Influence of Irradiating Bulk Soybeans on Their Future Functional and Sensory Properties in Soyfood Processing

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ABSTRACT

Soybeans were chosen for lunar and planetary missions, where soybeans will be supplied in bulk or grown locally, due to their nutritive value and ability to produce oil and protein for further food applications. However, soybeans must be processed into foods prior to consumption. Wilson et al. (2003) raised questions about the influence of radiation (on germination and functional properties) that the soybeans would be exposed to during bulk storage prior to and during a Mars mission. The influence of radiation can be broken down into two components: the affect of surface pasteurization to ensure the astronauts safety from food-borne illnesses (HACCP, CCP), and the affect of the amount of radiation the soybeans receive during a Mars mission. Decreases in the amount of natural antioxidants free radical formation, and oxidation-induced changes in the soybean will influence the nutritional value, texture, color, and aroma of soyfoods. The objective of this study was to determine the influence of surface radiation on whole soybeans using gamma and electron beam radiation at 0, 1, 5, 10, and 30 kGy (pasteurization and sterilization) on microbial load, germination rate, ease of processing, and quality of soymilk and tofu. Surface radiation of whole dry soybeans using electron beam or gamma rays from 1-30 kGy did provide microbial safety for the astronauts. However, the lower dose levels had surviving yeasts and molds. These doses caused oxidative changes that resulted in soymilk and tofu with rancid aromas. GC-MS of the aroma compounds using SPME Headspace confirmed the presence of lipid oxidation compounds. While lower doses may reduce these problems, we may lose the ability to insure microbial safety of bulk soybeans. Counter measures could include vacuum packaging, nitrogen flushing, added antioxidants, and radiating under freezing conditions. Doses below 1 kGy need to be investigated further to determine the influence of the radiation encountered during Mars missions.

INTRODUCTION

Soybeans were chosen for Lunar and Terrestrial missions due to their nutritive value and their ability to product oil and protein for further food applications. However, soybeans must be processed into foods so that the astronauts can consume it. Long-term storage of soybeans prior to and during Mars missions may result in degradation of soybeans through radiation induced oxidation reactions. Oxidative stress can influence the antioxidant level in the food, its shelf life, and the quantity and quality of food produced.

Irradiation is one of the approved methods to extend shelf life and preserve foods. Less than one kGy can be used to inhibit sprouting of potatoes, control insects in fruits and grains, and delay ripening. 1 to 10kGy treatments can kill pathogenic microorganisms, whereas 30kGy to 67kGy can be used to sterilize foods such as meats, dried spices, etc. (Bennion and Scheule, 2004; Potter and Hotchkiss, 1995). Ionizing radiation is a primary concern not only for human health but also for food quality and functionality. The foods shipped to Mars must be free from food-borne illness microorganisms and pathogens. An extended inter-planetary mission to Mars, as proposed by NASA, will require a 5-year shelf life for prepackaged foods for the return flight. In addition, any ingredients or food items used on the planetary surface will require a shelf life of close to 5 years. Understanding the effects of safety measures taken prior to transit, as well as adverse conditions to which these products will be exposed, it is necessary to enable development of a high quality nutritious food system.

While radiation can be used to pasteurize and sterilize foods, the radiation can influence the sensory and nutritive value of the food. Lipids may be the most affected by radiation because free radicals will participate in the initiation step of the lipid oxidation reaction. This oxidation leads to the development of short chain acids, aldehydes, ketones, carbonyls, and peroxides that impart off-odors, and off-flavors in lipids and foods containing lipids. Even small amounts of these compounds may leave foods, oils, or fats unfit for consumption.

Unsaturated fatty acids (containing double bonds) and other unsaturated compounds in foods are easily oxidized, which may make soybeans, which are high in unsaturated fatty acids, (oleic, linoleic and linolenic acids), very susceptible to this type of preservation technique. High temperatures, presence of oxygen, iron, UV light, and lipoxygenase are all known to catalyze the formation of free radicals and the ensuing autoxidation. Lipoxygenase enzymes catalyze the formation of hydroperoxides that break down unsaturated fatty acids into low molecular weight flavor compounds, described as green, grassy, beany, painty, and oxidized odors and flavors (Wilson, 1996; Torres-Penaranda et al., 1998). While soybeans contain Vitamin E, it can be 'used up' protecting the unsaturated fatty acids, thus allowing oxidation to start after the initial lag phase in this reaction. Since radiation has been used to create mutagens to develop new soybean oil cultivars, the use of irradiation as a CCP may alter the germination of treated soybeans, which would be a major problem if the soybeans were expected to produce crops on lunar and Mars missions.

The objective of this study was to determine the influence of surface radiation on whole soybeans as a HACCP step to ensure that bulk soybeans would not be vectors for food-borne illness microorganisms without deteriorating the quality of the soybeans and soyfoods made from them. Both electron beam and gamma radiation at 0, 1, 5, 10 and 30kGy (pasteurization and sterilization) on bulk soybeans were used as a Critical Control Point (CCP) to ensure the safety of the astronauts. Microbial load, germination rate, ease of processing, vitamin E content, and quality of soymilk and tofu were determined as measures of quality.

METHODS

Vinton 81 and IA 2032LS soybean cultivars were selected as food quality soybeans. All of the cultivars were non-GMO and grown at known locations. Vinton 81 is a high protein, large seeded cultivar that is considered the gold standard by the soyfoods industry around the world for soymilk and tofu production. IA 2032LS is a large seeded, high protein cultivar that is lacking all three lipoxygenase isozymes and thus was found to have a much milder aroma and flavor (Wilson et al. 2003; Wilson et al. 2004). It is used for soymilk, tofu and edamame soybean production. All soybeans were stored in the dark at 20°C prior to and after irradiation.

One pound of each cultivar was put into large Ziploc® bags, the air squeezed out, sealed, and labeled prior to being treated. The amount in the bag allowed a single layer of seeds to be exposed to the radiation treatment. Electron Beam irradiation of soybeans were shipped twice (replication) to each irradiator site (Texas A&M Electron Beam Facility for 0, 1, 5, 10 kGy, and 0, 10, and 30 kGy at Iowa State University's Linear Accelerator Facility). Dosimeters accompanied each bag to verify doses the soybeans received. One set of each cultivar was shipped to each location, but not irradiated to serve as a control. After each treatment, the soybeans were divided into four batches for: (1) chemical analyses [proximate analyses, peroxide value, thiobarbartic acid], Vitamin E content, and aroma by gas chromotagraphy (GC) (Boylston et. al., 2003); (2) microbial analyses (standard plate count, coliforms, Salmonella, yeasts and molds) using standard methods in the NASA Food Microbiology Lab (JSC, Houston, TX); (3) germination test (ISU Seed Lab SOP); and (4) soymilk and tofu production. All treatments were kept in the dark at 20° C until needed for analyses. All bags were sampled aseptically for microbial analyses prior to being sampled for composition and processing.

The functionality of the soybeans was evaluated by manufacturing soymilk and tofu. The standardized methods of Johnson and Wilson (1984), Moizuddin, et al. (1999), Moizuddin, Johnson, and Wilson (1999), Wilson et al. (2003) and Wilson et al. (2004) were used. The Japanese method of soymilk production (Wilson, 1995) from whole soybeans was utilized (soak beans 8-12 hours, grind beans, cook at 95 °C for 7 minutes, filter out okara, coagulate the soymilk, cut the curds to release the whey, press in tofu press, and refrigerate overnight prior to chemical and instrumental tests). Two different stainless steel tofu presses (10.5x4.5x9 cm; 5x4.5x9 cm), with press weights (Wilson, 2004) for 50g and 100g (dry beans) batches were used. 8% soluble solids soymilk (measured by a B&L refractometer) was produced and coagulated at 85°C using calcium sulfate dihydrate (Allied Custom Gypsum, Bessie, OK). The amount of coagulant needed was determined by the method of Moizuddin, Johnson, and Wilson (1999). After the arrival of the irradiated soybeans, control and treated samples were run in order to get an estimate of their behavior and the amount of coagulant needed per treatment. This preliminary data yielded information about the characteristics of the beans themselves as well as how they performed in soymilk, tofu, okara, and whey processing. The processing was done in on the stove in the JSC Space Food Systems Laboratory (Houston, TX) and CCUR Test Kitchen (Ames, IA) to allow for manipulation of a number of variables at once, and to maximize the use of the limited supply of soybeans.

Yields of soymilk, tofu, okara, and whey along with the color, texture, and aroma of the soymilk and tofu were determined utilizing instrumental methods. Color was measured by using a Hunter Color Difference Meter Model XE under D65 light with a 10-degree standard observer. Texture was determined by using a Texture Profile Analysis (TPA) procedure (Bourne, 1978) to determine hardness, brittleness, adhesiveness, cohesiveness, and elasticity of each sample. A 1 cm-cube of tofu was compressed (80%) using a compression head in a Texture Technology TA XT2ci instrument. pH and conductance measurement of the whey were used to determine the optimum coagulation of the milk (Moizuddin, Johnson, and Wilson, 1999b; Wilson et al., 2003). Volatile flavor compounds were isolated from the soymilk using solid phase micro extraction (Boylston et al., 2003). Gas-chromatography-mass spectrometry and gas chromatography-olfactometry with authentic flavor standards were used to confirm the identity, flavor characteristics and intensity of the volatile compounds. All procedures were replicated in triplicate. The results were analyzed statistically for treatment effects and correlations.

RESULTS AND DISCUSSION

Vitamin E Content

The control and irradiated beans were analyzed for Vitamin E content (Medallion laboratories, Minneapolis, MN). Significant decrease in vitamin E content was observed in irradiated soybeans. Figure 1 shows the highest vitamin E content in the control beans (0kGy) and with lower levels in all the irradiated soybeans (1, 5, 10, 30kGy).

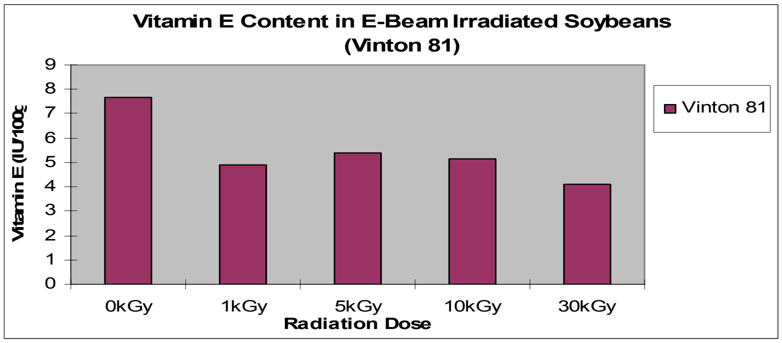

Figure 1. Vitamin E content in 0, 1, 5, 10 and 30kGy electron-beam irradiated soybeans (Vinton 81).

Thiobarbartic Acid (TBA) and Free Fatty Acid (FFA) Analysis

TBA values for the control and irradiated soybeans are presented in Figure 2 and 4. TBA values increased with increasing doses of irradiation for all the electron-beam and gamma treated cultivars. IA 2032LS, which is lacked lipoxygenase enzyme, has lower TBA values comparing to the Vinton 81 cultivars. Figure 3 and 5 shows the free fatty acids values in both electron beam and gamma-rays irradiated soybeans. An increase in free fatty acids values was observed in the gamma treated Vinton 81 as the radiation doses increased (Figure 4). However, radiation doses did not significantly affect the free fatty acids levels in the gamma treated IA2032LS cultivars. Some variation in free fatty acids values was observed in the electron beam treated soybeans and the high dose treated soybeans (10 and 30 kGy) still encountered the highest free fatty acids values in comparison to the control and lower doses (1 and 5kGy).

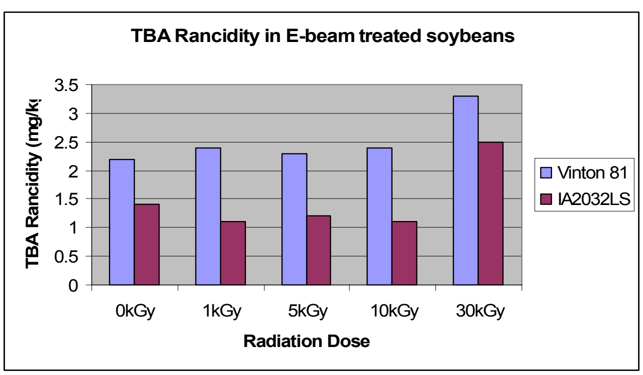

Figure 2. TBA rancidity in electron beam irradiated soybeans (Vinton 81, IA 2032LS) at 0, 1, 5, 10 and 30kGy.

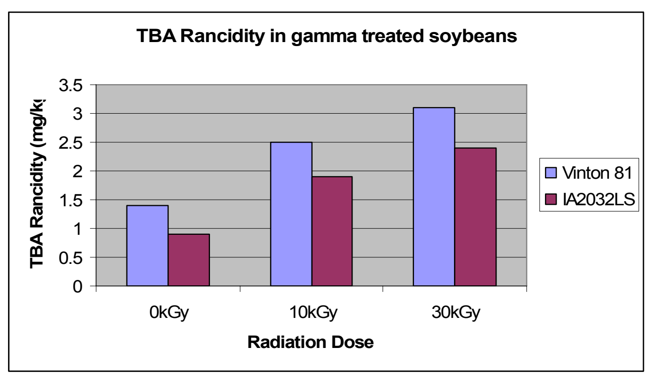

Figure 4. TBA rancidity in gamma irradiated soybeans (Vinton 81, IA 2032LS) at 0, 10,
30kGy.

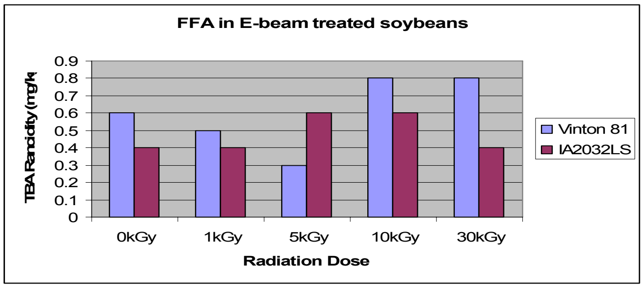

Figure 3. Free fatty acids values in electron beam irradiated soybeans (Vinton 81, IA 2032LS) at 0, 1, 5, 10 and 30kGy.

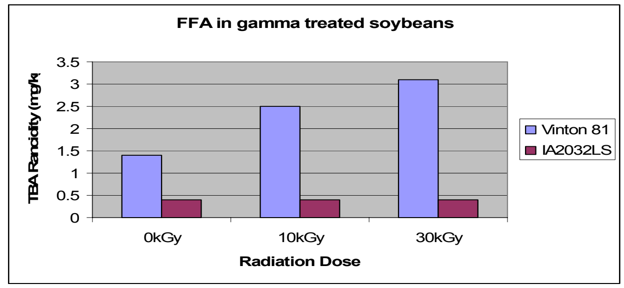

Figure 5. Free fatty acids values in gamma irradiated soybeans (Vinton 81, IA 2032LS) at 0, 10, 30kGy.

Appearance and aroma of soybeans, and soymilk

Irradiated raw soaked soybeans for all cultivars were visually damaged at the ends (sphere to bean shape) by the radiation treatment (electron and gamma), with the 30kGy treatment causing the most damage. The 30kGy treatments were all softer than the other treatments. The irradiated beans lost more solids (0.2% to 4% solids) into the soak water than the control beans (Control<1kGy<5kGy<10kGy<30kGy). This is of concern for waste water treatment and solid waste treatment. The 2004 IA 2032LS soybeans were found to contain some “hardshell” or “stone” soybeans, unlike 2003. These beans did not imbibe water, even after 24 hours of soaking. The percentage of “hardshell” beans did not change due to irradiation treatment or type of irradiation. The ‘hardshell’ soybeans were not influenced by irradiation or up to 1 year storage at 20C. Removal or damaging of the seedcoat allowed the cotyledons to take up water. Either soybeans should be screened for “hardshell” beans prior to shipping or the beans should have their seedcoats damaged prior to soaking to ensure higher processing yields.

There was some odor changes detected in the irradiated Vinton 81 soybeans increasing from 1kGy onwards. Both electron-beam and gamma 10kGy and 30kGy treated Vinton 81 soybeans had a rancid odor after soaking. The 30kGy treatment had the strongest aroma for all cultivars. The oxidized aromas increased with grinding and cooking for the 10 and 30 kGy treatments. The IA 2032LS cultivar had less of this aroma than the other cultivars for all treatments. After grinding and during cooking the off-odor increased and filled the room. This is an unacceptable situation, as the air would need to be filtered to keep the odor compounds at low levels, and the odor is not appetizing for most people. The IA 2032LS soybeans had less of this rancid-oxidized odor than the other irradiated cultivars. Our hypothesis for this result is that the irradiation damaged the tissue allowing the lipoxygenase enzymes in the Vinton 81 soybeans to come into contact with polyunsaturated compounds (lipids) during the soaking, grinding, and come-up time during heating. IA 2032LS lacks this enzyme system, and would only have these odor compounds produced through traditional, non-catalyzed autoxidation (slower). This hypothesis is supported by the state of the soaked soybeans, solids loss, changes in the okara (small particles passed the filtering system during pressing), textural changes, TBA, and the gas chromatographic results. In fact, tofu yield losses suggest that the damage further reduced yield loss compared to the stored control

Hexanal increased with increasing doses of e-beam and gamma radiation. Additional volatiles increased with radiation dose as noted for IA 2032LS (Figure 7), especially 1-octen-3-ol and hexanal. (Trans,trans)-2,4-decadienal (peak 28.59) increases with radiation dose for all treatments (Figure 6). However, this peak is much lower for IA 2032LS soymilk than for Vinton 81. Gas Chromotography-Olfactometry (GCO) and MS were used to further identify and characterize these compounds (Figure 7).

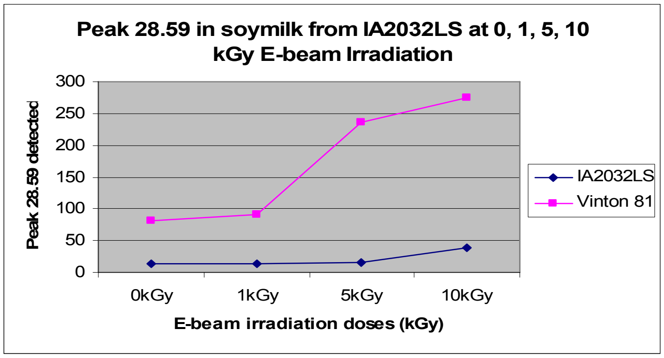

Figure 6. Peak 28.59 is identified as (trans,trans)-2,4-decadienal in e-beam irradiated IA2032LS and Vinton 81 beans.

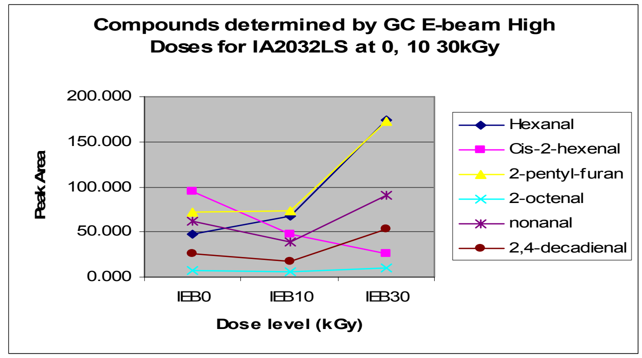

Figure 7. Volatile compounds determined by gas chromatography in electron beam irradiated IA2032LS cultivars at 0, 10, 30kGy.

Microbial Load of Soybean Cultivars

Average total aerobic counts across all cultivars ranged from 0 (30kGy) to 250 CFU/g (control). No coliforms or Salmonella were found in any of the cultivars or irradiated soybeans. Yeasts and molds ranged from 0 to 18 CFU/g. *Aspergillus flavus* was found on one sample, which may be due to contamination after irradiation (sampling). All of the soybean cultivars in these studies would meet NASA Flight Food Microbiological Requirements, meaning that they could fly on Shuttle and International Space Station missions.

Germination Study

Germination test from ISU Seed Lab shows that doses from 1 kGy to 10 kGy clearly reduced the ability of the soybeans to germinate (Figure 8). 10-30 kGy treatment stopped the soybeans from germinating (dead), while lower doses produced abnormal germination and dead beans.

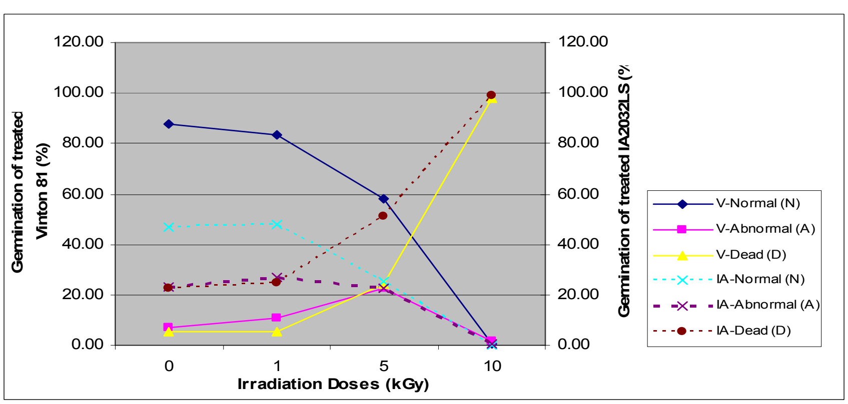

Figure 8. Germination study on medium dose electron beam irradiation (0-10kGy) for two different cultivars (Vinton 81 and IS2032LS).

Soymilk and tofu yield

The high dose irradiated beans lost more solids (0.2% to 4% solids) into the soak water than the control beans (Control < 10kGy < 30kGy). This is of concern for waste water treatment and solid waste treatment. Medium dose irradiation did not significantly change water absorption or solids loss during soaking. However, IA2032LS had a lower percentage of water absorption than the Vinton 81 cultivar due to the presence of “hardshell” beans. Initial runs of all of the control, treated, and treated stored soybeans were made to determine the amount of coagulant needed and there were no changes in the amount of coagulant need to coagulate these samples. This is important, as the coagulant is a resuppliable item on missions. All treated samples coagulated, but there were curd/whey and texture differences. Regardless of irradiation dosage, IA 2032LS produced the same amount of tofu from the soymilk (average of 22%), and Vinton 81 consistently produced a higher tofu yield from the soymilk at an average of 24%.

Appearance of soymilk and tofu

The color of the tofu was affected by both irradiation level and storage, as depicted in Figures 9-10. The Hunter scale of L, a, b are used which refers to the lightness (L), redness (+a) or greenness (-a), and yellowness (+b) or blueness (-b) of the sample. Medium irradiation doses (0-10 kGy) did not significantly influence the color of the tofu, but Vinton 81 consistently produced a darker tofu than IA 2032LS. However, high dose (10 and 30 kGy) treated cultivars (for pasteurization or sterilization) and stored for 1 year at 20 C, showed major decreases in lightness (L) and increases in redness ‘a’ (Figures 9 and 10 respectively). Both Vinton 81 and IA2032LS also slightly increased in redness at doses 0, 1, 5 10kGy, but not significantly.

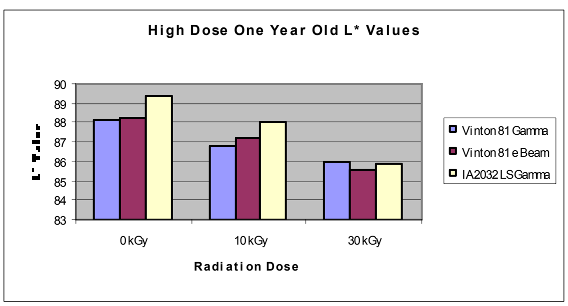

Figure 9. Influence of high dose irradiation of soybeans and storage on the lightness of their tofu.

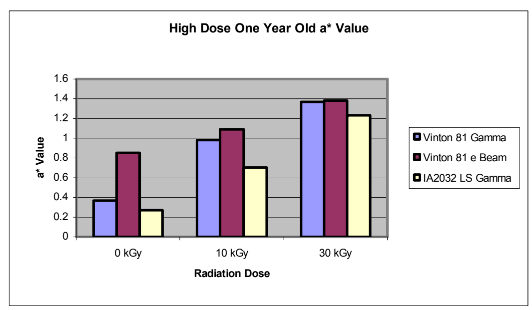

Figure 10. Influence of high dose irradiation of soybeans and storage on the redness (+a) of their tofu.

Texture of the tofus

Irradiation of the soybeans resulted in softer tofus as shown in Figure 11 and 13. As the irradiation dose increased for the whole dry bean, the tofu became softer, less adhesive, less springy, less cohesive, and less resilient. However, Vinton 81 held its firmness better than IA2032LS cultivar (Figure 13). At high doses (10 and 30 kGy), significantly softer tofus were produced from the irradiated soybeans (Figure 13). The irradiated (10 and 30 kGy) stored soybeans produced softer tofu, 40 to 64% softer for stored Vinton 81 respectively, than the freshly irradiated soybeans. Changes in yield and texture due to storage conditions have been reported, but not the influence on irradiation combined with storage. The tofu from the 30 kGy treatment was very granular, and its okara was very pasty with very small particles. Figure 13 shows a decrease in hardness as dose level increased and decrease in cohesiveness was observed in Vinton 81 (Figure 14).

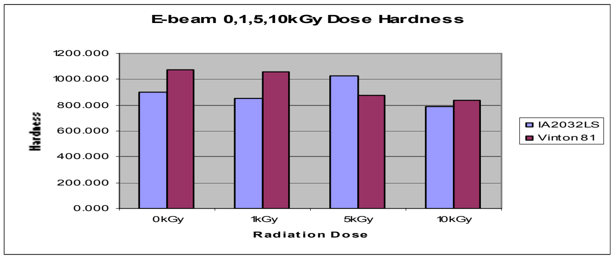

Figure 11. Influence of medium dose electron beam irradiation on the hardness of their tofus.

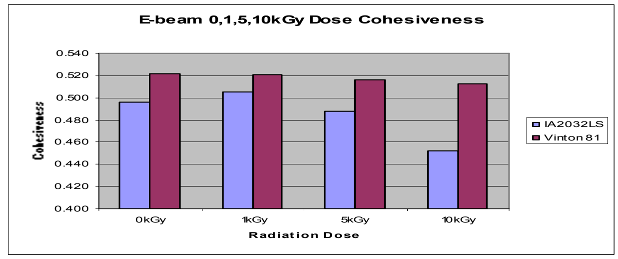

Figure 12. Influence of medium dose electron beam irradiation on the cohesiveness of their tofus.

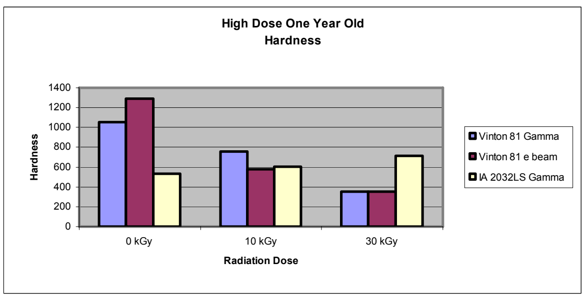

Figure 13. Influence of high dose irradiation and storage of soybeans on the hardness of their tofus.

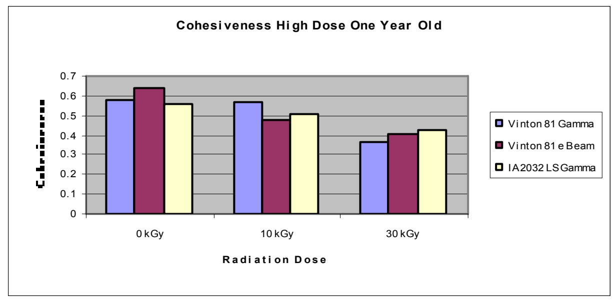

Figure 14. Influence of high dose irradiation and storage of soybeans on the cohesiveness of their tofu

CONCLUSION

The surface radiation of whole dry soybeans using electron beam or gamma rays at 1kGy to 30kGy did provide microbial safety for the astronauts. However, these doses caused oxidative changes that resulted in tofu with rancid aroma, darkening of the tofu, lower tofu yields, more solid waste, and loss of the ability of the seeds to germinate. Lower doses at 0, 1, 5 and 10kGy may reduce these problems but low level of oxidative changes was observed. Counter measures could include vacuum packaging or radiating under freezing conditions. Better estimates of the radiation that the food will be exposed to, needs to be determined and shared. Appropriate shielding for the food as well as the astronauts needs to be developed. Further study on a No Effect Dose needs to be determined for a better estimate of radiation of food.