days old sprouts were harvested and packed in pinhole surface perforated polyethylene pouches (size 18 cm × 15 cm, thickness 0.1 mm). Five hundred grams of sample in triplicates was packed in polyethylene bags and gamma irradiated in the dose range of 0.5–1.5 kGy using Co-60 gamma ray source (BRIT, BARC, Mumbai). The samples were kept at front middle position directly facing the source and irradiated at a dose rate of 285 Gy/h as determined using Ceric–Cereous dosimetry. To ensure that samples receive the exact dose, the dosimeters were placed in each sample for each treatment. The radiation treatment was carried out at a temperature of 5 ± 2°C under normal light conditions. After irradiation, the samples were stored under refrigerated conditions (3 ± 1°C, RH 85%) for 6 days. Samples were evaluated at intervals of 1 day for color values, sensory attributes, microbial load, bioactive contents, antioxidant and hypoglycemic activities. Triplicate samples were taken for each treatment. Samples which did not receive any gamma irradiation treatment served as control.

2.2. Physico-Chemical Analysis

Texture measurement of sprout samples was done using Instron, Universal Testing Instrument (TM Model, Instron Engineering Corp., Norwood, Mass, U.S.A.), which was equipped with 2-mm brass needle probe for penetration and a 100-kg load cell. Texture was measured at central edible portions of the sprouts and was calculated as maximum force (kg) measuring resistance to the penetration (2-mm fixed depth) imposed by the sample to the metal probe. Objective color values on the surface of un-irradiated (control) and

irradiated onion sprouts were determined using a Hunter Colorimeter (Hunter Assoc, Reston, VA, USA; McGuire, 1992). Color measurement were displayed in L (lightness), a (measure of browning) and b (yellowness) values. Hue and chroma were calculated from a and b values. Total phenols and flavonoids were determined according to the previously described method with slight modifications [23]. Total phenols and flavonoids were determined with the use of an external standard curve in the concentration range of 0.1–1.0 mg/ml and expressed as mg gallic acid and catechin equivalents per 100 g. Ascorbic and dehydroascorbic acid estimation was done by HPLC system of JASCO, Japan (model, LC-Net II/ADC), fitted with an automatic degassing unit, UV-2070 detector, PU-2080 pump and a HiQ-Sil C18 column (size 4.6 mm × 250 mm) as per the previously described method [24]. An external standard of $_{L}$-ascorbic acid and dehydroascorbic acid in 3% meta phosphoric acid was used for the identification and quantification of ascorbic and dehydroascorbic acid. Total ascorbic acid was calculated as sum of the ascorbic and dehydroascorbic acid. Chlorophyll was determined by spectrophotometrically with slight modifications [25]. Total carotenoids as beta carotene equivalents were determined as per the method described by Kimura and Rodriguez-Amaya [26] with slight modifications. Phenylalanine ammonia-lyase (PAL) activity was determined as per the earlier used method [27]. For the determination of Polyphenol oxidase (PPO) activity, the sprout samples were homogenized in a twofold amount of chilled 50 mM sodium phosphate buffer (pH 5.0) containing polyvinyl-polypyrrolidone (PVPP, 50g/l) for 2 min using a homogenizer (Phlips, India). The homogenate was filtered through cheese cloth and the filtrate was centrifuged at 14,000 rpm for 30 min at 4°C. Activity of PPO enzyme from the crude enzyme extract was determined spectrophotometrically by measuring the increase in absorbance at 500 nm using $_{L}$-tyrosine as substrate [28]. A group of five trained panelists was involved in assessing the overall acceptability of control and irradiated onion sprouts. OAA based on appearance (green color), texture and odor was evaluated using 4 point scale where 4 = excellent, 3 = good, 2 = fair and 1 = poor. Twenty to twenty five sprout samples were selected randomly, coded and served to judges for evaluation of color, crunchiness and odor. The limit of acceptability was kept as 2.5 and the samples whose acceptability values were below 2.5 corresponding to a particular storage period were rated unacceptable. Microbial load as yeast and mold count (YMC) was determined by the serial dilution method using pour plate technique [29]. For each measurement, triplicate samples were used.

2.3. In Vitro Antioxidant and Hypoglycemic Activity

Five different assays were used to evaluate the antioxidant activity of control and gamma irradiated onion sprouts. Ascorbic acid, gallic acid, butylatedhydroxy toluene (BHT) and ethylenediamine tertraacetic acid (EDTA) were used as positive controls for the studied assays and the antioxidant activities were compared in terms of EC_{50} values. DPPH radical scavenging activity was determined as per the previously used method with minor modifications [30]. The EC_{50} (DPPH) value, which represents the concentration of extract that gives 50% reduction in DPPH absorbance, was determined from the graph of inhibition percentage versus concentration. The reducing power of extracts was determined by evaluating the transformation of Fe^{3+}– Fe^{2+} according to the earlier used method [31]. β- Carotene bleaching assay (BCBA) was carried out by measuring the coupled autoxidation of β-carotene and linoleic acid [32]. Hydroxyl radical scavenging assay was carried out according to the previously used method with minor modifications [33]. Ferrous ion chelating activity was measured according to the method of Suter and Richter [34]. The alpha-amalyse and alpha-glucosidae inhibitory assay was carried out according the earlier described method [35]. The EC_{50} which represents the concentration that gives 50% inhibition in the activities of the enzyme was calculated from the graph. For both the activities, acarbose was used as standard.

3. Statistical Analysis

Mean values, standard deviation, analysis of variance (ANOVA) were computed using a commercial statistical package SPSS 10.1 (USA). The data was compared using Duncan's multiple range tests at 5% significance level.

4. Results and Discussion

4.1. Color Scores

The primary criterion that the consumers consider about a product is its appearance; color has been considered to have a key role in the choice of food, food preference and acceptability, and may even influence taste thresholds, sweetness, perception and pleasantness. Color is one of the main attributes, along with texture, that characterizes the freshness of most vegetables [36]. Color scores of control and irradiated onion sprouts are depicted in Table 1. The statistical analysis of color score data revealed that no significant ($p \geq 0.05$) difference existed among all color parameters for both control and irradiated sprouts up to 2 days of storage except in L values which were significantly ($p \leq 0.05$) higher in 1.0 kGy and 1.5 kGy irradiated samples. L value has been used by several researchers as an indicator of vegetable deterioration [37, 38]. During storage, L values decreased in all the treatments and decrease was significantly ($p \leq 0.05$) higher in control and 0.5 kGy irradiated samples. The order of decrease in L value was 8.3% in control and 5.5% in 1.5 kGy irradiated sprouts after 6 days of storage. Decrease in L values during storage have also been reported in fresh-cut green beans [37], fresh-cut cabbage [39], fresh-cut potatoes [40] and in quinoa sprouts [22]. The hue value, which represents true color and is considered as an effective parameter for visualizing the color appearance of food products [41], also exhibited a decreasing trend during

storage in all the treatments. However, among treatments; higher hue values were retained significantly ($p \leq 0.05$) in 1.5 kGy samples. Percentage decrease in hue value was 7.2% in control samples compared to 5.3% in 1.5 kGy irradiated samples after 6 days of storage. The decrease in hue values indicates that color of sprout samples changed from green to light green-yellow-orange as a result of chlorophyll degradation. Retention of higher hue values in 1.5 kGy treated sprouts indicated inhibition of chlorophyll degradation at higher doses. The other color parameters namely a (browning/red index), b (yellowness index) and chroma recorded an increasing trend during storage in all the treatments including control. After 6 days of storage, yellowness index increased by 18.8% in control and 11.2% in 1.5 kGy irradiated sprouts. Chroma, which represents saturation of color either due to browning or chlorophyll degradation was significantly ($p \leq 0.05$) higher in control sprouts compared to irradiated ones. Data analysis pertaining to chroma indicated that enhancement in color saturation was statistically non-significant ($p \geq 0.05$) up to 2 days in control and 0.5 kGy samples compared to 3 days in 1.0 kGy and 1.5 kGy sprouts. Increase in chroma after the end of 6 days of storage was 20.8% in control compared to 12.2% in 1.5 kGy sprouts. The changes in L and a value were correlated with polyphenol oxidase activity [42], while as changes in b value have been related to chlorophyll degradation [37]. The ability of the radiation treatment (1.5k Gy) in maintaining the higher L and hue value and preventing the increase in b value and chroma is attributed to inhibitory effect of the treatment against the polyphenol oxidase activity and on chlorophyll degradation [43].

Table 1. *Effect of radiation treatment on color score of onion sprouts during refrigerated storage.*

Dose (kGy)	Storage period (days)						
	1	2	3	4	5	6	LSD
	Color scores						
	a) L values						
Cont.	69.5±1.2 $^{a, 4}$	68.2±1.2 $^{a, 3}$	67.6±1.3 $^{a, 3}$	66.7±1.1 $^{a, 2}$	65.9±1.3 $^{a, 2}$	63.7±1.5 $^{a, 1}$	1.5
0.5	69.2±1.5 $^{a, 4}$	68.4±1.4 $^{a, 3}$	67.6±1.3 $^{a, 3}$	66.5±1.4 $^{a, 2}$	65.7±1.4 $^{a, 2}$	63.3±1.2 $^{a, 1}$	1.1
1.0	70.4±1.2 $^{a, 3}$	69.6±1.4 $^{b, 3}$	68.2±1.4 $^{a, 2}$	66.8±1.6 $^{a, 2}$	66.1±1.5 $^{a, 1}$	65.2±1.3 $^{b, 1}$	1.4
1.5	70.4±1.4 $^{a, 3}$	70.6±1.3 $^{b, 3}$	68.8±1.6 $^{b, 2}$	67.9±1.5 $^{b, 2}$	67.3±1.5 $^{b, 1}$	66.5±1.5 $^{c, 1}$	1.2
LSD	1.2	1.0	0.8	0.8	0.6	0.7	
	b) a values						
Cont.	1.4±0.4 $^{a, 1}$	1.6±0.4 $^{a, 1}$	2.3±0.3 $^{b, 2}$	2.9±0.5 $^{b, 2}$	3.5±0.5 $^{b, 3}$	4.2±0.6 $^{b, 3}$	0.7
0.5	1.3±0.5 $^{a, 1}$	1.5±0.4 $^{a, 1}$	2.2±0.3 $^{b, 2}$	2.7±0.4 $^{b, 2}$	3.2±0.4 $^{b, 3}$	3.9±0.5 $^{b, 3}$	0.7
1.0	1.2±0.4 $^{a, 1}$	1.5±0.5 $^{a, 1}$	1.9±0.4 $^{a, 2}$	2.4±0.5 $^{a, 2}$	2.9±0.6 $^{a, 3}$	3.5±0.3 $^{a, 3}$	0.6
1.5	1.2±0.4 $^{a, 1}$	1.5±0.4 $^{a, 1}$	1.7±0.5 $^{a, 1}$	2.1±0.5 $^{a, 2}$	2.5±0.6 $^{a, 2}$	3.1±0.5 $^{a, 3}$	0.5
LSD	0.3	0.4	0.3	0.4	0.4	0.4	
	c) b values						
Cont.	19.2±1.2 $^{a, 1}$	19.6±1.2 $^{a, 1}$	20.2±1.3 $^{b, 2}$	20.8±1.1 $^{b, 2}$	21.7±1.4 $^{b, 3}$	22.8±1.4 $^{c, 4}$	0.7
0.5	19.3±1.1 $^{a, 1}$	19.6±1.4 $^{a, 1}$	20.1±1.3 $^{b, 2}$	20.7±1.2 $^{b, 2}$	21.5±1.4 $^{b, 3}$	22.6±1.2 $^{c, 4}$	0.6
1.0	18.7±1.2 $^{a, 1}$	19.1±1.3 $^{a, 1}$	19.4±1.1 $^{a, 1}$	20.1±1.2 $^{a, 2}$	20.8±1.3 $^{a, 2}$	21.6±1.3 $^{b, 3}$	0.8
1.5	18.8±1.1 $^{a, 1}$	19.1±1.1 $^{a, 1}$	19.4±1.2 $^{a, 1}$	19.9±1.2 $^{a, 2}$	20.4±1.2 $^{a, 2}$	20.9±1.2 $^{a, 3}$	0.6
LSD	0.6	0.5	0.4	0.5	0.6	0.5	
	d) Hue						
Cont.	85.8±1.4 $^{a, 4}$	85.3±1.2 $^{a, 4}$	83.5±1.3 $^{a, 3}$	82.1±1.4 $^{a, 2}$	80.3±12.3 $^{a, 1}$	79.6±10.6 $^{a, 1}$	0.7
0.5	86.1±1.5 $^{a, 5}$	85.6±1.4 $^{a, 5}$	83.8±1.3 $^{a, 4}$	82.5±1.4 $^{a, 3}$	81.5±11.4 $^{b, 2}$	80.2±11.2 $^{a, 1}$	0.9
1.0	86.3±1.3 $^{a, 4}$	85.5±1.4 $^{a, 4}$	84.2±1.4 $^{b, 3}$	83.2±1.6 $^{b, 2}$	82.1±11.5 $^{b, 2}$	80.8±10.3 $^{c, 1}$	1.1
1.5	86.2±1.4 $^{a, 3}$	85.5±1.5 $^{a, 3}$	84.9±1.5 $^{b, 3}$	83.9±1.5 $^{b, 2}$	83.1±10.8 $^{c, 2}$	81.6±10.5 $^{d, 1}$	1.3
LSD	0.6	0.5	0.7	0.7	0.7	0.6	
	e) chroma						
Cont.	19.2±1.2 $^{a, 1}$	19.7±1.2 $^{a, 1}$	20.4±1.3 $^{b, 2}$	21.0±1.1 $^{b, 2}$	21.9±1.3 $^{b, 3}$	23.2±1.5 $^{c, 4}$	0.8
0.5	19.3±1.3 $^{a, 1}$	19.6±1.1 $^{a, 1}$	20.2±1.3 $^{b, 2}$	20.8±1.4 $^{b, 2}$	21.7±1.4 $^{b, 3}$	22.9±1.2 $^{c, 4}$	0.6
1.0	18.7±1.2 $^{a, 1}$	19.1±1.1 $^{a, 1}$	19.5±1.1 $^{a, 1}$	20.2±1.2 $^{a, 2}$	21.0±1.1 $^{a, 2}$	21.9±1.3 $^{b, 3}$	0.8
1.5	18.8±1.2 $^{a, 1}$	19.1±1.2 $^{a, 1}$	19.4±1.1 $^{a, 1}$	20.0±1.4 $^{a, 2}$	20.5±1.3 $^{a, 2}$	21.1±1.3 $^{a, 3}$	0.8
LSD	0.6	0.7	0.6	0.5	0.7	0.6	

Values are mean ± SD (n = 3); LSD = least significant difference
Values within treatments in a column with different superscript lowercase letters (a-c) differ significantly ($p \leq 0.05$)
Values within storage periods in a row with different superscript numerical (1-5) differ significantly ($p \leq 0.05$)

4.2. Texture, Overall Acceptability and Microbial Load

Texture is another fundamental character determining the acceptability of fresh-cut fruits and vegetables. Texture changes are very tightly linked to tissue deterioration and are used as measures of freshness and quality decline in fresh-cut research and industry [44]. Effect of radiation treatment on texture of onion sprouts is shown in Table 2. Statistical analysis of the data revealed that no significant ($p \geq 0.05$) difference existed in texture of onion sprouts among treatment up to first 3 days of storage. After 4 days of storage, texture was significantly lower in control and 0.5 kGy sprouts compared to irradiated 1.0 kGy and 1.5 kGy sprouts. This trend in texture continued up to 5 days of storage. However, after 6 days of storage, texture was

significantly higher in 1.5 kGy sprouts compared to all other treatments. Percentage decrease in texture recorded after 6 days of storage was 32.3% in control compared to 14.7% in 1.5 kGy sprouts. Thus radiation processing of onion sprouts at 1.5 kGy was significantly ($p \leq 0.05$) helpful in preventing the textural degradation in onion sprouts and resulted in more than two fold retention of texture in onion sprouts compared to control. Tissue softening is the major problem limiting the shelf-life of fresh-cut products including sprouts. Processes of plant senescence increase as soon as a tissue is harvested from the plant, and involves degradative changes in membranes, cell walls, subcellular organelles and texture [45]. Since gamma irradiation delays senescence and other physiological processes and inactivates the activities of enzymes responsible for cell wall degradation and hence maintains the texture of the produce.

Table 2. *Effect of radiation treatment on texture, overall acceptability and microbial load of onion sprouts during refrigerated storage.*

Dose (kGy)	Storage period (days)						
	1	2	3	4	5	6	LSD
Texture (kg)							
Cont.	9.6±0.6 $^{a, 4}$	9.3±0.5 $^{a, 4}$	9.0±0.4 $^{a, 3}$	8.6±0.7 $^{a, 3}$	7.6±0.7 $^{a, 2}$	6.5±0.6 $^{a, 1}$	0.6
0.5	9.6±0.5 $^{a, 4}$	9.3±0.4 $^{a, 4}$	9.1±0.5 $^{a, 3}$	8.6±0.5 $^{a, 3}$	7.8±0.8 $^{a, 2}$	6.5±0.7 $^{a, 1}$	0.6
1.0	9.5±0.4 $^{a, 3}$	9.3±0.3 $^{a, 3}$	9.3±0.5 $^{a, 3}$	9.1±0.6 $^{b, 3}$	8.3±0.5 $^{b, 2}$	7.4±0.5 $^{b, 1}$	0.4
1.5	9.5±0.4 $^{a, 3}$	9.3±0.5 $^{a, 3}$	9.3±0.6 $^{a, 3}$	9.2±0.5 $^{b, 3}$	8.6±0.8 $^{b, 2}$	8.1±0.5 $^{c, 1}$	0.4
LSD	0.20	0.25	0.3	0.3	0.5	0.5	
Overall acceptability							
Cont.	3.7±0.2 $^{a, 3}$	3.5±0.2 $^{a, 3}$	3.1±0.4 $^{a, 2}$	2.8±0.7 $^{a, 2}$	2.1±0.5 $^{a, 1}$	1.8±0.6 $^{a, 1}$	0.4
0.5	3.7±0.2 $^{a, 3}$	3.6±0.3 $^{a, 3}$	3.2±0.3 $^{a, 2}$	2.8±0.6 $^{a, 2}$	2.3±0.6 $^{a, 1}$	1.9±0.5 $^{a, 1}$	0.4
1.0	3.9±0.1 $^{a, 2}$	3.7±0.2 $^{a, 2}$	3.4±0.3 $^{b, 2}$	3.1±0.4 $^{b, 1}$	2.9±0.5 $^{b, 1}$	2.7±0.6 $^{b, 1}$	0.5
1.5	3.9±0.1 $^{a, 2}$	3.9±0.1 $^{b, 2}$	3.6±0.2 $^{b, 2}$	3.3±0.4 $^{b, 1}$	3.1±0.4 $^{b, 1}$	2.9±0.5 $^{c, 1}$	0.4
LSD	0.2	0.2	0.22	0.2	0.3	0.12	
Yeast and mold count (log cfu/g sample)							
Cont.	4.6±0.1 $^{b, 1}$	4.8±0.2 $^{b, 1}$	5.0±0.2 $^{c, 2}$	5.3±0.2 $^{c, 3}$	5.5±0.2 $^{c, 3}$	5.7±0.3 $^{d, 4}$	0.2
0.5	4.3±0.1 $^{a, 1}$	4.5±0.1 $^{a, 1}$	4.7±0.2 $^{b, 2}$	4.9±0.1 $^{b, 2}$	4.9±0.2 $^{b, 2}$	5.1±0.2 $^{c, 3}$	0.2
1.0	ND	ND	3.6±0.1 $^{a, 1}$	3.6±0.1 $^{a, 1}$	3.8±0.1 $^{a, 1}$	4.1±0.1 $^{b, 2}$	0.2
1.5	ND	ND	ND	3.6±0.1 $^{a, 1}$	3.6±0.2 $^{a, 1}$	3.8±0.1 $^{a, 1}$	0.3
LSD	0.2	0.15	0.2	0.2	0.2	0.15	

Values are mean ± SD (n = 3); LSD = least significant difference
Values within treatments in a column with different superscript lowercase letters (a-d) differ significantly ($p \leq 0.05$)
Values within storage periods in a row with different superscript numerical (1-4) differ significantly ($p \leq 0.05$)

Overall acceptability which is the sum of different quality attributes plays an important role in consumer perception towards the acceptance or rejection of a product. Overall acceptability based on color, texture and odor of onion sprouts treated with gamma irradiation is shown in Table 2. Data analysis indicated no significant difference in overall acceptability of onion sprouts among treatment after 1 day of storage. After 2 days of storage, overall acceptability of control, 0.5 kGy and 1.0 kGy treated sprouts differed non-significantly ($p \geq 0.05$) with respect to each other and was significantly lower compared to overall acceptability of 1.5 kGy treated sprouts. However, after 3 days of storage, overall acceptability of control and 0.5 kGy irradiated sprouts was significantly lower in comparison to values in 1.0 kGy and 1.5 kGy sprouts. This trend in overall acceptability continued up to 5 days of storage. After 6 days of storage, overall acceptability of sprouts irradiated at 1.5 kGy was significantly higher compared to all other treatments. Further based on limit of acceptability, control and 0.5 kGy treated sprouts were unacceptable after 4 days of storage, while as 1.0 kGy and 1.5 kGy were in acceptable condition even after 6 days of storage. Towards the end of the storage, 51.4% decrease in overall acceptability was recorded in control onion sprouts compared to 25.6% in 1.5 kGy irradiated sprouts. Therefore, it is inferred that irradiation of onion sprouts at doses above 1.0 kGy resulted in two fold retention of overall acceptability.

The importance of microbiological food safety is paramount because of the potential for harmful microorganisms to grow and multiply in food commodities. Entry of possible contaminants such as microbiological agents into food is a threat to the safety of food products. This can result in food poisoning, increase in food borne outbreaks and a decrease in food availability because of discarding the contaminated food products [46]. Effect of radiation processing on microbial load of onion sprouts is presented in Table 2. It is clear from the data that irradiation of onion sprouts at dose 1.0 kGy and 1.5 kGy was highly effective in inhibiting the yeast and mold growth in onion sprouts up 2 and 3 days of storage and resulted in 4.8 and 5.0 log reduction in microbial load. During further storage, yeast and mold count increased in dose dependent manner in all the treatments including control. After 4 days of storage, no significant difference existed in yeast and mold count of sprouts irradiated at 1.0 kGy and 1.5 kGy and this trend was observed even after 5 days of storage. After 6 days of storage, yeast and mold count was significantly ($p \leq 0.05$) lower in 1.5 kGy irradiated samples compared to other samples. Among treatments, dose of 1.5 kGy was effective in keeping the microbial load at a lower level during storage and resulted in about 1.9 log reduction in yeast and mold count after the end of 6 days of storage. The effectiveness of radiation in reducing the microbial load of onion sprouts is attributed to inactivation of microorganisms by causing

single strand or double strand breaking of DNA molecule, thus finally leading to death of the cell. Radiations also generate radiolytic products of water, which combine with cell components especially bases of DNA molecule, thereby leading to mutations in cell [47].

4.3. Total Phenols and Flavonoids

The total phenol and flavonoid content of control and gamma irradiated onion sprout samples is depicted in Table 3. The data analysis indicated an increase in total phenol and flavonoid content in irradiated sprouts compared to un-irradiated controls and the increase was significant ($p \leq 0.05$) at doses above 0.5 kGy. After 1 day of irradiation, total phenol and flavonoid content of control samples was 428.2±10.2 mg/100g and 206.2±10.1 mg/100 g and that of irradiated sprouts was in the range of 430.3±10.5 - 438.3±12.4 mg/100 g and 209.1±10.3 - 219.4±11.4 mg/100 g respectively. Percentage increase in total phenols and flavonoids over control as a result of irradiation at 1.5 kGy was 2.4% and 6.4% at 1 day of irradiation. During storage, total phenol and flavonoid content increased in both control as well as irradiated samples; the content remained significantly ($p \leq 0.05$) higher in irradiated sprouts. The maximum total phenol and flavonoid content was observed at 3 days of storage in both control and irradiated sprouts. In control samples, maximum increase in total phenols at 3 days of storage was 1.9% compared to 2.1% in 1.5 kGy irradiated sprouts. Similarly, maximum increase in total flavonoids at 3 days of storage was 3.4% in control and 4.1% in 1.5 kGy irradiated sprouts. This means that significant increase of total phenols and flavonoids in onion sprouts occurred mainly due to radiation treatment compared to storage. Increase in phenolic compound contents after irradiation with sanitization doses has also been reported for other plant foods such as Justicia Adhatoda, a medicinal plant [48]. A more recent study on black soybean extracts also demonstrated increase in total phenols during irradiation and the increase occurred in a dose dependent manner [49]. The observed increased total phenol and flavonoid content in irradiated onion sprouts could be attributed to the release of phenolic compounds from glycosidic components, degradation of larger phenolic compounds into smaller ones by gamma irradiation, with a consequent improvement in the extraction yield of the phenolic compounds because of the change in tissue structure by gamma irradiation [50]. Irradiation exerts its effects by direct and indirect mechanisms. In case of indirect mechanism, radiolysis of water results in the production of free radicals such as hydroxyl radicals, hydroperoxide radicals and hydrated electrons. These radicals may break the glycosidic bonds of procyanidin trimer, tetramer and hexamer that are present in fruits and vegetables; leading to the formation of procyanidin monomers or soluble phenols, which increase the total phenolic content in irradiated fruits [51]. Jamshidi et al. [52] reported that soluble phenols also result from breaking of covalent bonds of polyphenolic components which in turn increase the total phenolic content when subjected to irradiation. After 3 days of storage, both total phenols and flavonoids exhibited a declining trend and decrease was significantly ($p \leq 0.05$) higher in control samples than in irradiated ones. In control sprout samples at 6 days of storage, total phenols decreased by 6.8% from their maximum value at 3 days; while as in 1.5 kGy samples the corresponding decrease was 3.4%. Decrease in total flavonoids from their maximum value at 3 days was 13.1% in control samples compared to 7.1% in 1.5 kGy samples at 6 days of storage. The decrease in total phenols and flavonoids during storage is attributed to poplyphenol oxidase (PPO)-catalysed oxidation of phenolic compounds. During storage, process of senescence, solubilisation of cell wall pectic substances and microbial infestation result in subcellular decompartmentation, disruption of membrane integrity and oxygen penetration, thereby leading to enhanced activity of PPO responsible for oxidation of phenols.

Table 3. *Effect of radiation treatment on phenolic content, phenylalanine ammonialyase and polyphenol oxidase activity of onion sprouts during refrigerated storage.*

Dose (kGy)	Storage period (days)						
	1	2	3	4	5	6	LSD
Total phenols (mg/100 g GAE)							
Cont.	428.2±10.2 $^{a, 3}$	431.3±11.2 $^{a, 3}$	436.2±12.3 $^{a, 4}$	428.3±12.1 $^{a, 3}$	420.2±12.3 $^{a, 2}$	406.4±10.6 $^{a, 1}$	5.5
0.5	430.3±10.5 $^{a, 2}$	434.4±11.4 $^{a, 3}$	439.2±13.3 $^{a, 4}$	431.3±12.4 $^{a, 3}$	425.2±11.4 $^{b, 2}$	416.3±11.2 $^{b, 1}$	5.1
1.0	435.4±12.2 $^{b, 3}$	439.3±12.6 $^{b, 3}$	443.2±13.4 $^{b, 4}$	435.4±11.6 $^{b, 3}$	428.3±11.5 $^{b, 2}$	420.2±10.3 $^{c, 1}$	4.4
1.5	438.3±12.4 $^{b, 2}$	443.4±12.8 $^{b, 3}$	447.3±12.8 $^{c, 3}$	440.2±12.5 $^{c, 2}$	436.3±10.8 $^{c, 1}$	432.2±10.5 $^{d, 1}$	4.2
LSD	4.1	4.3	3.4	3.5	3.1	3.2	
Total flavonoids (mg/100 g CE)							
Cont.	206.2±10.1 $^{a, 2}$	210.2±10.6 $^{a, 3}$	213.3±11.3 $^{a, 3}$	205.3±10.1 $^{a, 2}$	201.2±10.3 $^{a, 2}$	185.4±10.6 $^{a, 1}$	5.8
0.5	209.1±10.3 $^{a, 2}$	214.2±10.4 $^{a, 3}$	217.2±11.3 $^{a, 3}$ 209.2±10.4 $^{a, 2}$	205.3±10.4 $^{a, 2}$	194.2±10.2 $^{a, 1}$	5.2	
1.0	214.3±11.2 $^{b, 2}$	219.1±11.6 $^{b, 3}$	222.3±12.4 $^{b, 3}$	214.1±11.1 $^{b, 2}$	209.2±10.6 $^{b, 2}$	201.3±10.5 $^{b, 1}$	5.1
1.5	219.4±11.4 $^{c, 2}$	224.3±11.8 $^{b, 2}$	228.4±11.8 $^{c, 3}$	221.2±11.5 $^{c, 2}$	216.2±10.8 $^{c, 1}$	212.3±11.5 $^{c, 1}$	4.8
LSD	4.3	4.5	4.2	5.1	5.2	5.8	
Phenylalanine ammonialyase (PAL) activity (u/g fw)							
Cont.	1.4±0.02 $^{a, 1}$	1.5±0.02 $^{a, 2}$	1.9±0.02 $^{a, 4}$	1.7±0.03 $^{a, 3}$	1.5±0.03 $^{a, 2}$	1.3±0.02 $^{a, 1}$	0.13
0.5	1.6±0.01 $^{a, 1}$	1.8±0.01 $^{b, 2}$	2.1±0.03 $^{b, 3}$	1.9±0.04 $^{a, 2}$	1.8±0.04 $^{b, 2}$	1.5±0.03 $^{b, 1}$	0.20
1.0	1.8±0.02 $^{b, 1}$	2.0±0.02 $^{b, 2}$	2.3±0.02 $^{c, 3}$	2.1±0.04 $^{b, 2}$	1.9±0.03 $^{b, 1}$	1.7±0.02 $^{c, 1}$	0.20
1.5	2.1±0.01 $^{c, 1}$	2.2±0.01 $^{c, 1}$	2.6±0.04 $^{d, 2}$	2.5±0.03 $^{c, 2}$	2.3±0.04 $^{c, 1}$	2.1±0.04 $^{d, 1}$	0.20

Dose (kGy)	Storage period (days)						
	1	2	3	4	5	6	LSD
LSD	0.2	0.2	0.13	0.21	0.20	0.11	
Polyphenol oxidase (POP) activity (u/g fw)							
Cont.	12.2±2.4 $^{b, 1}$	21.4±3.2 $^{c, 2}$	35.4±2.1 $^{c, 3}$	47.3±2.7 $^{c, 4}$	55.3±2.5 $^{c, 5}$	69.4±2.6 $^{c, 6}$	5.2
0.5	10.9±2.1 $^{b, 1}$	19.6±2.6 $^{b, 2}$	32.7±1.8 $^{c, 3}$	44.6±2.2 $^{c, 4}$	51.4±2.2 $^{c, 5}$	65.6±2.1 $^{c, 6}$	5.5
1.0	7.3±1.6 $^{a, 1}$	16.5±2.2 $^{b, 2}$	27.2.±1.6 $^{b, 3}$	35.4±1.8 $^{b, 4}$	42.4±1.7 $^{b, 5}$	50.4±1.5 $^{b, 6}$	5.1.
1.5	5.2±1.2 $^{a, 1}$	10.4±1.7 $^{a, 2}$	20.2±1.3 $^{a, 3}$	29.5±1.6 $^{a, 4}$	36.6±1.7 $^{a, 5}$	42.2±1.5 $^{a, 6}$	3.8
LSD	2.5	3.1	3.5	2.9	4.1	4.2	

Values are mean ± SD (n = 3); LSD = least significant difference
Values within treatments in a column with different superscript lowercase letters (a-d) differ significantly ($p \leq 0.05$)
Values within storage periods in a row with different superscript numerical (1-6) differ significantly ($p \leq 0.05$)

4.4. Phenylalanine Ammonialyase (PAL) and Polyphenol Oxidase (PPO) Activity

Phenylalanine ammonia-lyase (PAL) is the key enzyme involved in the biosynthesis of phenols. It is responsible for catalyzing the deamination of L-phenylalanine to yield ammonia and trans-cinnamic acid which serve as the precursors for phenolic compounds. The effect of gamma irradiation on PAL activity of onion sprouts is presented in Table 3. Data reveals that irradiation treatment at doses above 0.5 kGy significantly ($p \leq 0.05$) increased the PAL activity of sprout samples when compared with control at 1 day of storage. PAL activity exhibited almost a linear increase with increase in irradiation dose. PAL activity of 1.5 kGy irradiated samples at 1 day of storage was 2.1±0.01 ΔOD h^{-1} mg^{-1} protein compared to 1.4±0.02 ΔOD h^{-1} mg^{-1} protein in un-irradiated control. The PAL activity exhibited an increasing trend during storage and reached a maximum at 3 days. Control samples recorded a PAL activity of 1.95±0.1 Δ OD h^{-1} mg^{-1} protein while as 1.5 kGy irradiated samples recorded PAL activity of 2.6±0.2 ΔOD h^{-1} mg^{-1} protein at 3 days of storage. Beyond 3 days of storage, PAL activity exhibited a decreasing trend and decrease was significantly ($p \leq 0.05$) higher in control samples. Our results revealed a positive correlation ($r = 0.81$) between PAL activity and irradiation and PAL activity and total phenols ($r = 0.89$); indicating that concomitant increase in total phenols up to 3 days is accompanied by an enhancement in PAL activity which also reached a maximum at 3 days of storage. Earlier studies have also reported the existence of positive correlation between irradiation doses and PAL activity in calli of rosemary [53] and Agaricus bisporous mushrooms [54]. It was shown that the PAL activity exhibited almost linear increase with an increase in irradiation dose and about a 4-fold higher activity was observed at 2.5 kGy dose compared to control [55]. The observed positive correlation between the PAL activity and irradiation underlines the basis for the enhancement in total phenols.

The effect of gamma irradiation on PPO activity of onion sprouts is shown in Table 1. Data reveals that irradiation above 0.5 kGy had a significant ($p \leq 0.05$) effect on the inhibition of PPO activity in onion sprouts. At 1 day of storage, treatment of 1.5 kGy resulted in 58.4% inhibition in PPO activity of onion sprouts. During storage, the PPO activity in onion sprouts increased and the increase was significantly ($p \leq 0.05$) lower in samples treated with gamma irradiation at doses above 0.5 kGy compared to control. There was no significant difference in PPO activity of control and 0.5 kGy samples throughout the storage. Significant negative correlations ($r = -0.93$) existed between PPO activity and the treatments. Among the treatments, 1.5 kGy irradiation was significantly ($p \leq 0.05$) effective in inhibiting the PPO activity up to 6 days of storage compared to other treatments. In control and 0.5k Gy irradiated samples, the PPO activity at 6 days of storage was 69 and 65 u/g fw respectively. On the other hand, the PPO activity in samples treated with 1.5 kGy irradiation was 42 u/g fw at 6 days of storage, indicating an inhibition of about 88.1% in PPO activity. The inhibition in PPO activity due to irradiation has proved significantly ($p \leq 0.05$) beneficial in maintaining the higher levels of total phenols and flavonoids in onion sprouts during storage [56].

4.5. Total Ascorbic Acid Content

Total ascorbic acid content of onion sprouts is presented in Table 4. It is seen from the Table 1 that radiation processing of onion sprouts resulted in a non-significant ($p \geq 0.05$) decrease in ascorbic acid when compared with control samples at 1 day of storage. Statistical analysis of the data revealed that this decrease in ascorbic acid among treatments continued up to 4 days of storage. At 5^{th} and 6^{th} day of storage, decrease in ascorbic acid content was significantly ($p \leq 0.05$) lower in samples irradiated at 1.0 and 1.5 kGy compared to control and 0.5 kGy samples. Data analysis also revealed that in control samples, decrease in ascorbic acid during storage was non-significant ($p \geq 0.05$) up to 2 days; while as in 1.5 kGy irradiated samples, the decrease was non-significant ($p \geq 0.05$) up to 3 days beyond that significant decrease was observed in ascorbic acid. Thus, it can be inferred that main loss of ascorbic acid is due to environmental factors like storage temperature and oxygen rather than irradiation. However, the lower ascorbic acid found, just after irradiation, in samples treated at doses 0.5–1.5 kGy seems to indicate that radiolysis could accelerate the conversion of ascorbic acid to dehydroascorbic acid (DHA).

Table 4. *Effect of radiation treatment on total ascorbic acid, total chlorophyll and total carotenoids of onion sprouts during refrigerated storage.*

Dose (kGy)	Storage period (days)						
	1	2	3	4	5	6	LSD
Total ascorbic acid (mg/100 g)							
Cont.	9.2±1.2 $^{a, 4}$	9.1±1.2 $^{a, 4}$	8.4±1.1 $^{a, 3}$	7.6±0.7 $^{a, 2}$	7.1±0.5 $^{a, 2}$	6.4±0.6 $^{a, 1}$	0.50
0.5	8.9±1.1 $^{a, 4}$	8.7±1.1 $^{a, 3}$	8.3±1.3 $^{a, 3}$	7.6±0.6 $^{a, 2}$	7.2±0.4 $^{a, 2}$	6.6±0.5 $^{a, 1}$	0.42
1.0	8.7±1.2 $^{a, 4}$	8.5±1.2 $^{a, 4}$	8.2±1.2 $^{a, 3}$	7.9±0.8 $^{a, 3}$	7.4±0.5 $^{a, 2}$	6.9±0.5 $^{b, 1}$	0.40
1.5	8.5±1.1 $^{a, 3}$	8.4±1.1 $^{a, 3}$	8.2±1.1 $^{a, 3}$	7.9±0.6 $^{a, 2}$	7.6±0.7 $^{b, 2}$	7.2±0.5 $^{b, 1}$	0.35
LSD	0.71	0.62	0.41	0.41	0.30	0.32	
Total chlorophyll (mg/100 g)							
Cont.	112.2±7.5 $^{a, 3}$	112.2±7.6 $^{a, 3}$	111.9±6.3 $^{a, 3}$	111.4±6.1 $^{a, 2}$	110.7±6.3 $^{a, 2}$	109.4±6.6 $^{a, 1}$	0.8
0.5	112.7±7.3 $^{a, 2}$	112.5±6.4 $^{a, 2}$	112.3±6.3 $^{a, 2}$	111.9±6.4 $^{a, 2}$	111.2±6.4 $^{a, 1}$	110.6±6.2 $^{a, 1}$	0.8
1.0	113.2±7.2 $^{a, 2}$	113.3±6.6 $^{a, 3}$	113.1±6.4 $^{b, 2}$	112.6±6.1 $^{a, 2}$	111.9±5.6 $^{a, 1}$	111.2±5.5 $^{b, 1}$	0.7
1.5	113.5±7.4 $^{a, 2}$	113.5±7.8 $^{a, 2}$	113.2±5.8 $^{b, 2}$	112.9±5.5 $^{b, 2}$	112.4±5.8 $^{b, 1}$	111.8±4.5 $^{b, 1}$	0.6
LSD	1.7	1.5	1.5	1.2	1.5	1.4	
Total carotenoids (mg/100 g)							
Cont.	69.1±2.5 $^{a, 1}$	69.9±2.6 $^{a, 2}$	70.6±2.5 $^{a, 3}$	71.4±2.6 $^{b, 4}$	72.3±3.3 $^{b, 5}$	73.7±3.6 $^{c, 6}$	0.5
0.5	69.1±2.3 $^{a, 1}$	69.7±2.4 $^{a, 1}$	70.3±2.3 $^{a, 2}$	70.9±2.4 $^{b, 2}$	71.7±2.5 $^{b, 3}$	72.8±3.2 $^{b, 4}$	0.7
1.0	69.5±2.2 $^{a, 1}$	69.8±2.6 $^{a, 1}$	70.2±2.4 $^{a, 1}$	70.5±2.1 $^{a, 2}$	70.9±1.6 $^{a, 2}$	71.4±1.5 $^{a, 3}$	0.7
1.5	69.5±2.4 $^{a, 1}$	69.8±2.5 $^{a, 1}$	70.2±5.8 $^{a, 1}$	70.2±1.5 $^{a, 1}$	70.6±1.8 $^{a, 2}$	70.9±1.5 $^{a, 2}$	0.7
LSD	0.6	0.5	0.5	0.5	0.8	0.6	

Values are mean ± SD (n = 3); LSD = least significant difference
Values within treatments in a column with different superscript lowercase letters (a-c) differ significantly ($p \leq 0.05$)
Values within storage periods in a row with different superscript numerical (1-6) differ significantly ($p \leq 0.05$)

4.6. Total Chlorophyll and Carotenoids

Effect of gamma irradiation treatment on total chlorophyll and carotenoids is shown in Table 4. It can be seen from the data that no significant difference existed in both total chlorophyll and carotenoid content among treatments including control up to 2nd and 3rd day of storage, although the contents were slightly higher in irradiated (1.0 and 1.5 kGy) samples. This slight increase in the contents of chlorophyll and carotenoids in irradiated samples can be attributed to the enhancement in the extractability of the pigments due to irradiation [55]. Thus, irradiation treatment of onion sprouts has proved beneficial in enhancing the release of nutritionally rich and biologically active constituent rather than causing any significant degradation. Data analysis revealed a positive (r = 0.91) correlation between chlorophyll retention and irradiation doses and inverse correlation (r = - 0.88) between carotenoid accumulation and irradiation treatment. It is also clear from the data that decrease in chlorophyll content with concomitant increase in carotenoid accumolation was dose dependent and significantly ($p \leq 0.05$) lower in samples irradiated at 1.0 and 1.5 kGy compared to control and 0.5 kGy samples. As the storage of sprout samples progressed, the chlorophyll content decreased and the carotenoid content increased. At 6 days of storage, the decrease in chlorophyll content was 2.5% in control compared to 1.5% in 1.5 kGy samples. The increase in carotenoid content at 6 day of storage was significantly ($p \leq 0.05$) higher in control (6.6%) compared to 2.0% in 1.5 kGy irradiated sprouts. The loss of chlorophyll during storage is attributed to the change of chloroplasts into chromoplasts containing yellow and red pigments. The major loss of chlorophyll is mediated through an increase in the activity of the enzyme chlorophyllase during storage which degrades the molecule. The retention of higher values of chlorophyll in case of samples irradiated at 1.5 kGy can be attributed to the inhibitory effect of both irradiation as well as low temperature on the activity of chlorophyllase enzyme.

4.7. In Vitro Antioxidant Activity

Effect of gamma irradiation treatments on DPPH radical scavenging activity of onion sprouts in shown in Figure 1(A). It is clear from the figure that at 1 day of storage, inhibition in DPPH activity was significantly ($p \leq 0.05$) higher in samples irradiated at 1.0 and 1.5 kGy compared to control and 0.5 kGy samples. Maximum inhibition in DPPH activity was observed at 3 days of storage in all the samples including control and was significantly ($p \leq 0.05$) higher in 1.5 kGy irradiated sprouts. Beyond 3 days of storage inhibition of DPPH activity decreased in all the samples and was significantly ($p \leq 0.05$) lower in control and 0.5 kGy samples. Comparison of the data indicated an increase of 6.6% in inhibition of DPPH activity by onion sprouts subjected to 1.5 kGy irradiation over control at 3 days of storage. At 6 days of storage, decrease in inhibition of DPPH activity from the maximum value observed at 3 days of storage was 7.1% in control sprouts compared to 4.7% in 1.5 kGy irradiated sprouts. The reducing power of control and irradiated onion sprouts was assessed by the potassium ferricyanide reduction method and is shown in Figure 1(B). The data analysis revealed that reducing power recoded a dose dependent increase with irradiation. It is clear from the figure that at 1 day of storage, reducing power of control and irradiated samples differed significantly ($p \leq 0.05$). The percentage increases in reducing power over control due to irradiation in the dose range of 0.5–1.5 kGy was of the order of 3.7–12.3% at 1 day of storage. Maximum increase in reducing power was observed at 3 days of storage in all the samples including control and was significantly ($p \leq 0.05$)

higher in 1.5 kGy irradiated sprouts. At 3 day of storage, the reducing power of control and 0.5 kGy irradiated sprouts differed marginally ($p \geq 0.05$) with each other. After 3 days of storage reducing power decreased in all the samples and was significantly ($p \leq 0.05$) lower in control and 0.5 kGy samples. Comparison of the data indicated an increase of about 13% in reducing power of onion sprouts subjected to 1.5 kGy irradiation over control at 3 days of storage. At 6 days of storage, decrease in reducing power from the maximum value observed at 3 days of storage was 11.8% in control sprouts compared to 8.4% in 1.5 kGy irradiated sprouts.

Beta-carotene is physiologically an important compound and shows strong biological activity. If beta-carotene is decomposed before its intake, its biological functions in the body would not be observed. Effect of gamma irradiation treatments on beta-carotene bleaching activity of onion sprouts in shown in Figure 1(C). At 1 day of storage, there was no significant ($p \geq 0.05$) difference in inhibition of beta-carotene bleaching between control and 0.5 kGy irradiated sprouts. Sprout irradiated at 1.0 and 1.5 kGy showed significant inhibition in beta-carotene bleaching and contributed to about 5.8% and 10% in inhibition of beta-carotene bleaching over control at 1 day of storage. At 3 days of storage, inhibition in beta-carotene bleaching was maximum and significantly ($p \leq 0.05$) different among treatment. Dose of 1.5 kGy gave an inhibition of about 13.4% in beta-carotene bleaching over control at 3 days of storage. Beyond 3 days of storage inhibition in bleaching activity decreased in all the samples and was significantly ($p \leq 0.05$) lower in control sprouts. At 6 days of storage, decrease in inhibition of bleaching activity from the maximum value observed at 3 days of storage was 8.5% in control sprouts compared to 5.5% in 1.5 kGy irradiated sprouts.

The OH radical scavenging activity expressed as percentage inhibition of control and irradiated onion sprouts is shown in Figure 1(D). It is clear from the figure that OH radical scavenging activity was again higher for irradiated samples compared to control. Control samples of onion sprouts had OH radical scavenging activity of 71.6% compared to 73.5 – 77.6% of irradiated sprouts at 1 day of storage, implying that radiation processing enhanced the OH radical scavenging activity of sprouts by 2.7 – 8.4% over control. At 3 days of storage, OH radical scavenging was maximum in all the treatment including control and there was no significant ($p \geq 0.05$) difference in OH radical scavenging activity of sprouts irradiated at 0.5 and 1.0 kGy. Comparison of data indicated that dose of 1.5 kGy resulted in increase of 13.3% in OH radical scavenging activity over control at 3 days of storage. At 6 days of storage, OH radical scavenging recorded a decreasing trend and decrease was significantly ($p \leq 0.5$) higher in control samples compared to irradiated sprouts. Decrease in OH radical scavenging from their maximum value was significantly ($p \leq 0.05$) higher in control (8.7%) compared to 4.5% in 1.5 kGy irradiated sprouts.

Ferrous ion chelating activity expressed as percentage chelating effect of control and gamma irradiated onion sprouts are shown in Figure 1(E). Ferrous ion chelating effect of control sprouts was 79.4% compared to 82.4 – 87.6% in irradiated sprouts at 1 day of storage. Enhancement in ferrous ion chelating was dose dependent. Percentage increase in ferrous ion chelating effect of sprouts as a result of irradiation was of the order of 3.8 – 10.3% at 1 day of storage. Ferrous ion chelating effect increased during storage and reached its maximum at 3 days of storage. At 3 days of storage, increase in ferrous ion chelating effect over control due to irradiation was in the range of 4.4 – 13.2%. At 6 days of storage, ferrous ion chelating also recorded decreasing trend and decrease was significantly ($p \leq 0.5$) higher in control samples compared to irradiated sprouts. Decrease in ferrous ion chelating from their maximum value was significantly ($p \leq 0.05$) higher in control (7.8%) compared to 4.2% in 1.5 kGy irradiated sprouts.

Figure 2(A-E) shows the effect of various concentrations of control and irradiated onion sprouts on the antioxidant potentials as determined by various *in vitro* assays. The antioxidant effect of onion sprouts increased significantly ($p \leq 0.05$) with increase in concentration and was higher in irradiated samples compared to control at all concentrations used. Concentration data indicated that enhancement in DPPH radical scavenging, ferric reducing power, inhibition of beta-carotene bleaching, hydroxyl radical scavenging and ferrous ion chelating activity over control was increased by 58.9%, 33.8%, 107.1%, 75.6% and 51.5% respectively in 1.5 kGy sprouts applied at concentration of 200 µg/ml. The enhancement in antioxidant activities through ionizing radiations has been also reported by many authors [48, 49]. Gamma irradiation is capable of breaking the glycosidic bonds of polyphenols, thereby releasing soluble phenols of low molecular weight, leading to an increase of antioxidant rich phenolics responsible for higher antioxidant activities. Increased antioxidant activities of irradiated foods have also been attributed to increased enzymatic activity (phenylalanine ammonia-lyase and peroxidase) or to the increased extractability from the tissues [55]. Data analysis indicated strong positive correlation of total phenols for reducing power ($r = 0.89$), beta-carotene bleaching assay ($r = 0.95$), hydroxyl radical scavenging ($r = 0.92$) and ferrous ion chelating effect ($r = 0.93$). Positive correlation also existed between concentration and reducing power ($r = 0.83$ for control, $r = 0.87$ for 1.5 kGy), concentration and inhibition in beta-carotene bleaching ($r = 0.86$ for control, $r = 0.90$ for 1.5 kGy), concentration and hydroxyl radical scavenging ($r = 0.85$ for control, $r = 0.91$ for 1.5 kGy) and concentration and ferrous ion chelating ($r = 0.85$ for control, $r = 0.91$ for 1.5 kGy). For DPPH radical scavenging activity, positive to moderate correlation existed between phenols ($r = 0.80$) and concentration ($r = 0.76$ for control, $r = 0.81$ for 1.5 kGy).

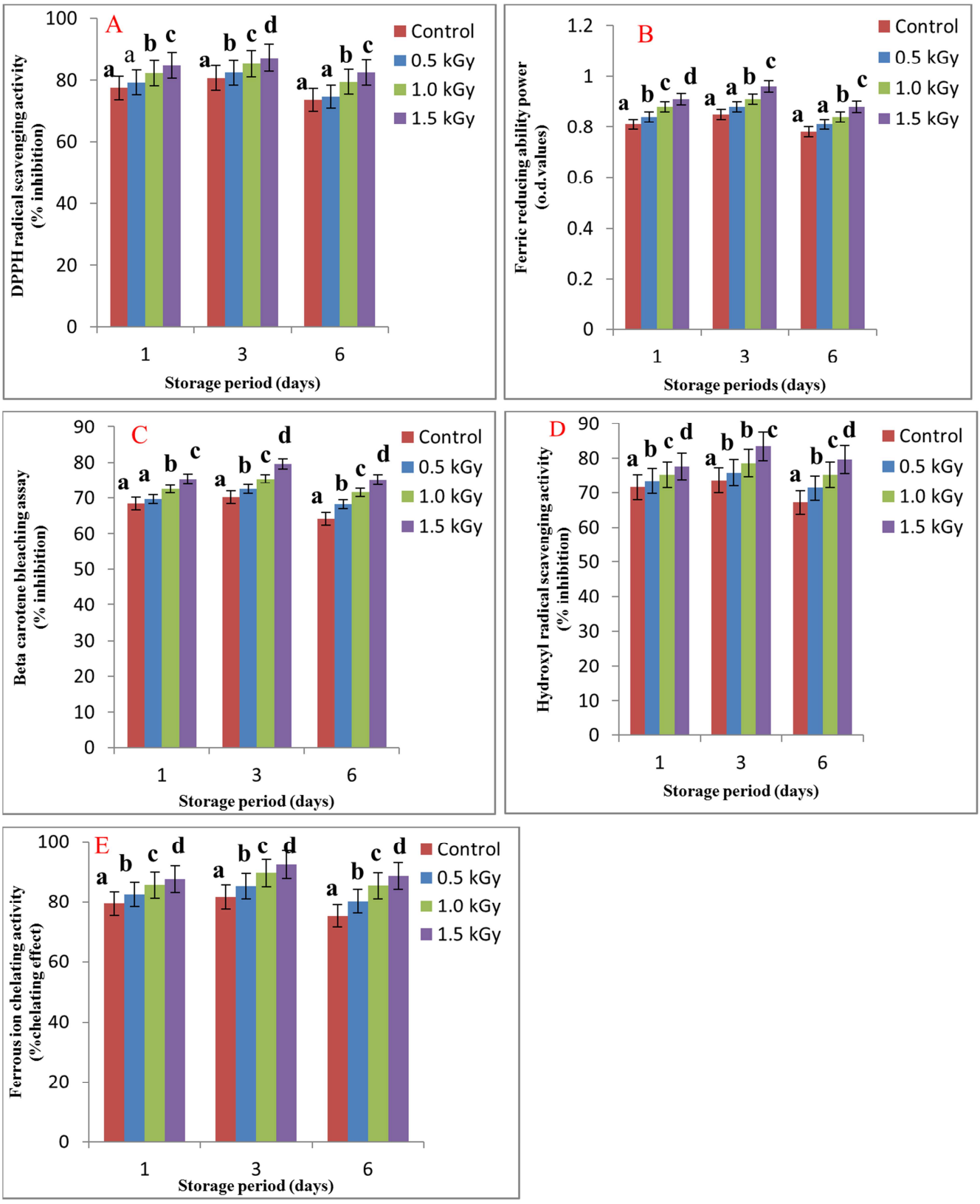


Figure 1. *Effect of radiation treatments on various antioxidant activities of onion sprouts during storage.*

(A) = DPPH activity, (B) = ferric reducing power, (C) = beta-carotene bleaching assay
(D) = hydroxyl radical scavenging activity, (E) = ferrous ion chelating activity

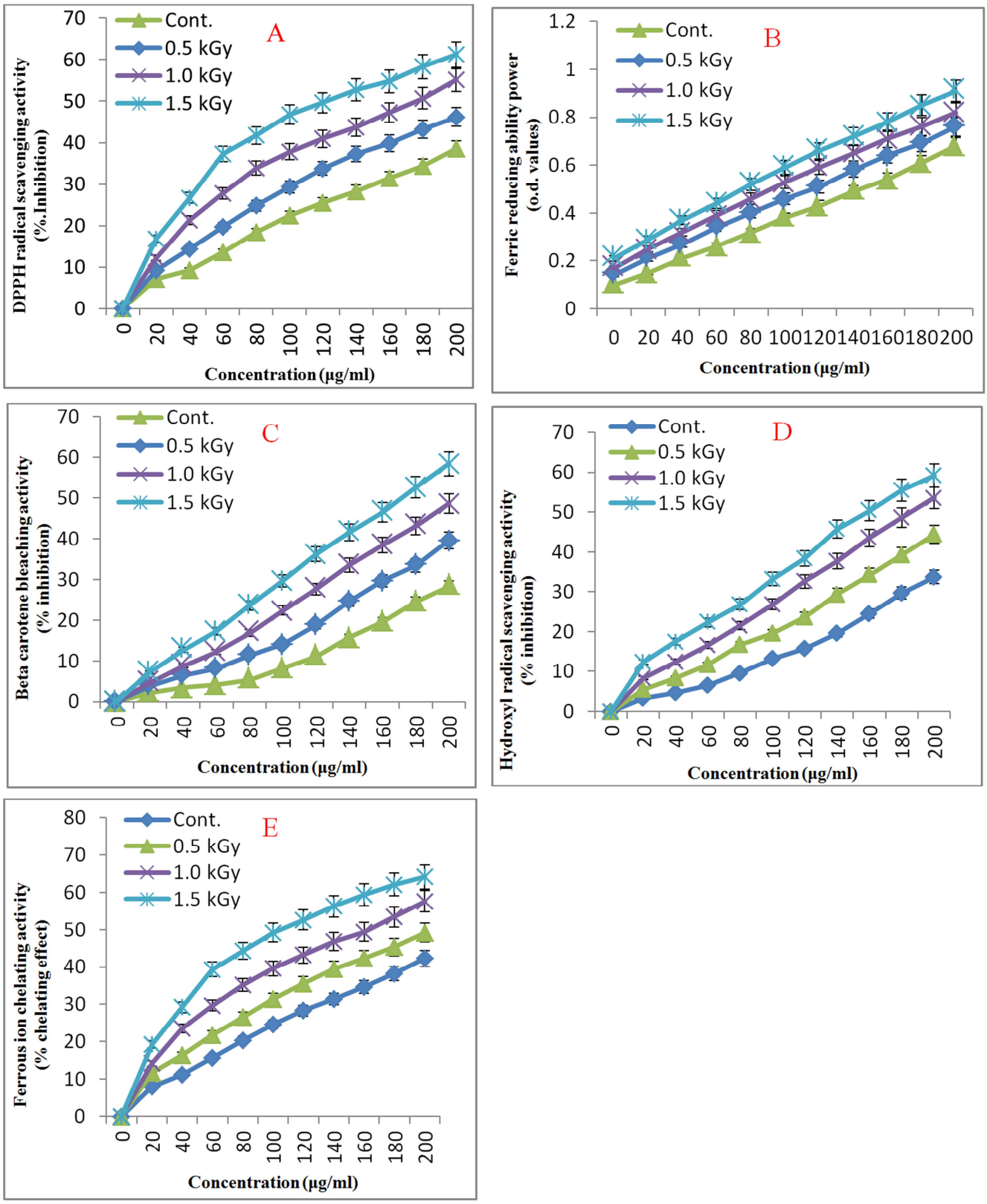
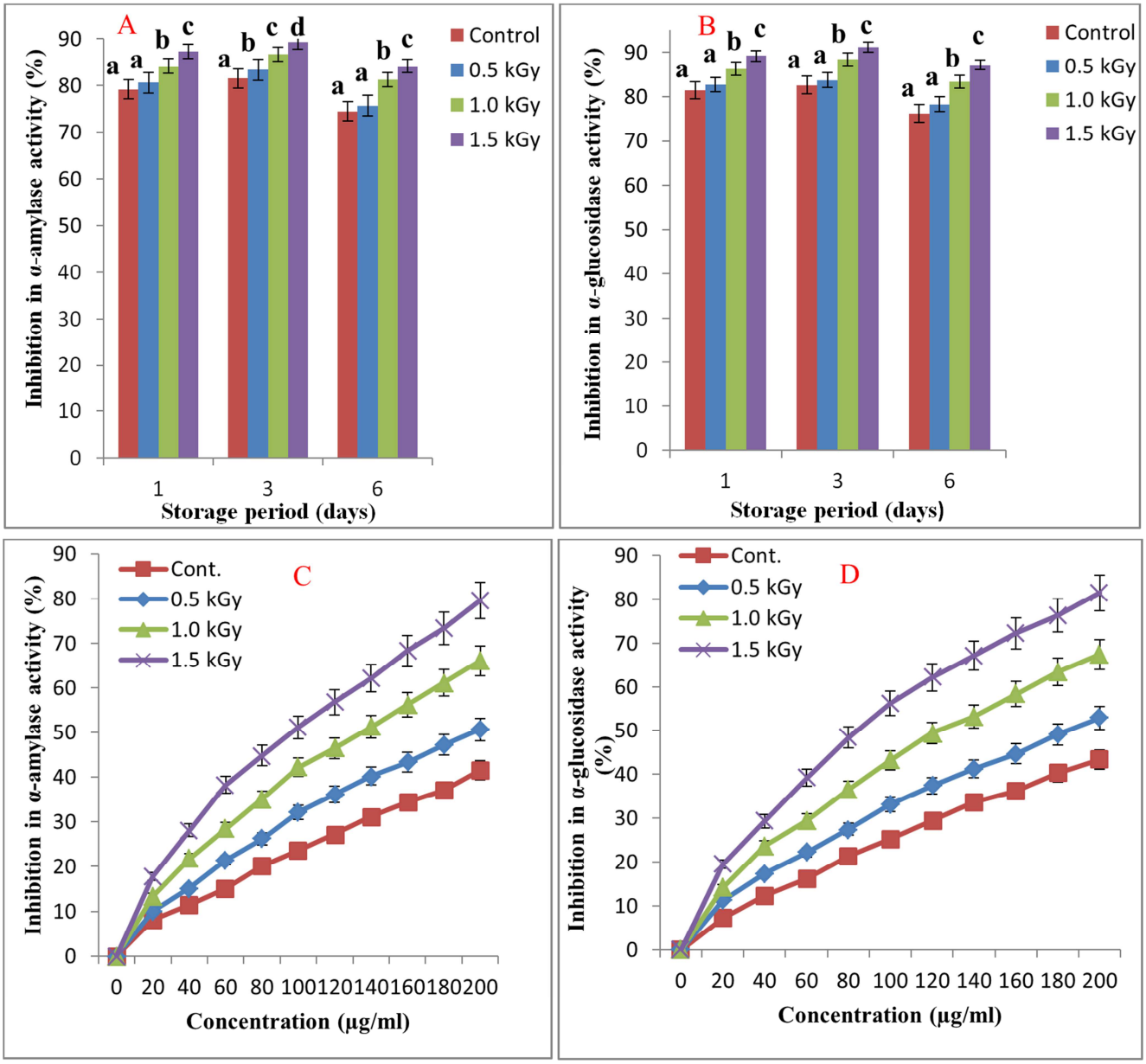


Figure 2. *Effect of control and irradiated sprout concentration on various antioxidant activities at 3 days of storage.*

(A) = DPPH activity, (B) = ferric reducing power, (C) = beta-carotene bleaching assay
(D) = hydroxyl radical scavenging activity, (E) = ferrous ion chelating activity



A and B = irradiation effect; C and D = concentration effect

Figure 3. *Inhibition effect of control and irradiated onion sprouts on activities of α-amylase and α-glucosidase enzymes.*

4.8. Hypoglycemic Activity

Inhibition of enzymes related to carbohydrate hydrolysis (α-amylase and α-glucosidase) has been accepted as one of the therapeutic approach in the management of type-2 diabetes. The inhibition of α-amylase and α-glucosidase delay carbohydrate digestion thereby reduces rate of glucose production and consequently causes reduction in postprandial hyperglycemia especially in patients with type-2 diabetes. Hypoglycemic/anti-diabetic activity of control and irradiated onion sprouts is shown in Figure 3 (A - D). It is seen from the figure that at 3 days of storage, hypoglycemic activity was significantly ($p \leq 0.05$) higher in samples irradiated at doses above 0.5 kGy compared to control. Positive correlations ($r = 0.93$) existed between hypoglycemic activity, gamma irradiation treatment and extract concentration; thereby indicating increase in inhibition of both alpha-glucosidase and alpha-amylase activity with increase in both irradiation dose and extract concentration. Among treatments, highest inhibition in alpha-glucosidase and alpha-amylase activity was observed in 1.5 kGy samples. Comparison of the hypoglycemic activity revealed that irradiation treatment resulted in significant ($p \leq 0.05$) inhibition of alpha-glucosidase activity compared to alpha-amylase activity. During storage, hypoglycemic activity increased and reached to its maximum at 3 days of storage. In samples irradiated at 1.5 kGy, percentage inhibition in enzymatic activity over control increased by 9.5% for alpha-amylase and 10.5% for alpha-glucosidae at 3 days of storage. Beyond 3 days of storage, hypoglycemic activity decreased in both control and

irradiated sprouts and decrease was significantly ($p \leq 0.05$) higher in control samples compared to irradiated sprouts. At 6 days of storage, decrease in inhibition of alpha-amylase activity from the maximum value was 8.6% for control and 5.7% for 1.5 kGy irradiated sprouts. On the other hand, decrease in inhibition of alpha-glucosidase activity was of the order of 7.7% for control and 4.4% for 1.5 kGy irradiated sprouts. From the concentration data it is inferred that for both alpha-amylase and alpha-glucosidase enzyme, inhibition in activity increased up to 79.6% and 81.5% for 1.5 kGy sprouts applied at 200 µg/ml concentration compared to 41.5% and 43.4% in control. α-Amylase is responsible for cleaving starch during the digestive process, which is important in the management of postprandial blood glucose levels. The α-amylase and α-glucosidase inhibition potential of the irradiated sprouts extract was significantly higher during 1-3 days which is correlated with higher phenolic content [57]. Therefore, enhancement in hypoglycemic activity of irradiated onion sprouts is attributed to the higher phenolic compounds in irradiated sprouts. Polyphenols such as phenolic acids (chlorogenic acid, gallic acid) and flavonoids such as quercetin, kaempferol and catechin have potent α-amylase and α-glucosidae inhibitory activities and play a vital role in retardation of carbohydrate digestion and mitigation of postprandial hyperglycemic excursions [57]. The results of the present study follows similar trend with previously published work that plant phytochemicals particularly polyphenols are mild inhibitors of α-amylase and strong inhibitors of α-glucosidase activities [58].

4.9. EC_{50} Values in Biological Assays

Antioxidant activity is the main physiological role of functional foods. In biological systems, reactive oxygen species (ROS) and radicals may oxidize nucleic acids, proteins or lipids and result in initiation of number of degenerative diseases. To reduce the incidence of chronic diseases including heart disease and other cancers, the antioxidant activity is the main factor governing the efficacy of the foods. For better comparison of the effect of gamma irradiation on antioxidant and hypoglycemic activity of onion sprouts, the results obtained from the DPPH radical scavenging, ferric reducing power, beta-carotene bleaching activity, hydroxyl radical scavenging activity, ferrous ion chelating assay and hypoglycemic activity were expressed as EC_{50} values. The EC_{50} values of control and irradiated onion sprouts for antioxidant activity were compared with standard ascorbic acid, gallic acid, BHT and EDTA while as EC50 values for hypoglycemic activity were compared with acarbose. A low EC_{50} value is the indication of strong biological activity. The EC_{50} values for various antioxidant assays and hypoglycemic activity for control and irradiated onion sprouts are presented in Table 5. Data analysis indicated that treatment of irradiation decreased the EC_{50} values for all the antioxidant assays and the decrease was significantly higher in 1.5 kGy samples. Comparison of the EC_{50} values revealed that DPPH radical scavenging activity of control and irradiated onion sprouts was significantly lower than standard ascorbic acid, gallic acid and BHT. Radiation processing of onion sprouts at 1.5 kGy significantly ($p \leq 0.05$) modified their reducing power; though the reducing power was again lower than ascorbic and gallic acid but at par with that of standard BHT. However, radiation processing of onion sprouts significantly ($p \leq 0.05$) enhanced the inhibition of beta-carotene bleaching, hydroxyl radical scavenging and ferrous ion chelating activity. The EC_{50} values of irradiated onion sprouts for these assays were significantly ($p \leq 0.05$) lower compared to the standards. Comparison of the EC_{50} values further supported the finding that antioxidant activity of onion sprouts was maximum at 3 days of storage. Data of EC_{50} values for hypoglycemic activities revealed that radiation processing of onion sprouts at 1.5 kGy was significantly ($p \leq 0.05$) beneficial to bring reduction in EC_{50} values thereby modifying the hypoglycemic activities compared to control samples. Results of hypoglycemic activity indicated that decrease in EC_{50} values over control due to radiation processing of onion sprouts at 1.5 kGy was 59.4% for inhibition in α-amylase activity and 61.4% for inhibition in α-glucosidase activity, thereby confirming the highest inhibition in α-glucosidase activity compared to α-amylase activity.

Table 5. *Effect of radiation treatments on the EC_{50} values (µg/ml) of onion sprouts for varios antioxidant and hypoglycemic assays during storage under refrigerated condition and comparison with standards.*

Storage period	Irradiation treatment (kGy)								
(days)	control	0.5	1.0	1.5	AA	GA	BHT	EDTA	Acarbose
DPPH radical scavenging assay									
1	340.4±5.1	311.2±4.6	271.1±3.3	222.4±3.1	19.6±0.45	16.1±0.36	18.9±0.41	-	-
3	259.7±3.3	216.4±3.2	177.5±2.2	132.8±2.1	-				
6	412.4±3.4	349.4±2.3	313.4±2.2	278.3±2.2	-				
Ferric reducing ability power (FRAP)									
1	191.2±2.1	145.2±1.6	115.1±1.3	96.4±1.1	30.6±0.55	9.7±0.16	57.7±1.1	-	-
3	148.1±1.5	117.6±1.4	94.4±1.2	76.9±1.1	-				
6	232.4±3.4	192.4±3.1	151.4±1.2	113.3±1.2	-				
Beta-carotene bleaching assay									
1	392.4±4.1	342.2±3.6	271.1±3.3	224.4±2.1	-	-	345.2±4.4	-	-
3	354.6±4.3	252.5±3.7	205.7±3.2	171.1±2.1	-				
6	482.4±4.4	449.4±3.3	363.4±3.6	314.3±2.2	-				
Hydroxyl radical scavenging assay									
1	362.4±3.5	333.2±3.1	291.1±2.3	264.4±1.7	237.2±2.1	216.6±1.6	-	-	-
3	296.7±2.3	225.2±2.1	186.5±1.6	159.1±1.1	-				
6	462.4±3.6	422.4±3.3	373.4±2.6	324.3±2.2	-				

Storage period	Irradiation treatment (kGy)								
(days)	control	0.5	1.0	1.5	AA	GA	BHT	EDTA	Acarbose
Ferrous ion chelating assay									
1	272.4±3.1	242.2±2.7	214.1±2.1	174.4±1.1	-	-	-	400.2±4.4	-
3	236.4±2.3	203.2±1.7	168.5±1.2	114.1±1.1	-				
6	322.4±3.1	285.4±2.5	253.4±1.6	214.3±1.2	-				
Inhibition in α-amylase activity									
1	317.2±3.1	280.4±2.5	215.4±2.1	170.2±1.5	-	-	-	-	20.2±1.4
3	240.9±2.3	197.6±1.7	136.7±1.2	97.8±1.1	-				
6	375.4±3.1	321.6±2.5	260.1±1.6	230.4±1.2	-				
Inhibition in α-glucosidase activity									
1	302.4±2.8	265.4±2.3	196.2±2.1	150.2±1.1	-	-	-	-	15.2±1. 1
3	230.4±2.3	188.4±1.7	131.5±1.2	88.9±1.1	-				
6	351.4±3.1	301.6±2.2	229.2±1.4	189.4±1.2	-				

AA = ascorbic acid; GA = gallic acid; BHT = butylatedhydroxt toluene; EDTA = ethylenediamine tetraacetic acid

5. Conclusion

Based on the results, it is concluded that radiation processing of onion sprouts at doses above 1.0 kGy besides maintaining the external appearance and appeal resulted in significant ($p \leq 0.05$) enhancement in the content of bioactive phytochemicals and functional properties. Inhibition in the activity of enzymes related to carbohydrate hydrolysis at doses above 1.0 kGy will increase the nutraceutical potential of irradiated onion sprouts and will serve as one of the therapeutic approach in the management of type-2 diabetes.

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