Food Sci. Biotechnol. Vol. 18, No. 2, pp. 390 ~ 395 (2009)

Food Science and Biotechnology

Effect of Irradiation of Red Radish Seeds on the Seed Viability and Functional Properties of Sprouts

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Abstract Red radish seeds were irradiated at doses up to 8 kGy using electron beam (e-beam) and gamma ray (γ-ray). The seed viability and functional properties (carotenoid, chlorophyll, ascorbic acid, and total phenol) of sprouts grown from these irradiated seeds were evaluated. High germination percentage (≥97%) was observed in seeds irradiated at ≤5 kGy, but the yield ratio and sprout length significantly decreased with increased irradiation dose. Irradiation at ≥6 kGy resulted in curling of the sprout roots. Sprouting enhanced the functional properties of red radish seeds as indicated by the increased carotenoid, chlorophyll, ascorbic acid, and total phenol contents during germination. However, radiation treatment hampered the growth of seeds resulting in underdeveloped sprouts with decreased carotenoid, chlorophyll, ascorbic acid, and total phenol contents. In general, e-beam and γ-ray irradiation of red radish seeds showed similar effects on the seed viability and functional properties of sprouts. Postharvest storage reduced the functional quality of sprouts.

Keywords: red radish sprout, irradiation, seed viability, functional property

Introduction

Fresh sprouts, such as alfalfa, broccoli, radish, and cabbage sprouts, have become popular worldwide due to their high nutritional values. They contain a substantial amount of vitamins, minerals, fiber, protein, and antioxidants (1-3). Radish sprout, in particular, exhibited the highest hydroxyl radical scavenging ability among 11 kinds of commonly available vegetables (4). Studies have also shown that radish sprout extract had high phase 2 enzyme induction potency (5) and consequently has potential anti-cancer action (6).

While seed sprouts are known as highly nutritious foods, they are also a recognized source of foodborne illness. From 1973 to 2006, at least 40 outbreaks of foodborne illness have been associated with the consumption of raw sprouts (7). Investigations revealed that the seeds were the most significant source of foodborne pathogens in most sprout-related outbreaks (8). In a recent survey on the microbiological quality of commercial seed sprouts in Korea, red radish sprout was found to be contaminated with *Listeria monocytogenes* (9). The US Food and Drug Administration (FDA) has approved the use of irradiation at doses up to 8 kGy to control microbial pathogens in seeds intended for sprout production (10). Radiation energy can completely eliminate foodborne pathogens due to its ability to penetrate the cracks, crevices, and intercellular spaces of the seeds that harbor the pathogens (11).

Based from the study conducted by Waje *et al.* (9), a γ-ray irradiation dose of 4.95 kGy for red radish seeds is needed to achieve the recommended 5-log reduction in the population of various foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella typhimurium*, *L. monocytogenes,* and *Bacillus cereus*. Although this dose is still within the allowable limit set by FDA for seeds, a high irradiation dose may have adverse effects on the germination and viability of the seeds. It was previously reported that an irradiation dose of 8 kGy completely eliminated *E. coli* O157:H7 from alfalfa seeds but was accompanied by a decrease in the yield of sprouts (12). Moreover, Rajkowski *et al.* (13) found that the yield ratio, length, and thickness of broccoli sprouts grown from seeds irradiated at 2 kGy significantly decreased.

To date, there is no published data on the effects of both electron beam (e-beam) and γ-ray irradiation at doses up to 8 kGy on the viability of red radish seeds. Furthermore, the changes on the functional properties of red radish sprouts as affected by irradiation of the seeds and subsequent postharvest storage of sprouts have not been investigated. Thus, this study was conducted to evaluate the effects of e-beam and γ-ray irradiation at different doses on the germination, yield, and growth of red radish seeds. The changes on the functional properties, such as carotenoids, chlorophyll, ascorbic acid, and total phenol contents, of red radish sprouts grown from e-beam and γ-ray irradiated seeds were also determined.

Materials and Methods

Sample preparation and irradiation Red radish seeds (*Raphanus sativus* L.) were purchased from a local sprout company in Seoul, Korea. The seeds were aerobically packed (ca. 200 g each) in oxygen-impermeable polyethylene bags and irradiated at doses 0, 1, 2, 3, 4, 5, 6, 7, and 8 kGy using an e-beam accelerator (FTR-125; Fuji Film, Tokyo,

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Received August 20, 2008; Revised November 4, 2008; Accepted November 11, 2008

Japan) with an acceleration voltage of 2.5 MeV and Co-60 gamma irradiator (100 kCi point source, AECL, IR-79, MDS; Nordion International Co., Ltd., Ottawa, ON, Canada). The absorbed doses were verified using a ceric-cerous and CTA dosimeters (Bruker Instruments, Rheinstetten, Germany). All seed samples were kept refrigerated at 4-8°C prior to use.

Germination test One-hundred seeds each from irradiated and non-irradiated samples were germinated according to the method described by the Association of Official Seed Analysts (14). Percent germination was determined by counting the number of germinated seeds. Each germination test was done in triplicates.

Yield ratio and sprout length A known amount of seeds were germinated in sterile petri dish lined with filter paper and added with distilled water. The seeds were germinated for 3 days at 25°C. Yield ratio is the equivalent weight of the sprouts divided by the weight of the seeds. At the end of each yield ratio determination, 100 sprouts were measured for sprout length with a digimatic caliper (CD-15CPX; Mitutoyo Corp., Kawasaki, Japan). All experiments were conducted in triplicates.

Sprout preparation for chemical analysis Red radish seeds irradiated at 0, 1, 3, and 5 kGy were sprouted in an EasyGreen Automatic Sprouter System (Model OV; Seed & Grain Tech, Inc., Las Vegas, NV, USA). Approximately 20 g each of irradiated and non-irradiated seeds was placed in small cartridges. The mist generator was set every 3 hr for 15 min and the seeds were grown for 5 days at 22-25 °C. After harvest, the sprouts were packed in commercial polyethylene packing container and immediately stored at 4-8°C for 1 week. Prior to germination, the non-irradiated seeds were analyzed for carotenoid, chlorophyll, ascorbic acid, and total phenol contents. Analyses were repeated for both irradiated and non-irradiated sprouts on the 3rd day of germination, immediately after harvest, and on the 4th and 7th day of postharvest storage. All experiments were done in triplicates.

Total carotenoid and chlorophyll analysis The carotenoids and chlorophyll contents of red radish sprouts were determined according to the method described by Fan and Thayer (15). The pigments were extracted with cold acetone (Duksan Pure Chemicals, Gyeonggi, Korea) and partitioned into diethyl ether (Duksan Pure Chemicals). The chlorophyll a, chlorophyll b, and carotenoid contents were calculated using the equations developed by Lichtenthaler and Wellburn (16). Total chlorophyll was calculated as the sum of chlorophylls a and b.

Ascorbic acid analysis The ascorbic acid content of broccoli sprouts was determined using titrimetric method as described by the AOAC (17).

Total phenol analysis The total phenol content of red radish sprouts was determined using the Folin-Denis colorimetric method (18). The results were calculated on the basis of a calibration curve of gallic acid (Sigma-Aldrich, Steinhein, Germany) and expressed as gallic acid equivalents (mg)/g dry weight of sprouts.

Statistical analysis Results from the measurements (n=3) were analyzed statistically using the SAS for Windows V8. (Statistical Analysis Systems Institute, Cary, NC, USA) Analysis of variance and Duncan's multiple-range test were employed to determine if the values were significantly different (p<0.05).

Results and Discussion

Seed viability of irradiated red radish seeds The % germination, yield ratio, and sprout length of red radish sprouts grown from irradiated seeds are shown in Table 1. The irradiated seeds exhibited high germination percentage with ≥97% for dose levels of ≥5 kGy in both irradiation treatments. Germination was still above 90% in seeds irradiated at 8 kGy using e-beam. In the case of γ-ray irradiation, seed germination was also relatively high (≥88%) at doses up to 7 kGy. However, the yield ratio and sprout length decreased linearly with increased irradiation dose regardless of the radiation source used. Furthermore, treatment of sprouts at a dose level of ≥6 kGy resulted in the curling of the sprout roots. Figure 1 illustrates the effect of irradiation of seeds on the growth of red radish sprout. At 5 kGy, the yield ratio and sprout length decreased by about 30 and 20%, respectively. At 8 kGy, a 53% reduction in the yield ratio

Table 1. Germination, yield ratio, and length of red radish sprouts grown from irradiated seeds[1)]

Dose (kGy)	e-Beam			γ-Ray		
	Germination (%)	Yield ratio (g/g seed)	Sprout length (mm)	Germination (%)	Yield ratio (g/g seed)	Sprout length (mm)
0	$99^{a2)}$	10.32 ± 0.64^{a}	30.3 ± 3.9^{a}	99^{a}	10.32 ± 0.64^{a}	30.3 ± 3.9^{a}
1	99^{a}	8.98 ± 0.49^{b}	29.3 ± 2.9^{b}	99^{ab}	8.93 ± 0.11^{b}	29.4 ± 2.4^{b}
2	99^{a}	8.30 ± 0.09^{c}	27.2 ± 2.8^{c}	99^{ab}	8.23 ± 0.29^{bc}	26.9 ± 3.5^{c}
3	99^{a}	7.99 ± 0.19^{c}	26.0 ± 3.2^{d}	98^{ab}	7.90 ± 0.38^{cd}	25.9 ± 2.9^{d}
4	98^{a}	7.20 ± 0.40^{d}	24.0 ± 3.0^{e}	98^{ab}	7.38 ± 0.50^{de}	25.5 ± 2.4^{d}
5	98^{ab}	6.97 ± 0.26^{d}	23.7 ± 2.2^{e}	97^{b}	6.67 ± 0.26^{ef}	23.5 ± 3.7^{e}
6	97^{bc}	6.61 ± 0.09^{d}	17.8 ± 2.6^{f}	90^{c}	6.29 ± 0.62^{fg}	16.6 ± 1.7^{f}
7	95^{cd}	5.88 ± 0.19^{e}	15.7 ± 2.6^{g}	88^{c}	5.69 ± 0.41^{g}	15.2 ± 1.4^{g}
8	94^{d}	4.54 ± 0.16^{f}	11.3 ± 2.3^{h}	81^{d}	4.84 ± 0.26^{h}	11.2 ± 1.9^{h}

[1)]Values represent mean±SD, n=3.
[2)]Means followed by different letters within the column are significantly different (p<0.05).

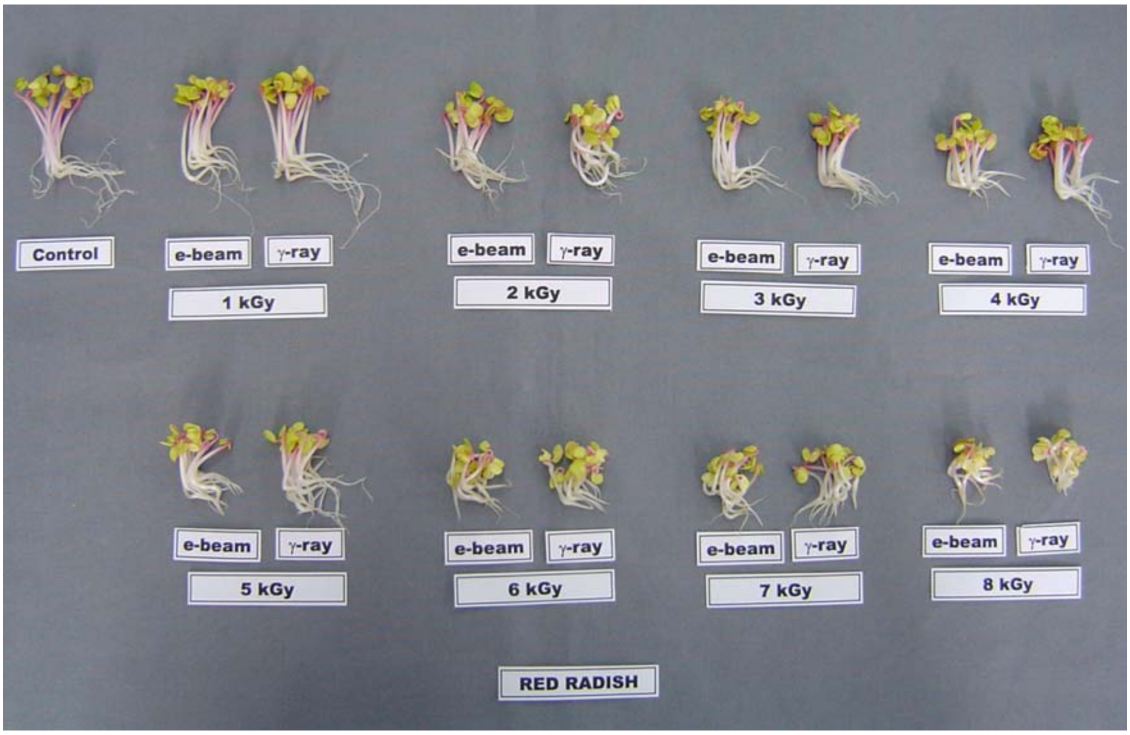

Fig. 1. Red radish sprouts grown for 3 days from irradiated seeds.

was observed. The decrease in length and yield ratio of red radish sprouts reflects the adverse effect of irradiation on the seeds as the irradiation dose increased. Rajkowski *et al.* (13) previously reported that irradiation of broccoli seeds also resulted in a decrease in yield ratio as the irradiation dose increased and the decrease corresponded to the decrease in the germination percentage. Likewise, Rajkowski and Thayer (19) found that as the dose increased above 3 kGy, the percent germination of alfalfa seeds was not affected but the yield ratio decreased. In this study, it appears that red radish seed has high resistance to irradiation in terms of its germinating ability but the growth rate is hampered. The slow growth of sprouts can be the result of stress response of seeds to irradiation (20). An irradiation dose of up to 5 kGy may not affect the germination of red radish seeds but it could result in a considerable reduction on the yield ratio and sprout length which may be unacceptable to sprout producers. To compensate for the yield loss, Fan *et al.* (20) suggested that sprouts be propagated for longer time to achieve the same yield as that in non-irradiated seeds. Further investigations are needed whether extending the germination time of irradiated red radish seeds would increase the yield ratio of sprouts.

Total carotenoid content Red radish seeds irradiated at 1, 3, and 5 kGy were germinated for 5 days to evaluate the effect of irradiation treatment on the functional properties of red radish sprouts during germination and postharvest storage at 4-8°C. The total carotenoid content of red radish seeds increased considerably during sprouting (Table 2). This shows that sprouting enhances the functional properties of red radish seeds. It was previously reported that sprouts have higher amounts of vitamins, minerals, and antioxidants than raw seeds (2,21-23). Khattak *et al.* (24) found that the carotenoid content of chickpea seeds significantly increased with increased germination time. The enhanced functional quality is probably due to the chemical changes that occur during sprouting which mobilize the stored carbohydrates and proteins into the growing sprout and simple carbohydrates, free amino acids, and essential nutrients are made in available form that can be readily used by the body (25).

Table 2. Total carotenoid content[1)] (μg/g, d.b.) of red radish sprouts grown from irradiated seeds

Stage	Time (day)	Control	e-Beam (kGy)			γ-Ray (kGy)		
			1	3	5	1	3	5
Seeds	0	0.32±0.02	-	-	-	-	-	-
Germination period	3	5.52±0.44$^{az2)}$	4.30±0.04^{bz}	2.55±0.10^{cz}	1.62±0.14^{ez}	4.41±0.10^{bz}	2.93±0.23^{cz}	2.00±0.23^{dz}
	5	21.45±1.56^{ax}	17.97±0.86^{bcx}	15.86±0.95^{cw}	10.82±1.83^{dx}	19.41±0.43^{bw}	17.28±0.28^{bcx}	15.79±1.33^{cx}
Postharvest storage	8	19.88±0.42^{ax}	15.04±0.46^{cy}	12.23±0.45^{dx}	8.92±0.43^{ey}	16.53±0.86^{bx}	14.25±1.06^{cy}	13.75±1.08^{cy}
	11	15.62±0.65^{ay}	13.41±1.52^{by}	10.80±0.29^{cy}	8.50±0.31^{dy}	14.01±0.44^{by}	13.88±1.39^{by}	13.25±0.46^{by}

[1)]Values represent mean±SD, n=3; -, not performed.
[2)]Means followed by different letters within the row (a-e) and within the column (w-z) are significantly different (p<0.05).

Table 3. Total chlorophyll content[1)] (μg/g, d.b.) of red radish sprouts grown from irradiated seeds

Stage	Time (day)	Control	e-Beam (kGy)			γ-Ray (kGy)		
			1	3	5	1	3	5
Seeds	0	3.81±0.19	-	-	-	-	-	-
Germination period	3	23.88±2.96[az2)]	20.89±0.62[abz]	17.06±0.24[cz]	12.90±0.50[dz]	22.36±1.20[az]	18.29±0.41[bcz]	16.37±3.58[cz]
	5	68.00±7.58[ax]	41.09±1.89[cx]	39.85±2.07[cw]	31.10±2.32[dx]	65.50±1.63[aw]	53.47±1.75[bx]	47.18±6.56[bcx]
Postharvest storage	8	66.65±7.49[ax]	39.68±2.06[dex]	35.64±0.95[efx]	29.89±2.58[fx]	57.60±2.89[bx]	48.74±5.97[cx]	43.50±3.18[cdx]
	11	40.70±2.64[ay]	32.33±4.66[bcy]	27.37±2.69[cdy]	23.32±3.52[dy]	37.88±5.45[aby]	30.20±1.48[cy]	28.58±1.37[cdy]

1)Values represent mean±SD, *n*=3; -, not performed.
2)Means followed by different letters within the row (a-f) and within the column (x-z) are significantly different ($p<0.05$).

Irradiation of red radish seeds resulted in a significant decrease of the carotenoid content in sprouts and the decrease was greater with increasing radiation dose. On the other hand, Fan *et al.* (26) reported that irradiation of alfalfa seeds up to 3 kGy did not influence the carotenoid content of sprouts. Because of the slow growth of irradiated red radish seeds, the harvested sprouts were not as developed as those grown from non-irradiated ones which would account for the lower carotenoid content in sprouts grown from irradiated seeds. Further research on the effect of seed irradiation on the carotenoid content of different types of sprouts may be necessary since it appears that red radish and alfalfa seeds have different responses to irradiation treatment. All red radish sprout samples exhibited a significant decrease in the carotenoid content after storage.

Total chlorophyll content A substantial increase was also observed in the chlorophyll content of red radish sprouts after seed germination (Table 3). This plant pigment, which contributes to the greenness of sprouts, decreased with increased irradiation dose and the decrease was greater in sprouts grown from seeds treated with e-beam. This suggests that sprouts from γ-ray irradiated seeds were relatively greener than those from e-beam treated ones. The difference in the chlorophyll content between the e-beam and γ-ray treated samples was still evident after 4 days of postharvest storage but disappeared after 7 days of storage. The chlorophyll content of red radish sprouts is dependent on the growth rate of the seeds. It was visually observed that sprouts grown from irradiated seeds were less developed and appeared lighter in color compared with that from non-irradiated ones which would account for the decrease in chlorophyll content of sprouts grown from irradiated seeds. The chlorophyll content decreased with storage time in all the samples and the values remained lower in sprouts grown from irradiated seeds than that from non-irradiated ones. In the case of alfalfa, irradiation of seeds did not affect the chlorophyll content of alfalfa sprouts but the subsequent storage of sprouts also resulted in a decrease of the chlorophyll content (26). In a color measurement of irradiated and non-irradiated alfalfa sprouts, it was found that the sprouts turned yellow during storage at 6°C (15) resulting in a decrease in chlorophyll content of sprouts.

Ascorbic acid content The ascorbic acid content of non-irradiated seeds was relatively low (35.4 μg/g), but it increased 21 times after germination and growth of sprouts (744.7 μg/g) on a dry weight basis (Table 4). Frias *et al.* (27) also reported that germinated lupins contain higher amounts of ascorbic acid than raw seeds. The ascorbic acid content of red radish sprouts grown from irradiated seeds decreased with increased irradiation dose regardless of the radiation source used. In contrast, earlier studies involving alfalfa sprouts grown from irradiated seeds revealed that ascorbic acid content was higher in samples grown from irradiated seeds than those grown from non-irradiated ones (20,26). It appears that the response of seeds to irradiation in relation to nutritional changes may vary depending on the type of seeds. Additional studies are needed to determine the effect of irradiation on the ascorbic acid content of sprouts grown from different varieties of irradiated seeds. The decrease in ascorbic acid content of red radish sprouts may probably be due to the slow growth of sprouts caused by the irradiation of seeds. After 4 and 7 days of postharvest storage, the ascorbic acid content decreased in all samples and the values remained lower in sprouts grown from irradiated seeds as compared to that from non-irradiated ones. The decrease in ascorbic acid content during storage was also observed in alfalfa sprouts (15,26).

Total phenol content The total phenol content of red

Table 4. Ascorbic acid content[1)] (μg/g, d.b.) of red radish sprouts grown from irradiated seeds

Stage	Time (day)	Control	e-Beam (kGy)			γ-Ray (kGy)		
			1	3	5	1	3	5
Seeds	0	35.4±1.4	-	-	-	-	-	-
Germination period	3	297.1±35.3[az2)]	235.8±16.8[bcz]	209.5±16.4[cdz]	169.8±8.3[ez]	254.1±8.3[bz]	223.1±15.1[bcdz]	197.1±7.5[dez]
	5	744.7±66.3[ax]	600.5±32.9[bw]	499.5±36.2[cw]	358.4±18.0[dx]	622.2±43.9[bx]	496.8±14.4[cx]	443.5±15.4[cw]
Postharvest storage	8	623.9±27.4[ay]	550.4±18.8[bx]	393.2±23.3[cx]	317.6±15.6[dy]	563.7±43.2[bxy]	416.2±36.1[cy]	397.5±13.8[cx]
	11	566.4±19.3[ay]	499.3±32.7[by]	323.3±15.4[dey]	308.5±11.2[ey]	541.6±32.3[ay]	394.9±18.8[cy]	363.0±23.8[cdy]

1)Values represent mean±SD, *n*=3; -, not performed.
2)Means followed by different letters within the row (a-e) and within the column (w-z) are significantly different ($p<0.05$).

Table 5. Total phenol content[1)] (mg of gallic acid/g sample, d.b.) of red radish sprouts grown from irradiated seeds

Stage	Time (day)	Control	e-Beam (kGy)			γ-Ray (kGy)		
			1	3	5	1	3	5
Seeds	0	7.99±0.40	-	-	-	-	-	-
Germination period	3	35.56±0.99$^{ay2)}$	32.72±0.74^{bz}	28.14±1.22^{dz}	22.99±0.50^{ey}	35.04±0.49^{ay}	30.30±0.54^{cz}	28.47±2.18^{cdy}
	5	45.76±1.18^{ax}	43.38±1.79^{abx}	42.62±2.74^{abcx}	39.11±0.98^{cx}	44.50±1.67^{abx}	43.83±1.50^{abw}	41.62±3.15^{bcx}
Postharvest storage	8	45.70±0.93^{ax}	43.09±0.18^{bx}	39.74±1.07^{cdxy}	37.94±2.58^{dx}	43.30±0.50^{bx}	41.78±0.64^{bcx}	40.15±1.75^{cdx}
	11	43.37±2.34^{ax}	39.35±2.09^{bcy}	37.06±2.17^{cy}	36.79±1.19^{cx}	41.43±0.40^{abx}	37.92±0.18^{cy}	37.51±0.95^{cx}

[1)]Values represent mean±SD, n=3; -, not performed.
[2)]Means followed by different letters within the row (a-d) and within the column (w-z) are significantly different (p<0.05).

radish seeds (7.99 mg/g) significantly increased after 3 (35.56 mg/g) and 5 (45.76 mg/g) days of germination on a dry weight basis (Table 5). Similarly, Lopez-Amoros *et al.* (28) reported an increase in phenolic compounds after germination of peas and beans. Fenugreek and mungbean sprouts also exhibited higher phenol content and antioxidant activity as compared to their respective seeds (2,29). Irradiation of seeds at 1 and 3 kGy did not significantly change the total phenol content of red radish sprouts. However, the sprouts grown from seeds irradiated at 5 kGy exhibited lower phenol content than those grown from non-irradiated seeds. As mentioned earlier, the slow development of sprouts during germination may have contributed to the reduced phenol content of samples. In contrast, previous studies of Fan *et al.* (20,26) showed that alfalfa sprouts grown from seeds irradiated up to 4 kGy had higher antioxidant content than those grown from non-irradiated ones. It was suggested that the increase in the antioxidant capacity of sprouts may be the result of the stress response of seeds to irradiation. Results of this study did not show any significant increase in the phenol content of sprouts grown from irradiated seeds. The total phenol contents of red radish sprouts were generally stable during 4 days of postharvest storage.

A number of studies have shown that irradiation treatment can eliminate the foodborne pathogens in sprout seeds thereby enhancing the food safety of sprouts (30-33). The effect of irradiation at doses that inactivate these pathogens on the seed viability and functional properties of red radish sprouts was investigated. Results showed that irradiation up to 5 kGy did not significantly affect the germination of seeds regardless of the radiation source used but the yield ratio and sprout length decreased. It was found that the functional properties of seeds increased during germination and since the radiation treatment hampered the growth of red radish seeds, a substantial reduction in the carotenoid, chlorophyll, ascorbic acid, and total phenol contents of sprouts was observed. The functional properties of sprouts further decreased during storage indicating the importance of consuming freshly harvested sprouts. Additional studies are needed whether extending the germination time of irradiated red radish seeds will improve the yield ratio and functional quality of the sprouts. In general, e-beam and γ-ray irradiation of red radish seeds had similar effects on the seed viability and functional properties of sprouts. Low dose irradiation of seeds, in combination with other treatments, such as chemical and heat treatments, may be needed to achieve the recommended 5-log reduction on the populations of foodborne pathogens with minimal effects on the quality of sprouts.

References

1. Barillari J, Cervellati R, Paolini, M, Tatibouet A, Rollin P, Iori R. Isolation of 4-methylthio-3-butenyl glucosinolate from *Raphanus sativus* sprouts (*Kaiware daikon*) and its redox properties. J. Agr. Food Chem. 53: 9890-9896 (2005)
2. Frias J, Zielinski H, Piskula MK, Kozlowska H, Vidal-Valverde C. Inositol phosphate content and trypsin inhibitor activity in ready-to-eat cruciferous sprouts. Food Chem. 93: 331-336 (2005)
3. Sikora E, Cieslik E, Leszczynska T, Filipiak-Florkiewicz A, Pisulewski PM. The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. Food Chem. 107: 55-59 (2008)
4. Takaya Y, Kondo Y, Furukawa T, Niwa M. Antioxidant constituents of radish sprouts (*Kaiware-daikon*), *Raphanus sativus* L. J. Agr. Food Chem. 51: 8061-8066 (2003)
5. Lee SO, Lee IS. Induction of quinone reductase, the phase 2 anticarcinogenic marker enzyme, in Hepa1c1c7 cells by radish sprouts, *Raphanus sativus* L. J. Food Sci. 71: S144-S148 (2006)
6. O'Hare TJ, Wong LS, Force LE, Gurung CB, Irving DE, Williams DJ. Glucosinolate composition and anti-cancer potential of daikon and radish sprouts. ISHS Acta Hort. 765: 237-244 (2008)
7. Waje CK, Kwon JH. Improving the food safety of seed sprouts through irradiation treatment. Food Sci. Biotechnol. 16: 171-176 (2007)
8. National Advisory Committee on Microbiological Criteria for Foods. Microbiological safety evaluations and recommendations on sprouted seeds. Int. J. Food Microbiol. 52: 123-153 (1999)
9. Waje CK, Jun SY, Lee YK, Kim BN, Han DH, Jo C, Kwon JH. Microbial quality assessment and pathogen inactivation by electron beam and gamma irradiation of commercial seed sprouts. Food Control 20: 200-204 (2009)
10. Code of Federal Regulations. 21 CFR part 179 irradiation in the production processing and handling of food. US Federal Register 65: 64605-64607 (2000)
11. Buck JW, Walcott RR, Beuchat LR. Recent trends in microbiological safety of fruits and vegetables. Plant Health Progress. Available from: http://www.plantmanagementnetwork.org. Accessed May, 2008.
12. Kim HJ, Feng H, Kushad MM, Fan X. Effects of ultrasound, irradiation, and acidic electrolyzed water on germination of alfalfa and broccoli seeds and *Escherichia coli* O157:H7. J. Food Sci. 71: 168-173 (2006)
13. Rajkowski KT, Boyd G, Thayer DW. Irradiation D-values for *Escherichia coli* O157:H7 and *Salmonella* sp. on inoculated broccoli seeds and effects of irradiation on broccoli sprout keeping quality and seed viability. J. Food Protect. 66: 760-790 (2003)
14. Association of Official Seed Analysts. Rules for Testing Seeds. Graphic Publishing Company, Inc., Boston, MA, USA. pp. 26-32 (1965)
15. Fan X, Thayer DW. Quality of irradiated alfalfa sprouts. J. Food Protect. 64: 1574-1578 (2001)

16. Lichtenthaler HK, Wellburn AR. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biochem. Soc. T. 11: 591-592 (1985)
17. AOAC. AOAC Official Methods of Analysis. 17th ed. Method 967.21. Association of Official Analytical Chemists, Arlington, VA, USA (2000)
18. Schanderl SH. Methods in Food Analysis. 2nd ed. Academic Press, London, Elgland. pp. 701-725 (1970)
19. Rajkowski KT, Thayer DW. Alfalfa seed germination and yield ratio and alfalfa sprout microbial keeping quality following irradiation of seeds and sprouts. J. Food Protect. 64: 1988-1995 (2001)
20. Fan X, Thayer DW, Sokorai K. Changes in growth and antioxidant status of alfalfa sprouts during sprouting as affected by gamma irradiation of seeds. J. Food Protect. 67: 561-566 (2004)
21. Randhir R, Lin YT, Shetty K. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pac. J. Clin. Nutr. 13: 295-307 (2004)
22. Vidal-Valverde C, Frias J, Sierra I, Blazquez I, Lambein F, Kuo YH. New functional legume food by germination: Effect on the nutritive value of beans, lentils, and peas. Eur. Food Res. Technol. 215: 472-477 (2002)
23. Urbano G, Lopez-Jurado M, Hernandez J, Fernandez M, Moren MC, Frias J, Diaz-Pollan C, Prodanov M, Vidal-Valverde C. Nutritional assessment of raw, heated, and germinated lentils. J. Agr. Food Chem. 43: 1871-1877 (1995)
24. Khattak AB, Zeb A, Bibi N. Impact of germination time and type of illumination on carotenoid content, protein solubility, and *in vitro* protein digestibility of chickpea (*Cicer arietinum* L.) sprouts. Food Chem. 109: 797-801 (2008)
25. Fernandez-Orozco R, Piskula MK, Zielinski H, Kozlowska H, Frias J, Vidal-Valverde C. Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. Zapaton. Eur. Food Res. Technol. 223: 495-502 (2006)
26. Fan X, Rajkowski KT, Thayer DW. Quality of alfalfa sprouts grown from irradiated seeds. J. Food Quality 26: 165-176 (2003)
27. Frias J, Miranda ML, Doblado R, Vidal-Valverde C. Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. Multolupa. Food Chem. 92: 211-220 (2005)
28. Lopez-Amoros ML, Hernandez T, Estrella I. Effect of germination on legume phenolic compounds and their antioxidant activity. J. Food Compos. Anal. 19: 277-283 (2006)
29. MacCue P, Shetty K. Clonal herbal extracts as elicitors of phenolic synthesis in dark germinated mungbean for improving nutritional value with implications for food safety. J. Food Biochem. 26: 209-232 (2002)
30. Bari ML, Al-Haq MI, Kawasaki T, Nakauma M, Todoriki S, Kawamoto S, Isshiki K. Irradiation to kill *Escherichia coli* O157:H7 and *Salmonella* on ready-to eat radish and mungbean sprouts. J. Food Protect. 67: 2263-2268 (2004)
31. Rajkowski KT, Thayer DW. Reduction of *Salmonella* spp. and strains of *Escherichia coli* O157:H7 by gamma irradiation of inoculated sprouts. J. Food Protect. 63: 871-875 (2000)
32. Thayer DW, Boyd G, Fett WF. Gamma irradiation decontamination of alfalfa seeds naturally contaminated with *Salmonella mbandaka*. J. Food Sci. 68: 2266-2270 (2002)
33. Schoeller NP, Ingham SC, Ingham BH. Assessment of the potential for *Listeria monocytogenes* survival and growth during alfalfa sprout production and use of ionizing radiation as a potential intervention treatment. J. Food Protect. 65: 1259-1266 (2002)