The Effect of Low-dose X-ray Irradiation on the Quality of Fresh-cut Asparagus in Microwaveable Vacuum Skin Packs

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Abstract. Low-dose x-ray irradiation was used on vacuum skin-packaged fresh-cut asparagus to extend its shelf life and improve its microbial safety. Freshly imported Peruvian asparagus (*Asparagus officinalis* L.) spears were obtained from a local source, cleaned, trimmed, and sanitized and then vacuum skin-packaged (VSP) in a tray pack system. A breathable lid film was vacuum-sealed to protect the product from contamination and allow for gas exchange. Asparagus were packaged in VSP, which modified the package atmosphere, and exposed to a target dose of 1.0 kGy or left untreated. Asparagus were also placed in low-density polyethylene (LDPE) bags as a control. All samples were then stored at 4 °C for 24 days. To determine the effectiveness of the irradiation treatment, sample packages were periodically removed from storage and several measurements were used to evaluate the product, including headspace gas content, microbial growth, water soluble sugar content, and enzyme activity. A dose of 1.0 kGy reduced initial microbial population up to 3.8 log colony-forming units (cfu)/g and maintained 20% more of the initial sugar content relative to the non-irradiated VSP asparagus. Phenylalanine ammonia-lyase (PAL) activity in VSP asparagus was lower than in the control package (LDPE bag) regardless of irradiation treatment ($P < 0.05$). However, x-ray treatment increased the PAL activity of asparagus in VSP up to Day 8 ($P < 0.05$).

Fresh-cut fruit and vegetable consumption has rapidly increased in the United States over the last decade. Currently, almost 30 kg/person of minimally processed fruits and vegetables are consumed per year in the United States (Abadias et al., 2008). Fresh-cut products are gaining in popularity as a result of consumer demand for convenience and nutri-

tion. Fresh green asparagus (*Asparagus officinalis* L.) is one of the most popular minimally processed vegetables in the United States as a result of its wealth of fiber and essential nutrients (Sothornvit and Kiatchanapaibul, 2009; Villanueva et al., 2005). However, it has a very limited shelf life because of its high respiration rate, rapid moisture loss, and microbial susceptibility (Simon et al., 2004). Minimally processed fresh-cut asparagus has relatively high initial microbial counts (10^4 to 10^6 cfu/g) (Garcia-Villanova et al., 1987; Simon et al., 2004). Therefore, it may be necessary to further treat fresh-cut asparagus before delivery to the consumer to reduce the microbial population. Various methods can be used to reduce microbial populations, including use of sanitizers and irradiation. Chlorine compounds are the most popular group of

Food and Drug Administration-approved sanitizers in the food industry but have limited effectiveness because of their rapid inactivation as a result of interaction with organic matter and difficulty in reaching into tissue crevices (Fan et al., 2003).

Ionizing radiation can enhance the safety and lengthen the shelf life of fresh-cut fruits and vegetables. Minimal product damage occurs because product temperature does not increase, and it is easily accomplished after packaging so that no recontamination of the product takes place (Farkas et al., 1997). The Food and Drug Administration (FDA) has approved ionizing radiation dose of up to 1.0 kGy for general fresh fruits and vegetables to control insects and to extend shelf life (FDA, 2009; Olson, 1998). Low-dose ionizing irradiation effectively reduces microbial populations on many fresh products and can extend product shelf life. Schmidt (2004) used 0.70 and 0.95 kGy irradiation to reduce the microbial population on fresh-cut tomatoes and obtained 1.8 and 2.2 log cfu/g reductions. Farkas (2006) showed that 1.0 kGy irradiation significantly reduced food spoilage bacteria and extended the sensorial quality of fresh-cut peppers and carrots. Hagenmaier and Baker (1997) found that chlorine sanitization in combination with x-ray irradiation (0.15 to 0.50 kGy) reduced the microbial contamination on lettuce.

The enzyme activity of fresh-cut products can be affected by irradiation. Specially, PAL activity is important because PAL is the main enzyme that causes the development of lignification and toughness (An et al., 2007; Powers and Drake, 2006; Waldron and Selvendran, 1990). Therefore, some studies showed that PAL response is closely correlated to decreased shelf life and visual quality of asparagus (Hennion et al., 1992; Howard et al., 1995). However, there are not many studies about PAL activity on asparagus after irradiation treatment. Previous research with lettuce (Fan et al., 2003), mushrooms (Benoit et al., 2000), and Clementines (Oufedjikh et al., 2000) have shown increased PAL activity after irradiation treatment. On the other hand, some enzyme activities were inhibited using irradiation. El Assi et al. (1997) determined that polygalacturonase activity was irreversibly suppressed but pectin methylesterase was significantly increased in tomato and pericarp after irradiation. Gautam et al. (1998) also reported reduction of polyphenol oxidase activity by irradiation at 0.5 and 1.0 kGy on mushrooms.

X-ray irradiation is the newest in ionizing irradiation technologies that is commercially used for foods. It is high-energy photons produced by the interaction of charged particles with matter, and it has essentially the same properties as gamma ray. However, x-ray is produced by a high-energy beam, which is generated from machine, whereas gamma ray is originated from radioisotopes (Farkas, 2006). It is generally lower in energy and therefore less penetrating than gamma ray (Environmental Protection Agency, 2007). Machine-generated x-ray can offer greater

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flexibility. For instance, it can be turned on and off unlike the constant emission of gamma ray from radioisotopes. Also, the x-ray generation does not involve radioactive substance and is free from harmful radioactive waste handling or disposal issue. On the other side, Robertson et al. (2006) said that x-ray irradiation has less negative consumer perception than gamma irradiation because consumers are familiar with medical x-ray.

Vacuum skin packaging is an advanced packaging technique and is used to maintain the quality of fresh meat and dairy products (Nychas and Tassou, 1996). The product is placed into a tray and film is brought into contact with the lid area. Rapid heating of the lid film at high temperature then takes place, and the thermally softened plastic is drawn over the product surface to form a "skin" over the product. Some lid films for VSP have high CO/CO₂ permeability and control the respiration rate of fresh vegetables, similar to what occurs with modified atmosphere packaging systems (Phillips, 1996). Vazquez et al. (2004) reported that the surface heat treatment during the packaging process slows bacterial growth more than traditional vacuum packaging. The combination with VSP with irradiation has the potential to maintain the quality of the asparagus. Thus, the objective of this research was to determine the effect of low-dose x-ray irradiation treatment on Peru asparagus. Microbial populations, PAL activity, and sugar content in the asparagus were monitored during 24 d of storage.

Materials and Methods

Materials

Asparagus and packaging. Untreated raw asparagus spears, imported from Peru, were purchased from a local grocery store (East Lansing, MI). Low density polyethylene bags (152.4 mm × 304.8 mm × 0.038 mm) were purchased from a local grocery store. Microwaveable multilayer barrier trays (Simple Steps™; 114.3 mm × 171.45 mm × 0.03175 mm) and a microwaveable lidding film (VP306K) were obtained from Cryovac (Duncan, SC). The oxygen transmission rate and carbon dioxide transmission rate of the film were 1.6×10^{-6} mol/m²/s (3024 cm³/m²/d) and 2.9×10^{-6} mol/m²/s (5523 cm³/m²/d), respectively. A sanitizer containing chlorine as the a.i. (Johnson CRS Fruits & Vegetable Wash) was purchased from S.C. Johnson (Sturtevant, WI).

Methods

Sample preparation. Asparagus spears without any visual defect were sorted to ≈8.5 mm in diameter and cut into ≈152.4-mm lengths using a stainless steel knife. Spears were washed with distilled water to remove soil and any contaminant, immersed in sanitizer solution, containing 200 ppm of chlorine, and rinsed twice with distilled water. The treatments were divided into three groups: 1) control (CON); 2) VSP (VSP); and 3) VSP plus x-ray irradiation (I-VSP). For CON, 210 g (≈18 asparagus) of fresh-cut asparagus were placed in LDPE bags, which

are commonly used for fresh fruits and vegetables in retail stores. The bags were not sealed to provide ambient air during the storage. For VSP samples, 210 g of fresh-cut asparagus was transferred under sanitary conditions into the trays and sealed using the Multivac T-200 tray sealer in VSP mode (Multivac, Kansas City, MO). The heat sealer temperature was 140 °C, the duration of heating was 2.5 s, and vacuum pressure was 91 kpa (910 mbar) for 1 s. All packaged samples were stored at 4 °C for 24 d.

Irradiation treatment. A low-energy x-ray food irradiator (Rayfresh Foods, Ann Arbor, MI) was used to irradiate the vacuum-packaged asparagus. Each packaged VSP was manually inserted into the irradiation chamber (420 mm × 420.75 mm × 550 mm) and irradiated. The irradiator operating condition was 145 KV with 19 mA, and the achieved rate of dosage was 1.0 kGy/30 min. For a preliminary test to determine the irradiation disinfectant effectiveness, VSP trays containing asparagus were irradiated at five different dose levels (0, 0.5, 0.8, 1.0, and 1.8 kGy). Based on the results of these tests, a dosage of 1.0 kGy was selected for all the irradiated samples (I-VSP), which were put into storage (4 °C). Each package was placed individually into the irradiation chamber and exposed to the x-rays. As a result of the characteristic pattern of x-rays and the irregular shape of the asparagus, the actual dose gradient was substantial in the sample. To help obtain a uniform irradiation treatment, all trays were treated twice (top and bottom side). Calculations for time of x-ray exposure were based on the maximum time to achieve the desired dose rate. Calibration was achieved by placing radiochromic dosimeters (Rayfresh Foods) on both top and bottom sides of the sample. For samples placed into storage, the target dose was 1.0 kGy, and the actual dose was 0.94 ± 0.075 kGy as a result of spatial variability. Three replications were performed (n = 3).

Gas analysis. The O₂ and CO₂ concentrations within the trays were monitored using an Illinois 6600 Head Space Analyzer (Illinois Instruments, Johnsburg, IL). A syringe needle was carefully inserted into the tiny gaps between the asparagus spears to extract headspace (10 mL) from the VSP packages and the headspace gas was injected into the instrument. A rubber septum was placed onto the lidding film to avoid gas exchange with the surrounding atmosphere during headspace gas extraction. Three samples were used for replication (n = 3).

Microbial analysis. Twenty-five grams of asparagus (approximately two whole asparagus spears) were aseptically removed from the trays, weighed, and homogenized in a stomacher bag (Whirl-Pack™; Nasco, Atkinson, WI) for 2 min with 225 mL of sterile peptone water. One milliliter of the asparagus/fluid mixture was removed and diluted in sterile tubes containing 9 mL of 0.1% peptone water to make serial dilutions (10^{-1} to 10^{-5}). The appropriate dilutions were plated on growth medium. Total aerobic bacteria (TPC) were determined on trypticase soy agar (Difco,

Detroit, MI) containing 0.6% yeast extract with 100 ppm cyclohexamide (Sigma-Aldrich Co., St. Louis, MO). The plates were counted after 72 h of incubation at 35 °C. Yeasts and Molds were determined on potato dextrose agar (Difco) containing 20 ppm streptomycin (Sigma) and 50 ppm ampicillin (Sigma). After 3 to 5 d of incubation at room temperature (22 °C), the number of microorganisms was determined. Three samples were used for replication (n = 3).

Determination of sugar content. Three asparagus spears were removed from each package and roughly ground using a blender (Oster 6640; Oster, Boca Raton, FL). Then, 10 g of the ground sample and 25 mL of distilled water were mixed and homogenized using another blender (Oster 6640; Oster) for 2 min. The mixture was then centrifuged at 6500 × g for 20 min at ambient temperature. The supernatant was filtered through a polytetrafluoroethylene membrane filter (0.45 μm), and the water-soluble sugar compounds (glucose, sucrose, fructose) were analyzed using a high-performance liquid chromatography (Waters 2695; Waters, Milford, MA) equipped with a refractive index detector (Waters 410; Waters). The mobile phase was made up of 100% high-performance liquid chromatography grade water with a Rezex RCM-monosaccharide column (Phenomenex, Torrance, CA) and a flow rate of 0.6 mL/min. The temperature of the column and detector were set at 85 and 40 °C, respectively. All tests were replicated three times (n = 3).

Phenylalanine ammonia-lyase enzyme assay. The PAL enzyme was extracted using the method of Lister et al. (1996). Three frozen asparagus spears were roughly grinded using a blender (Oster 6640; Oster), and 5 g of the samples was collected and homogenized with 20 mL of 50 mM phosphate buffer (pH 7.0) containing 5% polyvinylpyrrolidone (molecular weight 44,000), 50 mM sodium ascorbate, 18 mM mercaptoethanol, and 0.1% Triton X-100. The homogenized solution was filtered with cotton gauze and centrifuged at 12,000 × g for 30 min. During preparation, the temperature was kept at 4 °C. Then, 0.25 mL of the sample (crude enzyme) was mixed with 0.06 M borate buffer (pH 9.5, 0.875 mL), and 10 mg of L-phenylalanine was added to the 0.25-mL sample solution (final concentration of 11 mM) to initiate the reaction. Sample tubes were then incubated at 30 °C for 1 h and 0.125 mL of trifluoroacetic acid (35%) added to stop the reaction. The tubes were then centrifuged for 5 min at 3000 × g to pelletize the denatured protein. One milliliter of the supernatant was deposited into a 1-cm quartz cuvette, and PAL activity was estimated by measuring the changed absorbance at 290 nm using a Lambda 21 ultraviolet/visible spectrophotometer (Perkins Elmer, Waltham, MA). The PAL activity was defined as mmol of L-phenylalanine per milligram protein for 1 h (mmol/h/mg protein). The protein content was estimated using the Bradford method (Bradford, 1976). The binding of Coomassie Brilliant Blue G-250 dye to proteins was detected at 595 nm and calculated to the

concentration of protein in the sample solution above using a spectrophotometer (Perkins Elmer). Bovine serum albumin (BSA) was used for a protein standard. The protein assay kit (including reagent and BSA) was purchased from Bio-Rad Laboratories, Inc. (Quick Start™ Bradford protein assay; Hercules, CA).

Statistical analysis. Statistical evaluation of the different treatments (CON, VSP, I-VSP) and the different times (0, 4, 8, 12, 16, 20, 24) was performed using analysis of variance. Differences ($P < 0.05$) among means were analyzed with the Tukey's honestly significant difference test. The statistical software used was SPSS Version 13 (SPSS inc., Chicago, IL).

Results and Discussion

Headspace gas analysis. Samples irradiated at 1.0 kGy had slightly higher CO₂ levels than non-irradiated VSP asparagus through 8 d, whereas O₂ concentrations of each were statistically similar for the irradiated asparagus (Fig. 1). The differences in CO₂ content were greatest on Day 4. Headspace gas concentrations in both packages eventually became steady at 10% O₂ and 7% CO₂ (CON had ambient air during the storage). Atmospheres in both I-VSP and VSP were within the CO₂ and O₂ levels sufficient to maintain the quality of fresh asparagus, which achieved steady state at ≈10% O₂ and 15% CO₂ (Simon et al., 2004). These results agree with other studies in which fresh produce was treated with ionizing radiation. Han et al. (2004) observed that a 1.5-kGy treatment significantly increased CO₂ concentration in romaine lettuce packages 258.2% higher than the control (non-irradiated) at 0 d. Hagenmaier and Baker (1997) reported that the respiration of irradiated iceberg lettuce increased by 33% higher than the control. The mechanism responsible for the increased irradiation-induced respiration not well understood. Gunes et al. (2001) postulated that irradiation-stimulated catabolism of acetate may have increased CO₂ production.

Microbial analysis. The effect of x-ray irradiation on the growth of microorganisms in VSP asparagus is shown in Figure 2. The initial population of TPC and mold/yeast was reduced significantly by the different radiation levels (0.5, 0.8, 1.0, and 1.8 kGy). The highest dose (1.8 kGy) caused the greatest reduction in both TPC and mold/yeast (3.8 and 4.0 log cfu/g, respectively). As mentioned previously, 1.0 kGy is the maximum allowable to apply to fresh fruits and vegetables for the purpose of disinfestations and maturation delay (FDA, 2009). Thus, 1.0-kGy exposure was further characterized in this study. A microbial population profile during storage at 4 °C for 21 d is shown in Figure 3. The initial aerobic bacterial counts of the irradiated samples (at 0 d) had a 4-log reduction after 1.0 kGy of x-ray exposure. The imported (Peru) asparagus had relatively high initial bacterial counts of 7.15 log cfu/g for TPC and 7.19 log cfu/g for yeast/mold on the non-sanitized asparagus. Even after treatment with 200 ppm of a chlorine solution, the

Fig. 1. The percent CO₂ and O₂ in vacuum skin-packaged (VSP) and VSP plus irradiation (I-VSP) during storage at 4 °C. Irradiation level =1.0 kGy.

Fig. 2. Effect of irradiation dose on the total plate count (TPC) and mold/yeast count of vacuum skin-packaged asparagus immediately after irradiation. Different letters (a–b for TPC and A–E for mold/yeast) among irradiation dose indicate significant difference ($P < 0.05$).

Fig. 3. Microbial counts on asparagus spears at 4 °C during 24 d storage (A) total plate count [TPC, initial without sanitization = 7.15 log colony-forming units (cfu)/g]; (B) yeast/mold counts (initial without sanitization = 7.19 cfu/g). Irradiation level =1.0 kGy.

reductions were less than one log cycle for each microbial test. TPC was 6.67 log cfu/g and mold/yeast was 6.57 log cfu/g. Thus, the microbiological safety of the asparagus could be a potential health issue because the high initial contamination increases the risk of pathogens presence on the product as well as an impact on the shelf life of the asparagus. X-ray treatment had a significant effect on the reduction of microorganisms. Both the TPC and mold/yeast in the CON sample reached 8.0 log cfu/g after 4 d storage, and significant visual deterioration and odor was detected at 8 d (observation only). Microbial numbers above 8.0 log cfu/g of total bacteria correlate with food spoilage, which will result in the end of shelf life (Garcia-Gimeno et al., 1998). TPC and mold/yeast in VSP also reached levels of 8.0 log cfu/g within 16 d. A previous study with locally harvested asparagus reported that VSP maintained its shelf life (including microbial growth and sensory quality) up to 21 d (Shin et al., 2008). The counts in the imported asparagus may have increased dur-

ing transportation and handling depending on temperature and exposure. Results showed that the 1.0-kGy treatment significantly reduced the initial microbial contamination on the asparagus. After the treatment, TPC and mold/yeast were, respectively, 2.81 and 2.91 log cfu/g lower than untreated asparagus.

Change in sugar content. Initial glucose and fructose contents in the asparagus were 2.11 mg/g for glucose and 4.41 mg/g for fructose (Fig. 4), and the values corresponded to the range from Krzesinski et al. (2007). As a result of a significant level of decay of the asparagus in CON, glucose and fructose in the package were not measured after Day 16. The glucose and fructose levels declined most rapidly in CON, less rapidly in the VSP, and least rapidly in the I-VSP. For CON, the glucose reduction was $\approx 77\%$ (2.11 to 0.50 mg/g) and that of fructose was 30% (4.41 to 3.05 mg/g) during the storage. The total reduction of glucose and fructose in I-VSP was $\approx 28\%$ (from 2.17 to 1.56 mg/g) and 22% (from 4.92 to 3.85 mg/g), respectively. Non-

irradiated asparagus (VSP) lost $\approx 48\%$ (2.10 to 1.10) glucose and 42% (4.41 to 2.54 mg/g) fructose on Day 24. The quantity of sucrose detected in the asparagus was negligible over the storage (the data are not shown). The degree of sugar consumption in fruits and vegetable is closely related to respiration and temperature (Hirai, 1982). Even if I-VSP had higher respiration rate from Day 0 to 8 than VSP, I-VSP maintained higher sugar level during the storage time. Several studies have reported effects on sugar content of other fruits and vegetables treated with gamma irradiation, one of the ionized irradiation. Benkeblia et al. (2004) reported no significant decrease in sugars in onion bulb after 0.39 kGy treatment after 6 months storage at 4 and 10 °C. Fan and Sokorai (2002) reported no significant changes in the nutritional composition of iceberg lettuce subjected to a low dose (0.35 to 1.0 kGy) of irradiation treatment. Boylston et al. (2002) mentioned that changes in the nutrient composition and overall quality of fresh products after irradiation have frequently occurred at medium and high doses of treatment. The higher glucose and fructose reduction in VSP, relative to I-VSP, may have been caused by the higher microbial growth. Fermentation as a result of microbial growth uses sugar, which decreases its concentration and results in the production of lactic acid (Lamikanra et al., 2000). On the other hand, Chalker-Scott and Fuchigami (1989) observed that irradiation stimulated hydrolysis of reserved polysaccharides such as starches and increased sugar contents in mango and pineapple. The sugar concentration in VSP also maintained higher level than CON. VSP maintained a controlled atmosphere during the storage, and it is considered to delay sugar consumption from respiration and microbial growth.

Changes in phenylalanine ammonia-lyase activity. In the early of storage, PAL activity in CON rapidly increased up to five times higher than VSP on Day 8 (Fig. 5). The irradiation (x-ray) effect on PAL activity was also shown to be statistically significant. The activity in I-VSP was higher ($P < 0.05$) than VSP at Day 8. Then, PAL activity in I-VSP was decreased and the difference from VSP became negligible. PAL activity presented a peak at Day 8 of storage, but its activities on both VSP and I-VSP were significantly lower than the control group. It indicated that treatment in irradiation and/or vacuum skin packaging inhibited the increase in PAL activity. No information exists on the irradiation effect on PAL activity of asparagus to our knowledge. However, tissue injury and stress resulting from irradiation may increase enzyme synthesis or activity in fresh produce (Chalker-Scott and Fuchigami, 1989). Fan and Sokorai (2005) showed that ionizing radiation increased electrolyte leakage in all vegetables evaluated. Irradiation treatment may cause minor stress to the asparagus, and it may affect its PAL activity. Some studies with other fruits and vegetables have been observed the enhancement of PAL activity by irradiation. Benoit et al. (2000) observed up to four times higher PAL activity in 2.5 kGy-treated

Fig. 4. Sugar content (mg/g) in asparagus spears stored at 4 °C. (A) Glucose; (B) fructose. Irradiation level = 1.0 kGy.

Fig. 5. Phenylalanine ammonia lyase (PAL) activity profile on asparagus during storage at 4 °C. Irradiation level = 1.0 kGy.

mushrooms than a non-irradiated sample on Day 3. The PAL activities in the mushroom then gradually decreased, and the author postulated this was curing from the minor cell damage. More detailed study on the impact of irradiation on asparagus quality is needed.

Conclusion

Irradiation treatment reduced aerobic bacteria (TPC) and mold/yeast populations significantly and helped to maintain sugar (glucose and fructose levels) in asparagus. In this study, irradiation temporarily increased PAL activity. Further research should be conducted to determine if this increase affects product attributes such as lignification. A more detailed study, including analytical measurement of texture and fiber content, also needs to be done to evaluate the quality change. X-ray treatment is an effective disinfection process. It will enhance consumer safety by decreasing the number of viable microorganisms on the product. The commercial success of irradiated foods is at least partially dependent on the consumer's perception of product quality. Thus, a thorough examination of the sensory quality and an evaluation of any nutritional changes caused by x-ray treatment are recommended to determine its commercial feasibility.

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