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MINI REVIEW

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Improving the Food Safety of Seed Sprouts Through Irradiation Treatment

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Abstract Fresh sprouts such as alfalfa, mung bean, radish, broccoli, and soybean sprouts have become very popular due do their high nutritional value. However, there have been several outbreaks of illness in the last few years that have been attributed to sprout consumption. A number of methods have been used to improve the safety of seed sprouts. One promising technology is the use of ionizing radiation treatment. Irradiation with doses up to 8 kGy has been approved in the USA to control microbial pathogens in seeds intended for sprout production. This review focuses on the potential use of ionizing radiation in reducing the pathogen levels in seed sprouts. The effects of irradiation on seed germination and the nutritional quality of the sprouts are discussed.

Keywords: food safety, seed, sprout, irradiation, pathogen

Introduction

Seed sprouts have gained popularity worldwide in recent years as highly nutritious foods that cost less than many other types of fresh produce, resulting in an increased demand for the product. Such products include alfalfa, mung bean, radish, broccoli, Chinese cabbage, mustard, kidney bean, and soybean sprouts. These sprouts, which are commonly consumed raw, contain a substantial amount of protein, fiber, minerals, and vitamins (1, 2), and are low in energy and fat (3). In addition, the consumption of fresh sprouts has been associated with a lower risk of developing cancers and cardio- and cerebro-vascular diseases (3-5). However, as the popularity of sprouts increases, so does the potential for sprout-related illnesses. Sprouted seeds have been identified as a special problem that poses unique challenges in relation to microbial food safety concerns (6, 7) because of the exponential growth of microorganisms during the sprouting process (8-13). The humid environment and heat generated during sprout production create a favorable environment for both sprout and microbial growth (3, 9, 14). Furthermore, the increased nutrients in germinating seeds provide substrates with which microorganisms, including foodborne pathogens, can proliferate during sprouting (1, 8). Numerous outbreaks of foodborne illness have already been reported relating to consumption of raw sprouts, often involving *Escherichia coli* O157:H7 and *Salmonella* sp. (12, 15-18). Table 1 (12, 19-21) shows some of the reported international outbreaks associated with sprout consumption from 1973 to 2006.

A number of studies have been conducted regarding the use of heat (22-24) and chemical treatments to control microbial growth (Table 2). Although most of the studies reported significant reductions in the microbial population, no single treatment has completely eliminated the pathogens from seeds or been very effective in the decontamination of sprouts. This may be due to the inability of sanitizers to infiltrate cracks, crevices, and intercellular spaces of the seeds and sprouts that harbor the pathogens (8). The most effective chemical treatment involved the use of calcium hypochlorite, thus, the US Food and Drug Administration (FDA) has recommended that prior to sprouting, all seeds must be disinfected by washing with 20,000 ppm calcium hypochlorite solution (35, 36).

Due to the unreliability of chemical sanitizers alone in eliminating pathogens in sprouts, the use of ionizing radiation in enhancing food safety of sprouts has been investigated by a number of researchers. Radiation energy can penetrate seed and sprout tissues and potentially eliminate pathogens confined within the protective tissues of the product (8). Ionizing radiation has long been recognized as safe and very effective in reducing foodborne pathogens (37). The maximum allowable irradiation dose set by FDA for fresh produce, including sprouts, is 1 kGy. In 2000, the FDA approved the use of irradiation at doses up to 8 kGy to control microbial pathogens in seeds used for sprouting (38). The objective of this paper is to review the potential use of irradiation treatment for reducing the levels of pathogenic microorganisms in sprouts, thereby improving food safety while maintaining the quality of the product. The risks involved and the effects of irradiation on the nutrient quality of seed sprouts will be also reviewed.

Irradiation treatment of fresh sprouts

A summary of recent studies investigating the irradiation of sprouts is presented in Table 3. Rajkowski and Thayer (39) investigated the use of gamma irradiation to reduce or inactivate *Salmonella* and *E. coli* O157:H7 inoculated in raw sprouts. Results showed that the D_{10} values (the irradiation dose needed for a 1-log reduction of the microbial population) of *Salmonella* spp. and *E. coli* O157:H7 cocktails made from vegetable isolates and inoculated in radish sprouts were 0.46 and 0.30 kGy,

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Table 1. Reported international outbreaks of illness associated with sprouts, 1973-2006 (12, 19-21)

Year	Pathogen	Country	Type of sprout
1973	*Bacillus cereus*	USA	Soy, cress, mustard
1988	*S. saint-paul*	United Kingdom	Mung
1989	*S. gold-coast*	United Kingdom	Cress
1994	*S.* Bovismorbificans	Sweden, Finland	Alfalfa
1995	*S. stanley*	USA, Finland	Alfalfa
1995-96	*S. newport*	USA, Canada, Denmark	Alfalfa
1996	*S. montevideo* and *S. meleagridis*	USA	Alfalfa
1996	*E. coli* O157:H7	Japan	Radish
1997	*E. coli* O157:H7	Japan	Radish
1997	*S. meleagridis*	Canada	Alfalfa
1997	*S. infantis* and *S. anatum*	USA	Alfalfa, mung
1997	*E. coli* O157:H7	USA	Alfalfa
1997-98	*S. seftenberg*	USA	Clover, alfalfa
1998	*E. coli* O157:NM	USA	Clover, alfalfa
1998	*S. havana*, *S. cubana*, and *S. tennessee*	USA	Alfalfa
1999	*S. mbandaka*, *S. typhimurium*, and *S. muenchen*	USA	Alfalfa
1999	*S. saint-paul*	USA	Clover
1999	*S. paratyhpi*	Canada	Alfalfa
2000	*S. enteritidis*	Canada	Alfalfa
2000	*S. enteritidis*	USA	Mung
2001	*S. enteritidis*	Canada	Mung
2001	*S. kottbus*	USA	Alfalfa
2002	*E. coli* O157:H7	USA	Alfalfa
2003	*S. saint-paul*, *S. chester*, and *E. coli* O157:H7	USA	Alfalfa
2004	*S. bovismorbificans*	USA	Alfalfa
2005	*E. coli* O157:H7	USA	Alfalfa
2005	*Salmonella* sp.	Canada	Mung
2006	*S. oranienberg*	Australia	Alfalfa

respectively. Hence, to achieve the 5-log reduction in microbial population recommended by FDA (9), an irradiation dose of 2.3 kGy is needed. The study also showed that a 0.5-kGy exposure of raw alfalfa sprouts grown from seeds naturally contaminated with *Salmonella* could eliminate the pathogen from the sprouts. These authors concluded that ionizing radiation could be used to reduce pathogen levels in sprouts. Rajkowski and Thayer (36) also conducted a study to determine the effect of gamma irradiation in maintaining the quality of fresh alfalfa sprouts regarding the aerobic microbial load and total coliform count. They found out that an irradiation with 2 kGy could decrease the total aerobic count by 2-3 logs and the total coliform count by 5 logs. In addition, the shelf-life of the sprouts was extended by 10 days. In a similar study done by Rajkowski *et al.* (14), the irradiation of broccoli sprouts with 2 kGy reduced the count of *E. coli* O157:H7 by 2 logs and also increased the shelf-life of the sprouts by 10 days.

In another study, radish and mung bean sprouts were treated with ionizing radiation at a dose of 1.5 and 2.0 kGy (40). Results showed that populations of *E. coli* O157:H7 and *Salmonella* were reduced to undetectable levels. Schoeller *et al.* (10) studied the potential use of electron beams to irradiate alfalfa sprouts inoculated with *Listeria monocytogenes* and reported that a 3.3 or 5.3 kGy irradiation dose could eliminate the pathogen, whereas an irradiation dose of 1.5 kGy could not. The above findings demonstrate that there may be significant differences in the radiation sensitivities of pathogens depending on the type of seed sprout and the irradiation treatment used.

While studies on the use of irradiation in sprouts have been successful in eliminating pathogens, the dose required to inactivate these pathogens (≥2 kGy) exceeds the current allowable limit (1 kGy) set by FDA for the treatment of fresh produce.

Irradiation treatment of seeds for sprout production

Table 2. Recent studies conducted on the effect of chemical treatments on microbial counts in seed sprouts

Seed/Sprout	Treatment	Results	Ref.
Alfalfa seed	5% lactic acid; 5 % acetic acid; 20,000 ppm $Ca(OCl)_2$	*E. coli* O157:H7 was reduced by 2-4 logs, but pathogen not eliminated	25
	1,800 ppm $Ca(OCl)_2$; 2,000 ppm NaOCl; 6% H_2O_2; 80% ethanol	*Salmonella* populations reduced by 3 logs, but pathogen not eliminated	26
	20,000 ppm chlorine; 5% Na_3PO_4; 8% H_2O_2; 1% $Ca(OH)_2$; 1% calcianted Ca; 5% lactic acid; 5% citric acid	*Salmonella* was reduced by 2-3 logs, no treatment eliminated the pathogen	27
	Fatty acid-based santizers	Populations of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* were reduced by 3-6 logs	28
Alfalfa seeds & sprouts	Electrolyzed oxidizing water	38-97 and 91-99% reduction of *E. coli* O157:H7 in seeds and sprouts, respectively; germination reduced from 92 to 49%	29
	20,000 ppm $Ca(OCl)_2$; 100 ppm chlorine; calcinated calcium	*Salmonella* not eliminated	30
Alfalfa, radish & mungbean seeds	Electrolyzed oxidizing water; 200 ppm active chlorinated water	*E. coli* O157:H7 was reduced by 3-6 logs	31
Alfalfa & mungbean seeds	Fumigation with ammonia (180 or 300 mg of ammonia/L air)	Populations of *E. coli* O157:H7 and *S. typhimurium* were reduced by 2-3 logs and 5-6 logs in alfalfa and mungbean, respectively	32
Mungbean seeds	Gaseous acetic acid (242 μL/L air)	Complete eradication of *Salmonella* and *E. coli* O157:H7 but not *L. monocytogenes*	33
	3% $Ca(OCl)_2$	4-5 log reductions of *E. coli* O157:H7, but not completely eliminated	34

Table 3. Recent studies conducted on the effect of irradiation on microbial counts in sprouts

Sprout	Type of irradiation/ irradiation dose (kGy)	Results	Ref.
Alfalfa	Gamma irradiation/ 0.5	Eliminated *Salmonella* from sprouts grown from seeds naturally contaminated with the pathogen	39
	1.0-2.0	Total aerobic count and coliform count decreased by 2-3 logs and 5 logs, respectively	36
	Electron beam/ 1.5, 3.3, and 5.3	3.3 and 5.3 kGy eliminated *Listeria monocytogenes* from the sprouts	10
Broccoli	2.0	2-log population reduction of *E. coli* O157:H7	14
Radish	Gamma irradiation	D_{10} values: *Salmonella* sp. – 0.46 kGy *E. coli* O157:H7 – 0.30 kGy	39
Radish & mungbean	Gamma irradiation/ 1.5 and 2.0	*E. coli* O157:H7 and *Salmonella* populations were reduced to nondetectable limits	40

Since sprouts are very fragile and cannot withstand extensive physical washings (41, 42), treatment of the seeds to reduce pathogen levels has been the primary focus of most recent studies regarding the food safety of sprouts. Moreover, investigations of sprout-related outbreaks have shown that the seeds were the most significant source of foodborne pathogens in sprouts (8, 9). Table 4 summarizes the recent studies done on the irradiation of seeds intended for sprout production.

With the same *Salmonella* sp. and *E. coli* O157:H7 isolates used in an earlier study of irradiated radish sprouts (39), Rajkowski *et al.* (14) studied the radiation D_{10} values of these pathogens in broccoli seeds. It was found out that the D_{10} values of *Salmonella* sp. (1.10 kGy) and *E. coli* O157:H7 (1.11 kGy) were higher than the previously reported values using radish sprouts. The authors attributed this increase in radiation D_{10} values to the relatively lower water content of the dry seeds compared to the moist sprouts. However, contrary to the findings of Thayer *et al.* (17), there was no increase in the radiation resistance of the pathogens as the alfalfa seeds were dried to reduce the moisture content from 12.14 to 10.48%. The authors stated that the relatively high D values were not due to the low moisture content of seeds but perhaps due to the presence of antioxidants in the seeds. There are reports that antioxidants can protect pathogens from radiation (43, 44). The same study also showed that a 2-kGy dose of radiation could achieve 3.3- and 2-log reductions in the populations of *E. coli* O157:H7 and *Salmonella*, respectively.

In another study conducted by Thayer *et al.* (45), the ability of gamma irradiation to sanitize alfalfa seeds naturally contaminated with *Salmonella mbandaka* was evaluated. An absorbed dose of 4 kGy resulted in a complete elimination of the pathogen from the seed lot samples. However, they observed that the contamination was randomly distributed on the seeds, thus, the dose required to inactivate the contaminating pathogen on the seeds will depend on the maximum contamination per seed and not on the mean contamination of the lot samples. They suggested that the irradiation of seeds should be combined with other sanitation methods to achieve the desired 99.99% inactivation of the pathogen. An attempt to combine irradiation with dry heat was done by Bari *et al.* (31). Results showed that a 2.5-kGy dose of radiation was required for the complete elimination of *E. coli* O157:H7 from radish seeds, whereas if combined with dry heat, a 2-kGy dose was enough to completely eliminate the pathogen from alfalfa and mung bean seeds. Preliminary studies involving the irradiation of alfalfa seeds with electron beams demonstrated a linear reduction in the natural microflora and *Salmonella* population as the dose level increases (9). Low energy electron beams effectively reduced the microbial surface contaminants of some agricultural products, including seeds and grains, to an undetectable level with no detrimental effects on the chemical and physiological qualities of the samples (46, 47).

Effect of irradiation on the quality of seed sprouts

There have been varying reports with regard to the effect of irradiation on the nutrient quality of sprouts. Fan and Thayer (4) studied the effect of gamma irradiation on the quality of alfalfa sprouts. Samples were gamma-irradiated at 0.85, 1.71, and 2.57 kGy and the results showed that the antioxidant capacity of the sprouts increased linearly with the radiation dose after both 1 and 7 days of storage at 6°C. In addition, the carotenoid content of sprouts irradiated at 1.71 and 2.57 kGy was higher than that of the nonirradiated sprouts after 7 days of storage. It was also reported that irradiation had no consistent effect on the color or chlorophyll and total ascorbic acid contents. On the other hand, Bari *et al.* (40) reported that the vitamin C content of radish and mung bean sprouts irradiated at 1.5 and 2.0 kGy decreased as the irradiation dose increased, but the color, firmness, and overall visual quality of the irradiated sprouts were acceptable. Using electron beam irradiation at 3.3 and 5.3 kGy doses, Schoeller *et al.* (10) observed that there were no noticeable changes in the appearance or odor of irradiated alfalfa sprouts.

In a similar study of irradiated alfalfa seeds, Fan *et al.* (48) investigated the vitamin C, total antioxidant, carotenoid, and chlorophyll contents of alfalfa sprouts. Sprouts grown from seeds irradiated at 1, 2, and 3 kGy had higher vitamin C and total antioxidant contents compared to those grown from nonirradiated seeds. Furthermore, the increase in vitamin C content and antioxidant activity was greater with increasing radiation dose. Irradiation had no effect on the carotenoid or chlorophyll content. The same results were also obtained by Fan *et al.* (49) when they analyzed the nutrient content of alfalfa sprouts grown from seeds irradiated with gamma rays at doses of 1, 2, 3, and 4 kGy. Alfalfa sprouts grown from treated seeds exhibited greater antioxidant capacity and ascorbic acid contents than sprouts grown from nonirradiated seeds.

The above studies indicate that the low dose irradiation of seeds and sprouts has minimal negative effects on the nutritional quality of sprouts. In most cases, the nutritional quality was considerably enhanced.

With regards to the germination of irradiated seeds, Rajkowski and Thayer (36) reported that there was little or no effect on the germination of irradiated seeds, but as the gamma irradiation dose increased, the yield ratio of alfalfa sprouts decreased. The authors concluded that irradiating alfalfa seeds up to a dose of 2 kGy would not unacceptably decrease the yield ratio for the production of alfalfa sprouts. On the other hand, the yield ratio, length, and thickness of broccoli sprouts grown from seeds irradiated at 2 kGy decreased significantly, and seed germination was decreased at 4 kGy (14). Similarly, Fan *et al.* (49) found that the germination and growth rate of gamma irradiated alfalfa seeds decreased as the radiation dose increased.

Dry heat in combination with irradiation at 2 kGy did not unacceptably decrease the germination of alfalfa seeds

Table 4. Recent studies conducted on the effect of irradiation on the microbial counts in seeds intended for sprout production

Sprout	Type of irradiation/ irradiation dose (kGy)	Results	Ref.
Alfalfa	Gamma irradiation/ 4.0	Complete elimination of *Salmonella mbandaka*	17
	Electron beam/ 1.0-10.0	Linear reduction of natural microflora and *Salmonella* population as dose levels increase At 10.0 kGy, *Salmonella* was not detected	9
Alfalfa & mung bean	Gamma irradiation/ 2.0 (in combination with dry heat)	Complete elimination of *E. coli* O157:H7	40
Broccoli	Gamma irradiation/ 2.0	D_{10} values: *Salmonella* sp. – 1.10 kGy *E. coli* O157:H7 – 1.11 kGy 3.3- and 2-log reduction in *E. coli* O157:H7 and *Salmonella* populations, respectively	39
Radish	Gamma irradiation/ 2.5	Complete elimination of *E. coli* O157:H7	40

nor the length of alfalfa sprouts, however the lengths of radish and mung bean sprouts decreased considerably (31). The treatment of alfalfa seeds with electron beam radiation up to a dose of 10 kGy did not reduce the germination of seeds, but physiological changes such as shortening, curling, and thickening of the roots were apparent (9).

Conclusion

While treatment with ionizing radiation can completely eliminate the pathogens present in seeds and sprouts without much effect on nutrient quality, they can affect the germination of seeds and the physiological quality of sprouts. Although the maximum radiation dose allowed for seed treatment is 8 kGy, adverse effects on seed germination and the yield ratio of sprouts were observed when more than 2-kGy of radiation was applied. On the other hand, the 1-kGy maximum allowable dose for the treatment of sprouts is not enough to inactivate the pathogens. The recommended 5-log reduction in microbial counts cannot be achieved by irradiation alone without drastically affecting the quality of seed sprouts. A combination of heat or chemical and irradiation treatments of seeds and sprouts should be explored further. Electron beam irradiation appears to be a promising technology. Preliminary studies have shown that the ability of seeds to germinate was not substantially affected even when high irradiation doses at various accelerated voltages was applied. This is another area of research that requires continued investigation. Furthermore, additional studies on the effect of ionizing and electron beam radiation on the nutritional and physico-chemical qualities of seed sprouts are needed (50, 51). It is important to note that a successful decontamination treatment must improve the food safety of sprouts by the complete elimination of pathogens while preserving seed viability and not compromising the sensory and nutrient qualities of the sprouts produced.

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