
Influence of Low level Irradiation of Soybeans on the Quality of Soyfoods during Mars Missions

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ABSTRACT

Soybeans were chosen for Lunar and Terrestrial missions due to their nutritive value and their ability to produce oil and protein for further food applications. However, soybeans must be processed into foods prior to consumption by the crew. Long-term storage of soybeans during Mars missions may result in soybean degradation through radiation induced oxidation reactions influencing antioxidant levels, its shelf life, and the quantity and quality of food produced. Wilson (2004) and Chia (2006) found that surface radiation of whole dry soybeans using electron beam or gamma rays at 1 to 30 kGy provided microbial safety for the astronauts, but it caused oxidative changes resulting in unacceptable soymilk and tofu [rancid aromas, off-color tofu with low yields, more solid waste, and loss of the ability of the seeds to germinate (a problem if they are to be grown on Mars)]. No data exists on the influence of low doses (1-5 Gy) on the functional properties of soybeans. The objective of the study was to determine if low Gy doses, encountered during the transit to Mars, and storage would influence tofu yield, quality, Vitamin E level, and germination of soybeans. Vinton 81 (industry standard) and IA 2032 (lipoxygenase-null) soybeans were irradiated (0, 1, 2, 3, 5, 20 Gy) and stored at 20°C. Control samples were shipped with each treatment, but only the treatment samples were irradiated. Soybean cultivars irradiated in 2005 and stored at 20°C were evaluated in 2005 and 2006 to assess the influence of storage on tofu quality. Soymilk and tofu were manufactured with small-scale soymilk and tofu systems (Wilson 2004). Color, texture, aroma, yield, and composition of the tofu were determined using standard methods (Wilson 2004). Tofu can be produced from low dose irradiated soybeans, but yields were lower and oxidized aromas were produced at levels at or above 1 Gy. Off-aroma increased after storage sensory and gas chromatography. Hardness was not influenced by low doses, which is in contrast to the darkening, softening and strong oxidized aromas produced by high doses (1 to 30 kGy). Stored bulk soybeans, and all foods, should be shielded from radiation on long-term 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 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. Wilson et al. (2004) and Chia (2006) found that surface radiation of whole dry soybeans using electron beam or gamma rays at 1 to 30 kGy provided microbial safety for the astronauts, but it caused oxidative changes that resulted in unacceptable quality soymilk and tofu [rancid aromas, higher thiobarbituric acid reactive substances (TBARs), darker brown tofu, lower tofu yields, more solid waste, paste-like okara, and loss of the ability of the seeds to germinate (a problem if there are to be grown on Mars)]. While lower doses may reduce these problems, we lose the ability to insure microbial safety (cross-contamination) of bulk soybeans for the astronauts. In November 2004 NASA radiation experts informed us that radiation doses of 1-5 Gy might be more in line with averages doses expected on the mission (NASA, personal communication). This level is well below effective doses to ensure microbiological safety. No data exists on the influence of low Gy doses of radiation on the functional properties of soybeans. This project builds upon the earlier results of Wilson et al (2004) to test the following hypotheses: High doses of radiation have been found to cause oxidation of lipids, loss of germination, and alteration of protein gel formation. The objective of this research is to establish whether low-level irradiation of soybean cultivars would have an influence on germination, antioxidant potential, aroma, and the yield and texture of tofu.

METHODS

Vinton 81 and IA 2032LS soybean cultivars were selected based upon the results of Wilson and Zehr (2003) and Wilson et al. (2004). All of the cultivars selected 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 and Zehr 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. The bagged soybeans were irradiated with gamma rays at Purdue University at five doses (0, 1, 3, 5, 20Gy). 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). The latter two treatments were used by Wilson (2004) and Wilson (2005) and mentioned here for

comparison purposes. 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, thiobarbitic acid] using standard AOCS procedures, antioxidant potential (PHOTOCHEM®), and aroma by gas chromatography (GC) (Wilson, 1998, 2004); (2) microbial analyses (standard plate count, coliforms, Salmonella, yeasts and molds) using standard methods in the NASA Food Microbiology Lab (Johnson Space Center (JSC), Houston, TX); (3) germination test (Iowa State University 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 and Zehr (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). Stainless steel tofu presses (5x4.5x9 cm), with press weights (Wilson, 2004) for 50g (dry beans) batches were used. 8% soluble solids soymilk (measured by a Bausch and Lomb 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 on the stove in the JSC Space Food Systems Laboratory (Houston, TX) 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 using a Hunter Color Difference Meter Model XE under D65 light with a 10-degree standard observer. Texture was determined 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, 1999; Wilson and Zehr 2003). Volatile flavor compounds were isolated from the soymilk and tofu 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

Compositional

Compositional data for all cultivars are given in Table 1. The protein content of this year's cultivars was similar to those used in Wilson's 2003 and 2004 studies (Table 1). Protein ranged from 36.4 to 39.3 % on a 13% moisture basis. IA 2032 had higher oil content than Vinton 81 in 2004 and 2005.

Table 1. Soybean Composition

Sample	Moisture	Percentages		
		Protein**	Oil**	Fiber**
2003* Vinton 81	8.9a	38.4a	17.4a	4.6a
	IA 2032LS	10.8a	39.0a	18.2a
2004 Vinton 81	7.9b	39.3a	18.2a	4.6a
	IA 2032LS	7.8b	37.6a	20.2b
2005 Vinton 81	10.2c	38.8a	17.9a	4.5a
	IA 2032LS	11.1c	36.4b	19.9b

*Crop year

**13% moisture basis

* A different letter in a column indicates statistical significance ($p < 0.05$).

Vitamin E Levels

Vitamin E levels ranged from 4.65 to 5.84 IU/100 g for IA 2032LS and 3.69 to 5.25 IU/g for Vinton 81 (13% moisture) for the low dose treatments (Figure 1). In comparison, vitamin E levels are slightly lower for the mid-dose (1-10 kGy) treatments 4.00- 7.37 IU/100g (Wilson 2005). There were no significant differences between the 0 – 5 Gy treatments after irradiation.

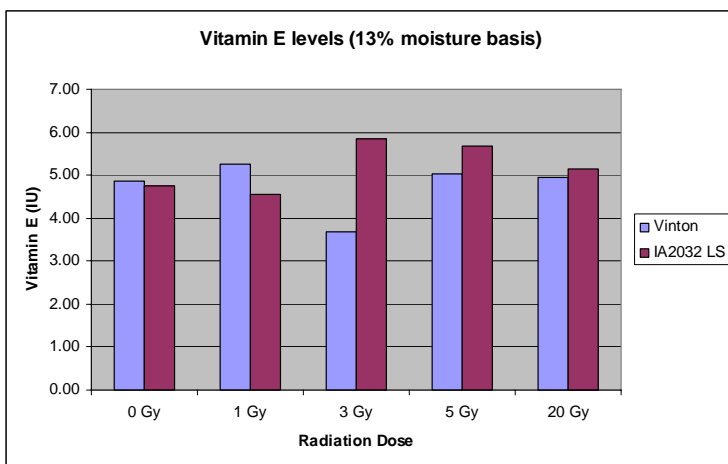


Figure 1. Vitamin E levels in low dose irradiated soybean cultivars.

Germination Tests

Germination tests on the low dose treated soybeans were not different from the control soybeans. However, doses from 1 kGy to 10 kGy clearly reduced the ability of the soybeans to germinate doses producing abnormal germination and dead beans (Wilson et al. 2005).

Microbial Load of Soybean Cultivars

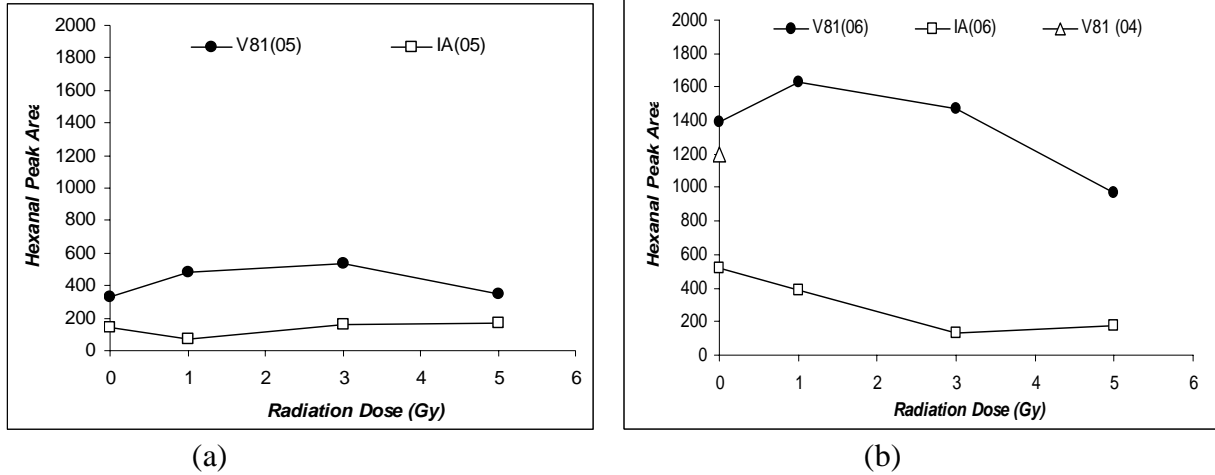
The microbial load of very low dose (0-5 Gy) treated soybeans were similar to their control (0 Gy).

Appearance and aroma of soybeans, and soymilk

The irradiated soaked (0-20 Gy) soybeans looked and smelled normal compared to their controls prior to processing, unlike the 10 and 30 kGy soybeans that were damaged and had oxidized aromas (Wilson et al. 2004). After grinding, the 3, 5, and 20 Gy soymilk samples had slight oxidized aromas. As noted by Wilson in 2003 and 2004, the lipoxygenase-free IA 2032LS soybeans were bland compared to the 'beany' aroma of the Vinton 81 cultivar. These aromas were all much lower than the kGy treated soybeans. Table 2 shows an example of selected volatile compounds detected by GC-MS (mass spectrometry) for soymilks. Hexanal increased with increasing doses of e-beam and gamma radiation (Figures 2a, 2b). Additional volatiles increased with radiation dose and storage. IA 2032LS had lower volatile levels than Vinton 81 across all treatments (60 to 64% lower across 0-20 Gy).

Table 2. Average peak area of volatile components detected by gas-chromatography-olfactometry (GCO) in 0, 1, 3, 5, 20Gy soymilk samples for both cultivars (Vinton 81, IA2032LS)

Compound	Average Peak Area									
	0 Gy	Vinton 81				IA2032LS				
	0 Gy	1 Gy	3 Gy	5 Gy	20 Gy	0 Gy	1 Gy	3 Gy	5 Gy	20 Gy
Hexanal	334.5	484.7	533.0	346.9	470.0	147.0	74.8	162.3	171.9	205.4
Cis-2-hexenal	218.3	337.3	255.9	247.0	233.5	102.1	68.9	85.8	77.6	77.4
1-octen-3-ol	143.4	187.6	158.9	155.0	162.3	224.1	364.6	282.3	255.2	213.8
2-pentyl-furan	282.9	312.9	428.0	341.1	333.8	75.8	40.3	48.7	47.6	52.0
2-octenal	42.9	52.6	57.2	50.6	52.0	9.2	5.2	5.1	7.0	6.7
Nonanal	148.0	183.6	207.0	181.3	187.9	84.3	42.9	52.4	51.2	58.2
2-hexyl furan	50.7	42.8	55.7	50.3	45.9	44.6	88.1	56.9	61.0	56.8



Figures 2. Peak area of hexanal detected by gas chromatography in soymilk from (a) stored Vinton 81 and IA2032LS irradiated and control soybeans (2005); (b) Vinton 81 and IA2032LS irradiated and control soybeans (2006)

Sensory:

Panelists noted a significant ($p < 0.05$) color difference (2 out of 5 difference test) in tofu between cultivars (Figure 3), and panelists also found a significant difference ($p < 0.05$) between IA2032LS whey and Vinton whey: IA 2032 LS whey was a clear lighter yellow vs. semi-opaque light orange Vinton whey. Panelists found that the beany/oxidized aroma increased at 1 Gy for the IA 2032LS, and panelists found that IA 2032LS was significantly less beany/oxidized than Vinton. (Intensity Scaling) Figure 4.

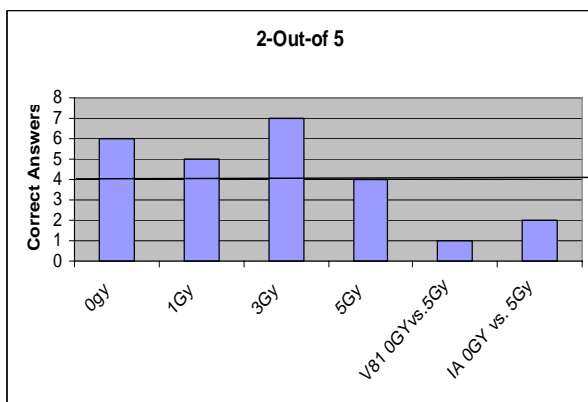


Figure 3. 2-out-of-5 test on tofu from Vinton 81 and IA2032LS irradiated and control soybeans (2006).

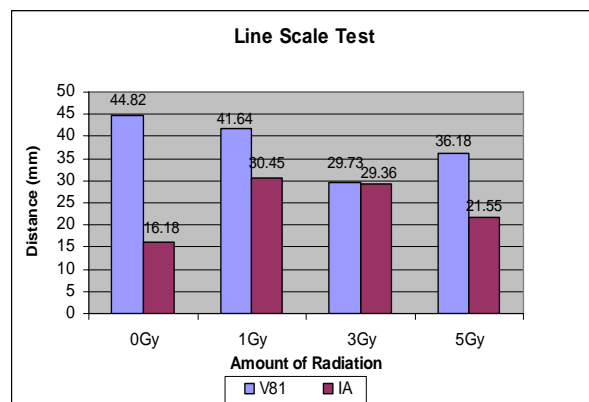


Figure 4. Line scale test of tofu from Vinton 81 and IA2032LS irradiated and control soybeans (2006).

Soy milk and tofu yield

Initial runs of all of the control, treated, and treated stored soybeans were made to determine the amount of coagulant needed, as was done by Wilson and Zehr 2003 and Wilson et al. 2004. 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. Irradiation of both soybean cultivars above 1 Gy, decreased the amount of tofu obtained from the soy milk (Figure 5). Likewise, Vinton 81 had a decreased tofu yield for all irradiation doses (Figure 6). Regardless of irradiation dosage, IA 2032LS produced the same amount of tofu from the beans, and Vinton 81 consistently produced a high tofu yield from the beans. Low dose irradiation did not significantly change water absorption or solids loss during soaking.

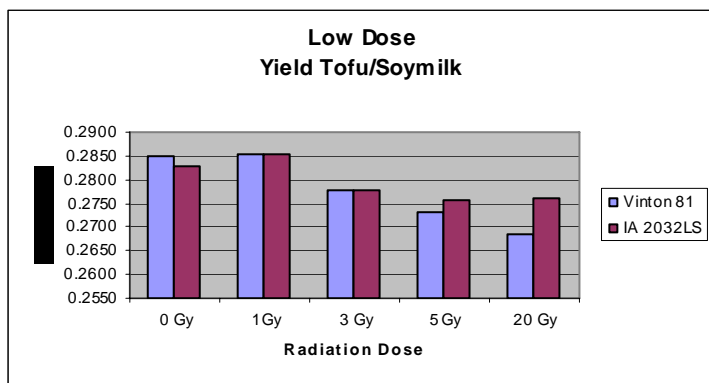


Figure 5. Tofu yield from irradiated (0-20 Gy) soybeans, based upon the amount of soymilk.

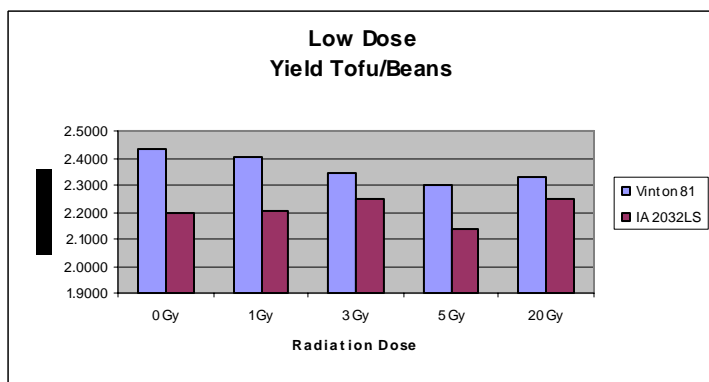


Figure 6. Tofu yield from irradiated (0-20 Gy) soybeans, based upon the amount of dry soybeans used to make the soymilk.

Appearance of soymilk and tofu

The color of the tofu was affected by both irradiation level and storage, as depicted in Figures 8-10. 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. Low irradiation doses (0-20 Gy) did not influence the color of the tofu, but Vinton 81 consistently produced a darker tofu than IA 2032LS (Figure 8). Vinton 81 had a slight increase in lightness at 3 Gy and in redness at 1 Gy; these differences were very small, and not significant. There were no significant differences due to low dose irradiation for yellowness from 0-20 Gy (Figure 10).

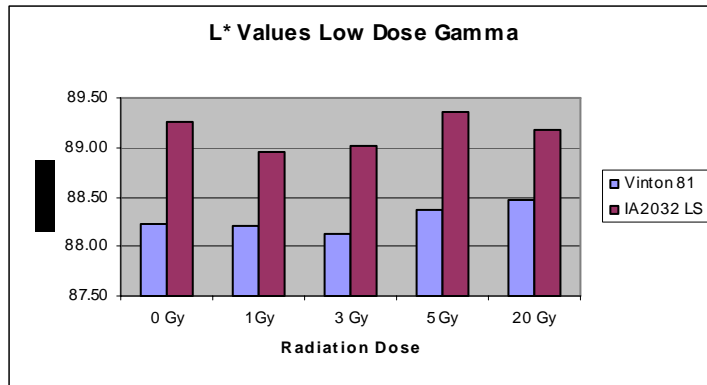


Figure 8. Influence of irradiating soybeans at low doses on the lightness of their tofu.

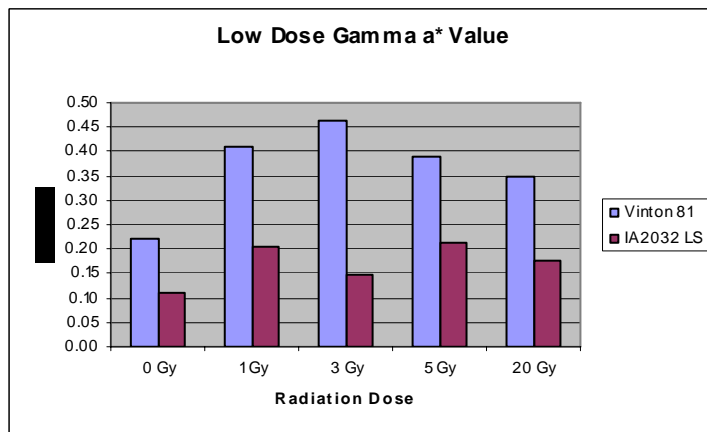


Figure 9. Influence of irradiating soybeans at low doses on the redness (+a) of their tofu.

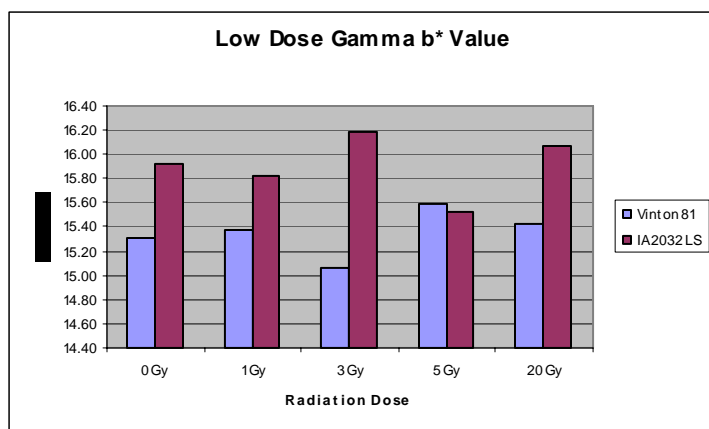


Figure 10. Influence of irradiating soybeans at low doses on the yellowness (+b) of their tofu.

Texture of the tofus

There were no significant effects of low dose irradiation on the hardness of the tofus (Figure 11). However, a trend was seen for increasing hardness with irradiated Vinton 81 soybeans. IA 2032LS was softer than Vinton 81 tofus until the 20 Gy treatment. At high doses (10 and 30 kGy), significantly softer tofus were produced from the irradiated soybeans (Wilson 2004). IA 2032LS tofus were less cohesive than the control for 1-20 Gy treatments (Figure 12). There were no significant differences in adhesiveness or springiness due to low dose irradiation.

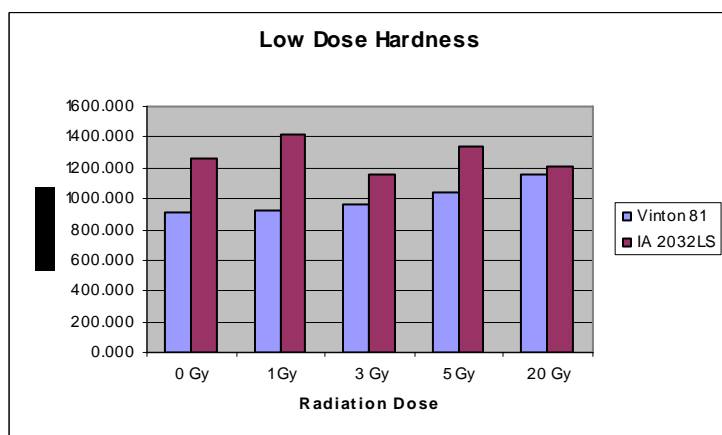


Figure 11. Influence of low dose irradiation of soybeans on the hardness of their tofus.

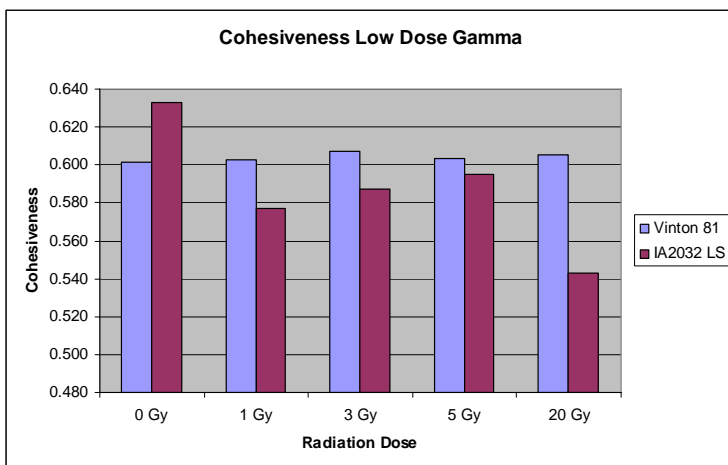


Figure 12. Influence of low dose irradiation of soybeans on the cohesiveness of their tofus

Summary

- Tofus could be produced from low dose irradiated soybeans, simulating the dose received on a Mars Mission.
- Tofu yields were decreased from soymilk due to gamma irradiation above 1 Gy.
- Vinton 81 had decreased tofu yields from soybeans, but still yielded more tofu than IA 2032LS.
- There was no effect on tofu hardness, but cohesiveness was decreased for IA 2032LS with increasing irradiation doses.
- Tofus were similar in color, with a slight increase in redness for low dose treated soybeans.
- Oxidized aromas were detected in soymilks above 1 Gy.
- The lipoxygenase-free soybean cultivar was less oxidized, even at low dose levels than the Vinton 81 irradiated cultivar. This is due to (Hypothesis) the lack of enzyme-substrate interaction after the radiation has, essentially, punched holes in the membranes and structural material (Wilson et al. 2004).

Conclusions and Recommendations

- Low doses expected to be encountered on a Mars mission will influence sensory properties and yields of tofu.
- The choice of soybeans for Mars missions must include cultivar, crop year, storage conditions/time, composition, and packaging requirements.

- Soybeans exposed to 1-5 Gy doses of gamma radiation and stored for one year can be used for soymilk and tofu production, but a flavor/aroma masking agent may be needed to hide the oxidized aroma.
- More beans may be needed to offset the yield losses.
- If doses higher than 5 Gy occur, all desirable characteristics of these soybeans will be decreased.
- Counter measures: Bulk soybeans, and all foods, that are going to be shipped to Mars should be protected from radiation, just like the astronauts, in order to maintain maximum sensory, functionality, nutritional, and yield (reduced waste) characteristics.
- While no pathogens were found on control samples from this study, ways to ensure that pathogens are not sent with bulk items need to be determined.
- This study only covers the first year of a 3 yr mission. A longer shelf life study is needed.

Acknowledgements

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