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Influence of radiation encountered on Mars missions on the yield and quality of soymilk and tofu from bulk soybeans

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Influence of radiation encountered on Mars missions on the yield and quality of soymilk and tofu from bulk soybeans

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This is to certify that the master's thesis of

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Signatures have been redacted for privacy

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Dedicated to the memory of my grandfather, Chia Ser Teik.

TABLE OF CONTENTS

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. Radiation that soybeans would be exposed to during bulk storage prior to and during a Mars mission may influence their germination and functional properties. The influence of radiation includes the affect of surface pasteurization to ensure the astronauts' safety from food-borne illnesses (HACCP, CCP), and the effect of the amount of radiation the soybeans receive during a Mars mission. Decreases in the amount of natural antioxidant, 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 pasteurization surface radiation on whole soybeans using electron beam radiation. The influence of 0, 1, 5, and 10 kGy on microbial load, germination rate, ease of processing, and quality of soymilk and tofu were determined. Surface radiation of whole dry soybeans using electron beam from 1-10 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. Soybean germination ability was reduced as radiation dosage increased. While lower doses may reduce these problems, the ability to insure microbial safety of bulk soybeans will be lost. Counter measures could include vacuum packaging, nitrogen flushing, adding 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.

CHAPTER 1

INTRODUCTION

Food safety and security have always been of concern and it is even more so after September 11, 2001. The Office of Homeland Security has encouraged the United State Department of Agriculture to increase food protection against bioterrorism (Rawson 2003; Nabhan 2003). Safety of foodborne illness microorganisms and pathogens are highly priorities for both Homeland Security and NASA. Soybeans were chosen by NASA for lunar and planetary 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 astronauts' consumption. Long-term storage of soybeans during Mars missions may result in degradation of soybeans through radiation induced oxidation reactions and deterioration of composition over storage time. Oxidative stress can influence the antioxidant level in the food, its shelf life, and the quantity and quality of food produced. Surface radiation of whole dry soybeans using electron beam or gamma rays at 10 or 30 kGy provided microbial safe food for the astronauts, but caused oxidative changes that resulted in unacceptable quality of soymilk and tofu with rancid aromas, darker color, lower tofu yields, more solid waste, paste-like okara, and loss of seed germination ability compared to the non-irradiated seeds and their products, which could be a problem if they are to be grown on Mars (Wilson and others 2005). Therefore, lower doses were suggested in reducing these problems. This study, funded by Wilson's NASA Directors Grant, is to determine the following hypotheses: Hypothesis 1: Radiation as a Critical Control Point (CCP) and storage conditions, prior to and during transit to Mars, interact to decrease the amount of natural antioxidants in the soybean, induce lipid oxidation and decrease the yield and quality of soybeans. Hypothesis 2: Radiation at 1, 5, 10 kGy will alter the germination rate of the soybeans. Gamma-irradiation at similar dose levels had shown increase in soymilk and tofu yield, and had minimal effect on their quality (Byun and Kang 1995). The objective of this research is to establish if the Hazard Analysis Critical Control Point (HACCP) CCP step of irradiating whole soybeans at 1, 5, 10 kGy using electron beam affects microbial load, antioxidant potential, seed germination, yield and quality of soymilk and tofu.

CHAPTER 2

LITERATURE REVIEW

SOYBEANS AND COMPOSITION

Soybean, *Glycine max* (L.), belongs to the family Leguminosae. It originated in northern China and it was cultivated as early as 5000 years ago (Wang 1997). Soybeans had been transformed into various types of soyfoods including soymilk, tofu, soy sprouts, soy paste and soy sauce by the Chinese. It was later brought to Japan, Korea, and other Far East countries about 1,100 years ago, and then to Europe and North America in the 18th century (Liu 2004a). In 1954, the United States became the world leader soybean production producing 40% of total production, followed by Brazil (24%), Argentina (18%), China (8%) and India (3%) (Liu 2004a; Soyatech, Inc. 2004).

Soybean is a good rotational crop due to its nitrogen fixing ability and adaptability to wide range of soils and climate. It produces more edible protein per acre of land than other crops due to its high protein content (approximately 40% protein, 20% oil, 35% carbohydrates and 5% ash on dry basis). It has the highest protein content comparing to cereal and other legume species, and the second-highest oil content among all food legumes. Soybeans are widely used as human food, animal feed and industrial material. The majority of soybeans are crushed into oil for food uses and defatted meal for animal feed uses. Only a small fraction is processed for direct human consumption including various soyfoods and soy-based ingredients [soy flour, soy concentrate, soy isolate, soy nuts, soy isoflavone (germ) and more] (Soyatech, Inc. 2004).

Soybean oil contains more than 99% triglycerides with high proportion of unsaturated fatty acids (approximately 85%). It is a good source of two essential fatty acids that cannot be synthesized in human body: linoleic and linolenic acids, where the latter is also an omega-3 fatty acid (Liu 2004a). Hence, it has been shown that mono- and polyunsaturation in soybean oil is beneficial to human health due to its cholesterol lowering effects (Martin and others 1986; Chow 1992). Table 1 shows the average and ranges of the fatty acid profile in soybean oil including oil from the new low linoleic soybean cultivar (~1% C18:3). Minor components present also include phospholipids, unsaponifiable materials (such as tocopherols, phytosterols, and hydrocarbons), free fatty acids and trace metals.

Table 1. Fatty acid composition of soybean oil extracted.

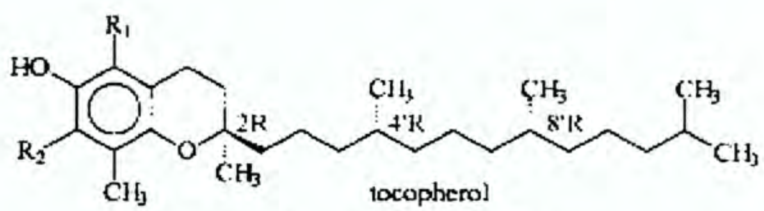
Fatty Acid Profile	Range	Average
Palmitic acid (C16:0)	4-23%	11%
Stearic acid (C18:0)	3-30%	4%
Oleic acid (C18:1)	25-86%	24%
Linoleic acid (C18:2)	25-60%	53%
Linolenic acid (C18:3)	1-15%	7%

Source: Liu 2001; Hammond and Glatz 1988.

The major storage proteins in soybeans are globulins with two major proteins: glycinin, an 11S fraction, and β-conglycinin, a 7S fraction. Both fractions account for approximately 70% of the total storage protein in soybeans (Nielsen 1985; Nishizawa and others 1994). Glycinin, the major seed storage protein in most varieties (>50%), is composed of one acidic and one basic polypeptide connected by disulphide linkages while β-

conglycinin is composed of α, α' and β-subunits but lacks disulphide linkages. Both of these protein subunits play important role in the quality of soyfoods.

Both water-soluble and fat-soluble vitamins are found in soybeans. The water-soluble vitamins include thiamin, riboflavin, niacin, pantothenic acid and folic acid while the fat-soluble vitamins include vitamin A and E (no vitamins D and K present). Water-soluble vitamins are lost during processing of soybeans such as tofu making. Ascorbic acid (Vitamin C) and Vitamin A, present as β-carotene, in mature soybeans is essentially negligible, but a measurable amount is present in immature and germinated beans (Bates and Matthews 1975). Vitamin E, the most sensitive fat-soluble vitamin, is present in four isomers: α-, β-, γ-, δ-tocopherols (Figure 1) (Knapp and Tappel 1961, Jiang and others 2001). Guzman and Murphy (1986) found that the concentration of different tocopherol isomers varies depending on the soybean cultivar (data reported as range of different soybean variety in Table 2). Even though 30-47% of vitamin E is loss during processing of soybeans into tofu, tofu is still considered a greater source of vitamin E than whole soybeans on a dry basis (Pryde 1980; Guzman and Murphy 1986).



α-tocopherol	$R_1=R_2=CH_3$
β-tocopherol	$R_1=CH_3$, $R_2=H$
γ-tocopherol	$R_1=H$, $R_2=CH_3$
δ-tocopherol	$R_1=R_2=H$

Figure 1. Chemical structure of vitamin E (tocopherols) in soybeans (Source: Jiang and others 2001).

Table 2. Vitamin E content in soybeans

Component	Range (μg/g)
α-tocopherol	10.9-28.4
β-tocopherol	less than 3% of total
γ-tocopherol	150-190
δ-tocopherol	24.6-72.5

Source: modified after Pryde 1980; Guzman and Murphy 1986.

Other major constituents of interest in soybeans include soy lecithin (0.5-1.5% of soybean seed), isoflavones (0.1-0.4% dry weight of soybeans), soy saponins, phytosterols (approximately 0.3-0.6mg/g of soybeans), phytate (1-1.7% dry basis), trypsin inhibitors, lectins or hemagglutinins and lunasin, a natural bioactive peptide in soybeans (Liu 2004a; Lolas 1976; Wang and Wixon 1999).

HEALTH BENEFITS OF SOY

Soy protein is low in sulfur-containing amino acids, but it does contain all 11 of the essential amino acids required for human or animal nutrition, including isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, and histidine (Zarkadas and others 1993). It is rated the highest *protein digestibility-corrected amino acid score* (PDCAAS) possible among plant proteins (PDCAAS=0.91) (Liu 2004a). Due to its comparable protein quality to cow's and human milk protein (PDCAAS=1.21), soymilk is an alternative choice for lactose-intolerant patients and good substitution for animal source proteins (Liu 2004b; Quak and Tan 1998; Schaafsma 2000).

Soy protein is also cholesterolemic. It is highly effective in lowering total cholesterol as well as low-density lipoprotein (LDL) by increasing the high-density lipoprotein (HDL)

(Anderson and others 1995). This has been shown in various studies as well as meta-analysis, which confirmed that soy protein lowers blood cholesterol concentrations in both animals and human (Anderson and others 1995; Hamilton and others 1976; Sirtori and others 1997). Therefore, the U.S. Food and Drug Administration (FDA) approved a health claim, for qualified soy products, that diets low in saturated fat and cholesterol that included 25g of soy protein a day may reduce the risk of heart disease (FDA 1999). Other active components including amino acids, isoflavones, saponins, phytic acid, trypsin inhibitors, fiber, and globulins may also contribute to the cholesterol lowering effect. Studies also have shown that soy protein plays significant role in lowering risk of cardiovascular disease, preventing hypertriglyceridemia, hyperinsulinemia, hyperglycemia and impaired renal function as well as decreasing urinary calcium excretion (Watkins and other 1985; Erdman 2000; Spence and others 2002; Stephenson and others 2002; Moriyama and others 2004).

Soy lecithin, the main by-product of soybean oil refining, is an important source of choline, which regulates signaling functions and structural integrity of cells. Lecithin helps to lower cardiovascular disease risk, prevent abnormal fetal development, reduce some forms of male infertility, promote healthy liver function, improve in memory and cognition, reduce adverse reactions to various drugs, as well as decrease risk of coronary heart disease and stroke by reducing plasma homocysteine levels (Zeisel and Blusztain 1994; Wald and others 1998; Liu 2004a). Studies also showed that consumption of soyfoods helps in preventing and treating chronic diseases, and reducing incidence of breast, colon, and prostate cancers, heart disease, osteoporosis, and possibly menopausal symptoms (Anderson and others 1995; Kennedy 1995; Barnes 1998; Setchell and Cassidy1999).

CONSUMPTION OF SOYFOODS

Soybeans have been consumed as food for thousands of years. They are traditionally consumed in a variety of soyfoods such as tofu, soy sauce, miso, soy sprouts and vegetable soybeans (Liu 2004b). Production of soybeans continues to grow to meet the market demand, and they have reached 57% of the total world oilseed production. The United States contributed to approximately 40% of the world soybean production in 2004 (Soy Stats™ 2005). Nowadays, soybeans have been transformed into various edible soy products for both human consumptions and animal feed, which are categorized as soy oil products, soy protein products, modern soyfoods, soy-enriched foods and soy dietary supplements and nutraceuticals by Liu (2004a) (Table 3).

Table 3. Variety of soyfoods in the current market.

Category	Product Examples
Traditional soyfoods	Non-fermented soyfoods • soymilk, tofu, soymilk film (yuba), okara, soybean sprouts, vegetable soybeans (edemame) Fermented soyfoods • Fermented soy paste, soy sauce, Japanese Natto, tempeh, sufu, soy nuggets
Soy oil products	Salad and cooking oils, shortening, margarine, mayonaise, salad dressings, confectionery coatings.
Soy protein products	Soy flour, soy protein concentrate, soy protein isolate, textured soy proteins
Modern soyfoods	Soy ice-cream, soy yogurts, soy cheese, soy burgers, meatless meatballs, imitation bacon bites, soy butter, soy puddings, tofu spreads and dressings
Soy-enriched foods	Soy bread, soy pastas, soy cereal, soy snacks, soy-enriched bakery products (muffins, pancakes, cookies, etc.)
Functional soy ingredients/ dietary supplements	Soy lecithin, oligosaccharides, isoflavones, tocopherols, phytosterols, trypsin inhibitors

Source: Liu 2004b.

SOYMILK AND TOFU PRODUCTION

Soymilk, a water extract from soybeans, and tofu, soybean curd, is believed to have originated in China and is made through the traditional thousand-year-old home-scale method (traditional Chinese method) (Liu 2004b). Different methods have been used in soymilk and tofu manufacturing to improve the flavor (reduce beaniness) of the soymilk., The traditional Chinese method, and newer methods that reduce the beany flavor of soy such as Japanese, Cornell, Illinois, Rapid Hydration HydroThermal Cooking (RHHTC), cold-grind under vacuum (ProSoya), deodorization, antioxidant addition and alkali treatment methods had been developed (Wilson 1995; Prawiradjaja 2003). The traditional Japanese method of soymilk and tofu making (Figure 2) varies from the traditional Chinese method in that the soymilk is separated from the okara before heating in the traditional Chinese method. The heating step after grinding in the traditional Japanese method is critical to inactivate enzymes such as lipoxygenase in raw soybeans, thus producing less beany soymilk. Soymilk is then further processed into tofu by coagulating the soy proteins using coagulants such as calcium sulfate, glucono-delta-lactone (GDL) or magnesium chloride (Watanabe and others 1964; Liu 2004b).

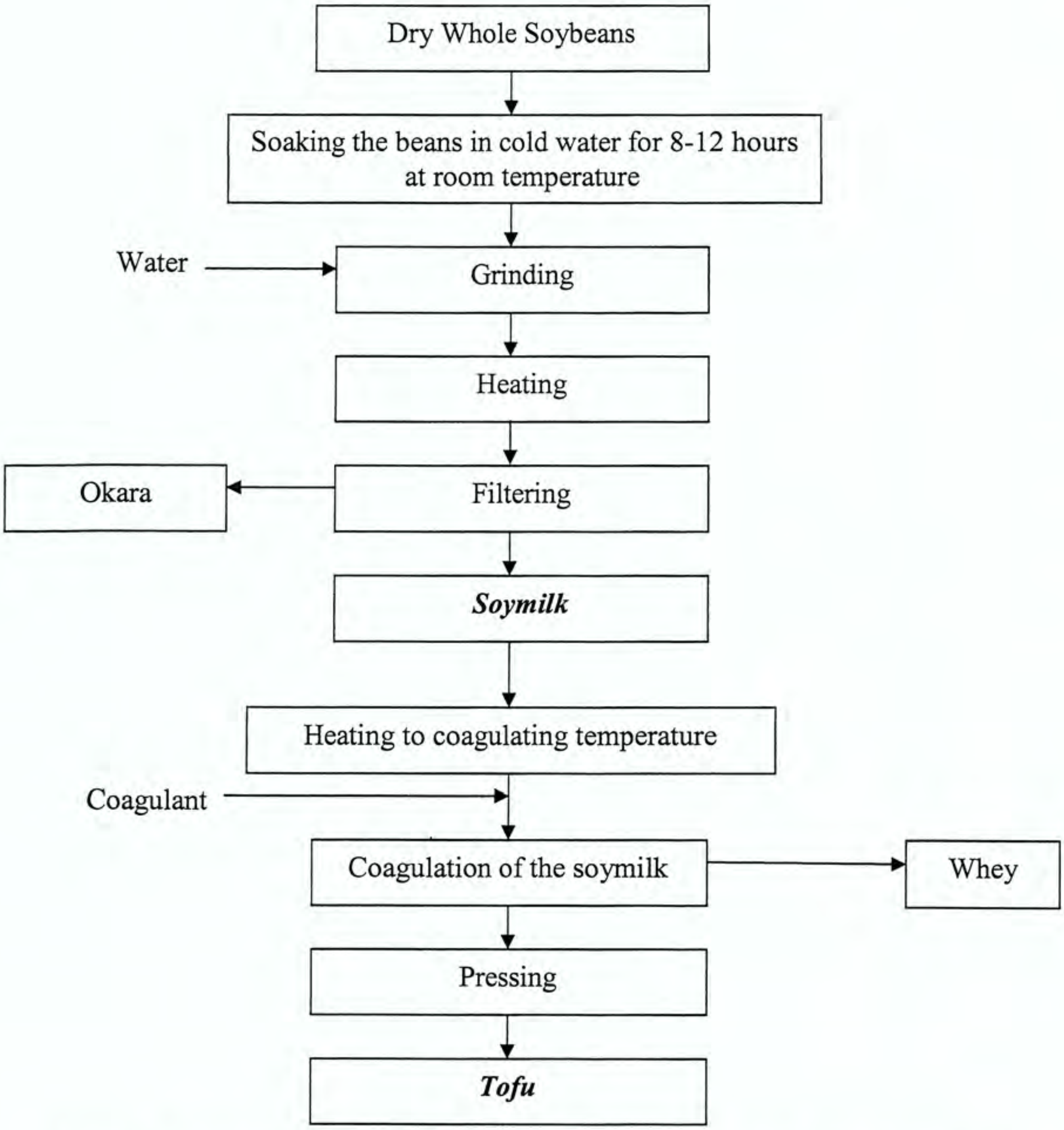


Figure 2. Traditional Japanese Method for Soymilk and Tofu Manufacturing

SOYBEANS AS ASTRONAUTS' FOOD

Since the first space mission of the National Aeronautics and Space Administration (NASA), food for astronauts has been studied and improved to provide better nutrition, increase the variety of foods, and make them easier to consume. Duration of space missions range from one or two weeks to years if permanent bases are to be set up on Mars (Karel 1989). It is increasingly important as the missions became longer that the astronauts should consume safe, palatable, healthy, and high quality food. Hence, research and development of space food and processing methods have been actively conducted to improve the safety, shelf-life and variety of space food (Nanz and others 1967; Klicka and Smith 1982; Barta and others 1999; Zasypkin and Lee 1999; Perchonok and others 2001; Toerne and others 2001; Wilson and others 2005). Implementation of the Hazard Analysis and Critical Control Point (HACCP) system for the space food program started with the Gemini program (1965 to 1966) (Perchonok and Bourland 2002). Application of radiation to space food started with the Apollo program (1968-1972), where the first irradiated food was consumed by the Apollo astronauts. It has been suggested that a HACCP, CCP step be used to ensure the food safety of astronauts (Bourland and others 2000; Perchonok and Bourland 2002; Wilson and others 2005). Foods can be pasteurized or sterilized through exposure to ionizing radiation. Increasing the variety of irradiated food is continuously being studied. The use of irradiation at 10-50kGy to sterilize frozen, packaged meats, including beefsteaks, was approved by the Food and Drug Administration (FDA) for use in NASA's space flight program in 1995 (FDA 1995; Crawford and Ruff 1996).

As space missions get longer, it has been suggested that crops be grown in the permanent base for (1) substitution of prepackaged food with ingredients processed from harvested crops in the future as well as (2) their contribution to bioregeneration of oxygen and carbon dioxide (Barta and Henninger 1994). Due to soybeans' high nutrient content, it has been chosen as one of the crops food for the Lunar and Mars missions. It can be processed into soy flour, tofu, tempeh, soymilk, whey, okara and soybean oil in space.

However, more efforts in design and development of food-processing procedures and equipment for conversion of crops into bulk ingredients, such as the STOW (Soymilk, Tofu, Okara, Whey) processor prototype for soybeans, need to be investigated to fully utilize the crops (Perchonok and Bourland 2002; Wilson and others 2004b).

FOOD IRRADIATION AS A STEP IN THE HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) SYSTEM

Application of ionizing radiation in food to ensure quality and shelf life has existed since the late 1800s according to Josephson (1983) and Diehl (1990b). The history of radiation started with discovery of X-rays by Roentgen in 1895 followed by observation of radiation effects on microorganisms by Pracronotti and Procelli in 1898. Extensive research on the influences of ionizing radiation on different foods and the development of accelerator machines followed these findings. In 1958, the United States Food Additives Amendment to the FDCA act defined food irradiation as an 'additive'. Laws and regulations on food irradiation were established to monitor applications of ionizing radiation on foods (Molins 2001). In 1980, the Joint Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA)/ World Health Organization (WHO) Expert Committee on Food Irradiation (JECFI) declared that "irradiation of any food up to an average dose of 10kGy pose no toxicological hazards to humans, no special nutritional or microbiological problems in foods" (Anonymous 1981; Ahmad 1995; Crawford and Ruff 1996; Molins 2001). This safe and acceptable dose level has been shown to be effective in various applications including delay or accelerate ripening, delay aging, enhance shelf-life, control microbial load, destroy insect infestation, eliminate unwanted sprouting, and sometimes improve flavor and texture of foods without imparting any radioactivity to the food. Due to the effectiveness of radiation in food prevention and controlling microbial contamination, the WHO declared that radiation is "a powerful tool against preventable food losses and foodborne illnesses" (Ahmad 1995; Crawford and Ruff 1996).

In 1997, due to the concern on the wholesomeness of food irradiated at 10 kGy and above, the joint Study Group on High Dose Food Irradiation reviewed data on high-dose food irradiation (10-100 kGy) and concluded that food treated with doses greater than 10 kGy can be considered safe and nutritionally adequate when produced under established Good Manufacturing Practices (GMPs). This is due to the fact that radiation at the levels will not lead to changes in the food composition and nutrient loss that would cause adverse effect on human health but would greatly reduce any potential microbiological risk to consumers (WHO 1999). Application of radiation to foods at three different dose ranges is shown in Table 4: low dose, medium dose and high dose radiation levels (Ahmad 1995; Crawford and Ruff 1996).

Cobalt-60 or caesium-137 and machine sources of electrons up to 10MeV and X-rays from electrons up to 5 MeV are the only radiation sources that are accepted as a Codex Alimentarius General Standard for food irradiation (WHO 1999). Table 5 shows the comparison of these three different sources of radiation (Deeley 2004).

Different types of radiation (Gamma, X-ray, E-beam, etc) may have different effects on the food depending on the dosage as well as the food matrix (Tables 4 and 5). Gamma rays are obtained from the decay of ^{60}Co, while electrons are generated by high energy electron beam accelerators. Both differ greatly in their ability to penetrate matter: gamma rays exhibit much higher penetration than electron beams (Cleland and Pageau 1985; Blank

Table 4. Levels of radiation subjected to food applications.

Level of radiation	Radiation dosage	Applications	Examples
Low dose application (disinfestation/ delay in ripening)	< 1 kGy	Inhibit sprouting in potatoes, onions, etc. Allow long-term storage Cause disinfestation by insects, etc. in pulses, cereal grains, dried fruits Destroy parasites in foods, e.g. nematods (Trichinella) in pork Delay other physiological processes	Potatoes, onions, garlic, root ginger, bananas, mangoes, and certain other non-citrus fruit, cereals and pulses, dehydrated vegetables, dried fish and meat, fresh pork
Medium dose application (pasteurization)	1-10 kGy	Reduce or eliminate spoilage bacteria, moulds and yeast, improve keeping properties Reduce pathogenic organisms such as Salmonella and Listeria.	Fresh fish, strawberries, grapes, dehydrated vegetables, fresh or frozen seafood, raw or frozen poultry and meat
High dose application (sterilization)	10-50 kGy	Sterilization for commercial purposes Eliminate some disease-causing viruses Sterilize herbs, spices and other ingredients, foods for immuno-compromised hospital patients and for astronauts during space flight. Decontaminate certain food additives and ingredients	Industrial products and medical supplies, etc. Meat, poultry, seafood and other food prepared for sterilized hospital diets, spices, enzymes preparations, natural gum

***Source*: modified after Ahmad 1995; Crawford and Ruff 1996.

and Corrigan 1995) (Table 5). However, electron beam irradiation was shown to be more efficient in decontamination or disinfestation of food products because the electron beam can be directed at the product or microorganism while the gamma sources emit radiation in all directions (Diehl 1990a; Chelack and other 1991; Blank and Corrigan 1995). X-rays have similar penetration ability as gamma and was first patented in the US to kill the nematod *Trichinella spiralis* in meat. Therefore, application of radiation on food may vary on types and dose level depending on its effectiveness and primary intentions (disinfestations, pasteurization or sterilization).

Table 5. Comparison of the primary functional parameters that differentiate radiation technologies.

	Gamma	*X-ray*	*E-beam*
On/off technology	No	Yes	Yes
Penetration depth	50-80cm	50-80cm	5.8cm
Dose rate	kGy/h	kGy/s	kGy/s
Dose homogeneity	High	High	Low

Source: Deeley 2004.

EFFECTS OF IRRADIATION ON FOOD SAFETY AND QUALITY

The use of food irradiation is most frequently focused on (i) microbiological safety of irradiated foods; (ii) nutrient loss during irradiation; (iii) free radical formation and radiolytic by-products of irradiation; and (iv) product quality-taste, texture, odor and other sensory attributes, (v) consumer acceptance (Bruhn and Noell 1987; Crawford and Ruff 1996; Nayga and others 2005). Hence, influences of radiation on food components that may affect the safety and overall quality of food have been widely studied.

According to Dickson (2001), radiation sensitivity of various organic compounds is proportional to their molecular weight. On the basis of this assumption, it has been estimated that a dose of 0.1kGy would damage 0.005% of the amino acids, 0.14% of the enzymes, and 2.8% of the DNA within a given cell (Pollard 1966; Dickson 2001). Studies have shown that irradiation is a safe and effective alternative to insure food safety other than heat and chemical treatments. Radiation inactivates microorganisms, thus improves the shelf-life of various products including meat products, herds, fruits and others (Byun and other 2002b; Kamat and others 2003; Palekar and others 2004; Wen H-W 2006). Radiation cause genetic material damage in microorganisms by generating radicals from water molecules which then react with the nucleic acids causing lesions in the DNA. However, sensitivity of the microorganisms to radiation is highly dependent on the irradiation condition and the food matrix, especially the availability of water in the systems (Dickson 2001).

Even though no major compositional changes and nutrient loss in food due to radiation has been known, studies have shown that ionizing radiation does alter the quality of food by affecting the major food components. Ionizing radiation can cause chemical changes in food components in both "direct", where the components are directly damaged by ionizing particles, and "indirect" actions, in which chemical changes are induced by the reactions of the products of water radiolysis with the food components. Irradiation of food containing fat in the presence of oxygen will lead to the development of rancid off-flavors due to lipid oxidation (Stewart 2001).

Irradiation of carbohydrate-containing food causes browning in sugar, which changes the color of food, and reduces the viscosity of food by reducing degree of polymerization of starch. In some carbohydrate foods, radiation also causes degradation of polysaccharides, such as starch, by inducing cleavage of glycosidic bond forming lower-molecular-weight sugars such as glucose, maltose, erythrose, ribose and mannose (Dauphin and Saint-Lebe 1977; Simic 1983; Sokhey and Hanna 1993). Researchers found that gamma irradiation caused modification of starch microstructure, reduction in paste viscosity of wheat, corn and rice and tenderization of rice curd texture (Ciesla and others 1991; Grant and D'Appolonia 1991; Sabularse and others 1991; Sung 2005; Kang and Byun 1996; Wu and others 2002).

Studies have shown that radiation at certain levels changes the structure of proteins, including structural modification of allergens using gamma irradiation to reduce its allergenicity (Byun and others 2002a). Radiation also introduced variability into seed storage proteins, which then affected their functional properties (Sabato and Lacroix 2002; Manjaya and others 2006). Individual or combined methods of radiation had been used to create mutant lines in crops breeding such as low-linolenic and high-oleic content (Rahman and others 1994, 1995; Patil and others 2004).

EFFECTS OF IRRADIATION ON SEED GERMINATION

Seed germination involves three major stages: imbibition of water, cell elongation followed by increase in cell number (Toole and others 1956). The imbibition stage is significant for water uptake of the seed prior to growth. Hence, the seed coat plays an important role in regulating the hydration of the seed, thus affecting its germination ability. Hardseed coats of stone beans (hardseeds) cause impermeability of the coats to water and dissolved gases, particularly oxygen and carbon dioxide, which then restrain the enlargement of the embryo. However, this condition can be improved by breaking or removing the seed coats (Toole and others 1956). The formations of hard seeds in legumes are believed to be due to the low moisture and/or high heat endured by the crop during final maturation prior to harvest (Mullin and Xu 2001).

Germination of the soybeans was also proved to be affected by radiation in Sakkar's study (1984). Increased doses of gamma radiation of the soybeans at 10, 20, 30, 40 KR (0.1, 0.2, 0.3, 0.4 kGy) increasingly affected the internode length and delayed the maturation process during germination where the maximum depression in germination was observed at the 20 KR dose (0.3 kGy) (Sakkar 1984). Manjaya also found the same radiation effect on gamma-irradiated soybeans - the plant height, flower color, sterility, leaf shape, early and late maturity had been affected (Manjaya and others 2006). Radiation above 0.2kGy reduced germination efficiency of lentil seeds while radiation above 1kGy caused loss of its germination ability (Chaudhuri 2002).

FLAVOR AND AROMAS OF SOYFOODS

The major issue in soybeans is the consumer acceptability of the beany flavor. Research on flavor characteristics of soybean products has been studied for decades with the intention to improve the flavor. Flavor compounds of raw soybeans have been extracted and volatile compounds were isolated and identified (Fujimaki and others 1965; Arai and others 1966, 1967; 1970) (Table 6). Volatile compounds from hexane extracted and Supercritical Fluid Extracted (SFE) soybean oils were also analyzed by Snyder and King (1994). Components identified includes C2 to C9 saturated aldehydes and the monounsaturated aldehydes, 2,4-heptadienal and 2,4-decadienal isomers, C5 to C8 saturated and unsaturated hydrocarbons, ethylfuran and penthylfuran, fatty acids and traces amount of ketones and alcohols (Table 7) (Snyder and King 1994). Part of the undesirable aroma in soy products may be due to lipid oxidation in soybeans.

Table 6. Flavor compounds isolated from raw soybeans.

Previous studies	Flavor compounds
Volatile neutral compounds from ground raw soybeans (Arai and others 1967)	Methanol, ethanol, 2-pentanol, isopentanol, pentanol, hexanol, heptanol, and pentanol acetate
Volatile fatty acids and volatile amines from raw soybeans (Arai and others 1966)	Acetic, propionic, isovaleric, valeric, isocaproic, caprylic, nonanoic, and capric acids
Volatiles from ground raw soybean (Fujimaki and others 1965)	Ethanol, 2-propanone, hexanal
Volatiles from raw soybean cotyledons (Arai and others 1970)	Pentanol, heptanol and heptanal
Volatiles in aqueous solution of soy protein isolates (Boatright and Lei 1999)	Dimethyl trisulfide, *trans,trans*-2,4-decadienal, 2-pentyl pyridine, *trans,trans*-2,4-nonadienal, hexanal, acetophnone, 1-octen-3-one.

Source: modified after Maga JA 1973; Boatright and Lei 1999.

Table 7. Headspace volatiles from hexane-extracted and supercritical fluid extracted (SFE) soybean oils.

Aldehydes	Hydrocarbons	Ketones and Alcohols	Others
Ethanal	Pentane	Ethanol	2-Ethylfuran
Propanal	2-Methylpentane	1-Propanol	2-Pentylfuran
Butanal	Hexane	A Butenol	Hexanoic acid
2-Butenal	Methyl cyclopentane	2-Butanone	
Pentanal	Heptane	1-Penten-3-one	
2-Methylbutanal	Octane	1-pentanol	
2-Pentenal	1,3-Nonadiene	A butendiol	
Hexanal		3-Pentenol	
2-Hexenal		1-Hexanol	
Heptanal		1-Octen-3-ol	
Octanal			
2t,4c-Heptadienal			
2t,4t-Heptadienal			
2-Octenal			
Nonanal			
2-Nonenal			
2t,4c-Decadienal			
2t,4t-Decadienal			

Source: Snyder and King 1994

Due to high unsaturation of soybean's fatty acids, which contain as high as 85% unsaturated fatty acids (Table 8), it is very susceptible to oxidation. Lipid oxidation in soybeans may be induced by natural enzymes that are present including lipoxygenases, lipases and some proteases. Non-enzymatic oxidation or autoxidation of lipids in soymilk may occur with the absence of natural enzymes depending on the unsaturation of fatty acids in the product and their exposure to oxygen, presences of metal ions (iron and copper), heat and UV light. Peñalvo and others found no major differences in the fatty acid profile of soybeans and soymilk after heat treatment, which indicated the possibility of lipid oxidation in both soybeans and its products (Peñalvo and others 2004) (Table 8).

Table 8. Fatty acid profile comparison of soybeans and traditional soymilk

Fatty acid *	*Soybean*	*Soymilk*
%SFA	13.8	15.3
% MUFA	21.2	22.1
% PUFA	64.6	62.4

* Based on percent of total fatty acids.
Source: Peñalvo and others 2004.

LIPOXYGENASE AND LIPID OXIDATION

Lipid oxidation is the process of the lipid component (unsaturated fatty acids or triglycerides) being oxidized in the presence of oxygen forming breakdown products that cause undesirable flavors and aromas. The overall mechanism of lipid oxidation involves three steps: (1) initiation, the formation of free radicals; (2) propagation, the free-radical chain reactions; and (3) termination, the formation of non-radical products. Each step of the oxidation is presented in Figure 9. Formation of free radicals and hydroperoxides accelerate the oxidation rate, which leads to oxidation of pigments and vitamins, polymerization of free radicals, formation of breakdown products that cause unpleasant off-flavor compounds such as ketones, aldehydes, alcohols, hydrocarbons, acids and epoxides, as well as causing insolubilization of proteins (Figure 3).

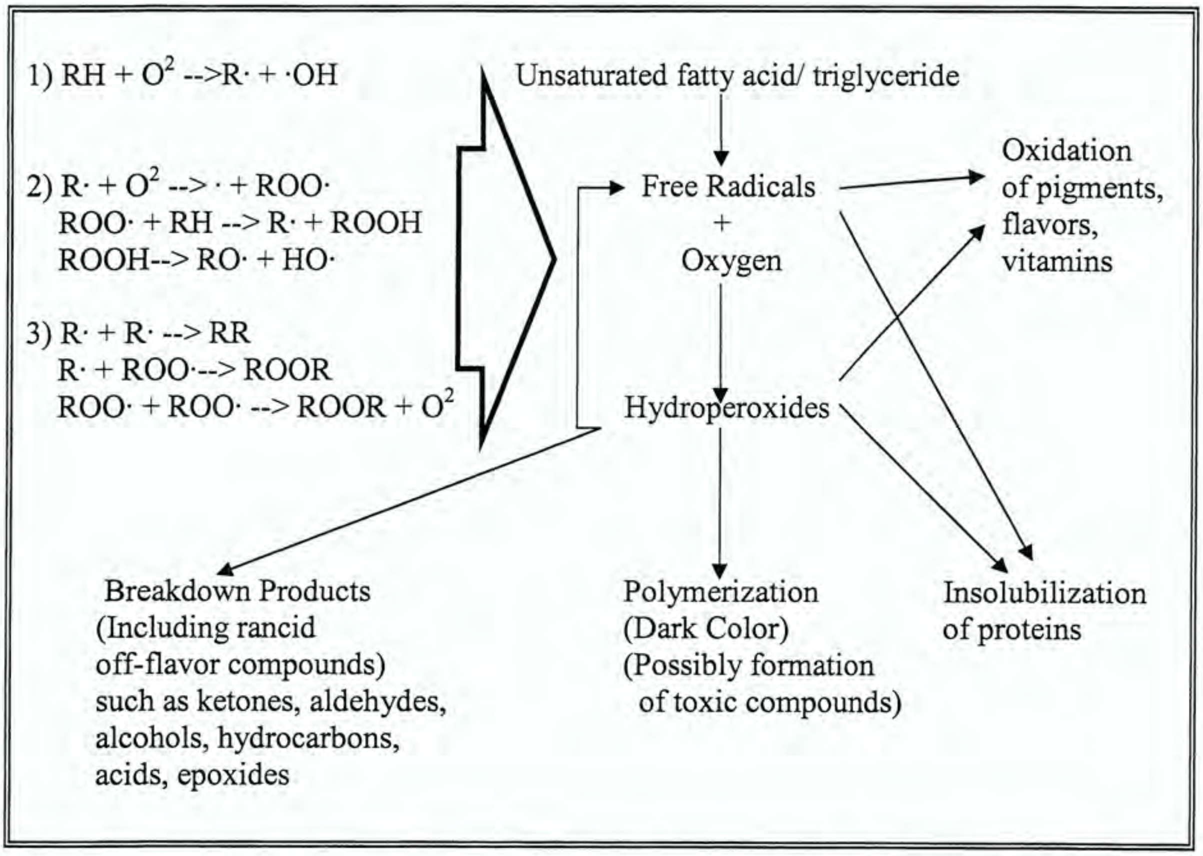


Figure 3. Mechanism of Lipid Oxidation (Source: Chemistry of Food Systems. University of British Columbia.)

Lipoxygenase, a monomeric polypeptides with a single non-heme iron cofactor, consists of three isozymes: Lipoxygenase-1 (L-1), Lipoxygenase-2 (L-2) and Lipoxygenase-3 (L-3) (Pistorius and Axelrod 1974; Axelrod and others 1981; Siedow 1991). Studies have shown that lipoxygenases play significant role in lipid oxidation in both soybeans and peanuts (St. Angelo and others 1979; Hildebrand and Kito 1984). The action of lipoxygenase in the presence of oxygen molecules on polyunsaturated fatty acids (linoleic and linolenic acids) or triglycerides that contain *cis*, *cis*-1,4-pentadiene moiety (Figure 4), forms hydroperoxide products that decomposed into acids, ketones, aldehydes, or other substances

(Rackis and others 1979). These break-down products further react with amino acids (lysine and threonine) and proteins to form new reaction products that may impair flavor (Kuck and others 1978). The flavor compounds can also interact through non-covalent interactions such as hydrophobic, hydrophilic and van der Waals forces, resulting in adsorption of off-flavor compounds onto soy protein (Aspelund and Wilson 1983). The principal cause of the objectionable flavors is short chain volatile carbonyl compounds, particularly hexanal, that bind to the soy protein (Fujimaki and others 1965, Sasaki and others 1981). It also has been shown that generation of *n*-hexanal from linoleic acid is due to lipoxygenase-2 isozyme (Matoba and others 1985).

The presence of lipoxygenase in soybeans is a major concern due to its ability to oxidize the polyunsaturated fatty acids (linoleic acids and linolenic acids), thus developing beany and grassy off-flavor in soy products by oxidizing polyunsaturated fatty acids (Siedow 1991; Wilson 1996). However, oxidation of linoleic acid maybe also be catalyzed by soy protein aggregation induced by lipoxygenase (Huang and others 2006). Twenty-one compounds (11 aldehydes, 3 alcohols, 4 ketones, 1 furan, one alkane and 1 alkene) were found in normal soybean line (BouÉ and others 2005). Studies have been done to improve the flavor of tofu and soymilk using lipoxygenase-free soybean (Torres-Penaranda and others 1998). In order to compare the effect of radiation on products of soybeans (soymilk and tofus), IA2032LS, the triple-null genotype soybean, and Vinton 81, lipoxygenase-present soybean, were used in this study to verify the effect of radiation versus effect of lipoxygenase on off-flavor development in soymilk.

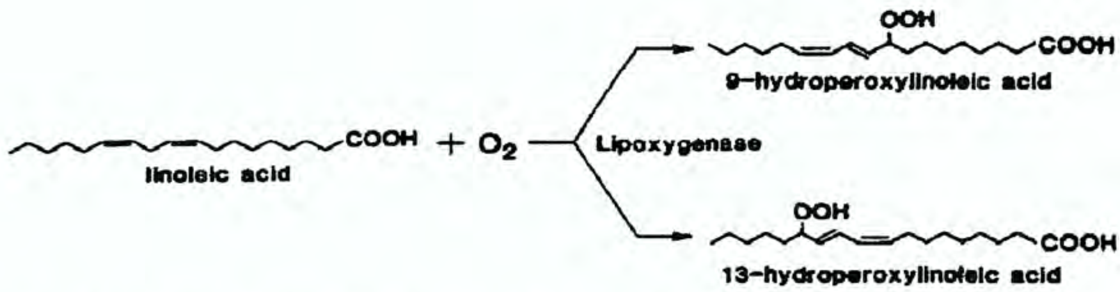
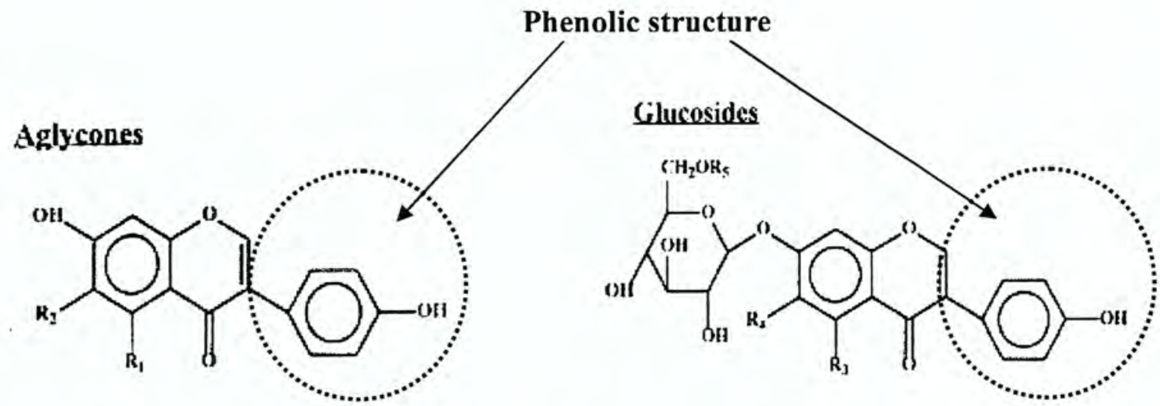


Figure 4. The oxidation of linoleic acid (*cis, cis*-9,12-octadecadienoic acid) catalyzed by lipoxygenase indicating two possible reaction products: 9- and 13-hydroperoxylinoleic acid. (Source: Siedow 1991.)

ANTIOXIDANT IN SOYBEANS

Natural antioxidants found in soybeans such as tocopherols (vitamin E), vitamin C, carotenoids, and phenolic compounds are known to be protective against oxidative stresses in the soybeans (White and Xing 1997). Antioxidants play a significant role in reducing the lipid oxidation level in the soy food products. Several studies reported that aroma compounds extracted from soybeans had antioxidant properties comparable to the antioxidant capacity of Vitamin E (Pratt and Birac 1979; Lee and Shibamoto 2000). Aroma compounds such as eugenol, maltol, benzyl alcohol and 1-octen-3-ol inhibited the oxidation of hexanal in the beans and malonaldehyde formation in cod liver oil (Lee and Shibamoto 2000).

Studies have shown that isoflavones demonstrate a weak antioxidant defense mechanism. Flavonoids are a group of plant polyphenols that have the common skeleton of the flavan nucleus (Pietta and Mauri 2001). Flavonoids can exert their antioxidant activity by inhibiting the activities of enzymes, including lipoxygenase and cyclooxygenase, by chelating metal ions, and most importantly, by scavenging free radicals (Shi and others 2001). Isoflavones, a significant flavonoid compound in soybeans, has been studied and shown to be a possible weak antioxidant in soybeans due to its phenolic structure (Figure 5). Table 9 shows the isoflavone concentration in different soy products and that processing leads to a wide range of isoflavone concentrations in soyfoods (Coward and others 1993, 1998; Wang and Murphy 1994).



R_1	R_2	Compound
H	H	Daidzein
OH	H	Genistein
H	OCH_3	Glycitein

R_3	R_4	R_5	Compound
H	H	H	Daidzin
OH	H	H	Genistin
H	OCH_3	H	Glycitin
H	H	$COCH_3$	Acetyldaidzin
OH	H	$COCH_3$	Acetylgenistin
H	OCH_3	$COCH_3$	Acetylglycitin
H	H	$COCH_2COOH$	Malonyldaidzin
OH	H	$COCH_2COOH$	Malonylgenistin
H	OCH_3	$COCH_2COOH$	Malonylglycitin

Figure 5. Chemical structure of 12 isoflavone isomers. (Source: Song and others 1998.)

Table 9. Isoflavone concentration in different soy products.

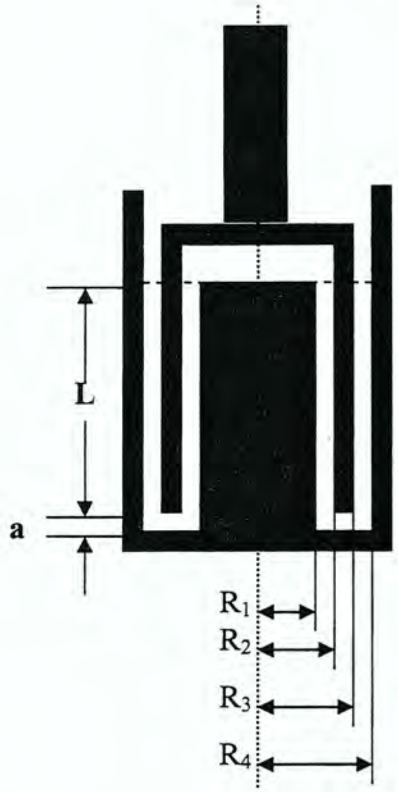
Product		Total Isoflavone (μg/g)
Isoflavone in soybean		
Range	0.1-0.4%	
Average	2.5%	
Soybean		1271.2
Toasted soy flakes		3095.4
soy flour		1807.0
Isolated soy protein		2161.5
Textured vegetable protein		2110.2
Regular soymilk		96.0
Low-fat soymilk		41.9
Non-fat soymilk		11.6
Regular tofu		323.0
Low-fat tofu		260.9

Source: Coward and others 1993, 1998; Wang and Murphy 1994.

APPEARANCE AND TEXTURAL QUALITY OF SOYFOODS

Quality of soyfoods needs to be monitored and maintained at high levels to ensure consistent characteristic of the products. Viscosity of soymilk and texture of tofu are significant in determining specific characteristic of the food products as well as providing acceptable mouthfeel for consumers. As the quality attributes of soymilk and tofu, measurement on rheological properties and textural characteristics are widely done in the food industry for quality control.

Viscosity of soymilk can be measured using a double gap cylinder sensor system Model DG41 (Haake Waltham, MA), which is made up of the bell-shaped rotor and a beaker or cup. Figure 6 shows the geometry design of the sensor system, where the radii relationship of the shear surfaces is almost equal to create identical shearing conditions in both gaps.



$$\frac{R_2}{R_1} = \frac{R_4}{R_3}$$

R_1 = Radius Beaker (inside)
R_4 = Radius Beaker (outside)
R_3 = Radius Rotor (outside)
R_2 = Radius Rotor (inside)
L = Length of shear Surface
a = Distance

Figure 6. Geometry of Sensor System DG41 made up of a beaker and a bell-shaped rotor. (Source: Haake 1997)

Viscosity of the soymilk can be determined by plotting the change of shear stress (τ) and shear rate ($\gamma.$) with time (s) by using Haake Software RheoWin-RS150 (Haake 1998) (Figure 7). Shear stress is proportional to the torque (Md) and to the stress factor (A) (Equation 1). Shear rate is proportional to the angular velocity or speed and the shear factor (M) (Equation 3).

Shear Stress (τ) = Stress Factor (A) · Torque (Md) -----------(1)

Where as

$$\text{Stress Factor (A)} = \frac{1}{2 \times \pi \times Ri^2 \times L} \quad \text{------------------------(2)}$$

Shear Rate ($\gamma.$) = Shear factor (M) x Angular speed (Ω) ------(3)

Where as Shear factor (M) = $\frac{2 \times R_a^2}{R_a^2 - R_i^2}$ ---------------------(4)

Angular speed (Ω) = $\frac{2\pi}{60} \times n$ ---------------------------(5)

Source: Haake 1997.

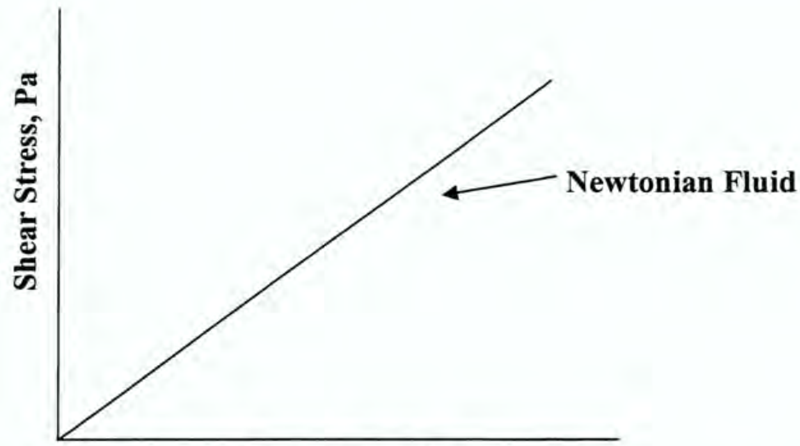


Figure 7. Plot of shear stress vs shear rate for determination of viscosity.

Rheological properties of the soymilk and other liquids can be were determined based on Power's Law ($\tau = K \cdot \gamma.^n$) where as n is the flow behavior index and K is flow consistent coefficient (unit of $Pa \cdot s^n$) of the fluid. The type of fluid can be determined based on these rheological properties as shown in Table 10. Newtonian fluids have a flow consistent coefficient (K) greater than zero, flow behavior index (n) equal to 1, and no shear stress (σ) (Steffe 1996).

Table 10. Rheological characteristics of different fluid types.

Fluid Type	*K*	*n*	*σ*
Herschel-Bulkley	> 0	0 < n< ∞	> 0
Newtonian	> 0	1	0
shear-thinning (pseudoplastic)	> 0	0 < n < 1	0
Shear-thickening (dilatent)	> 0	1 < n < ∞	0
Bingham plastic	> 0	1	> 0

Source: Steffe 1996.

Texture of tofu can be measured using texture profile analysis (TPA). It uses double compression to imitate the first bite and second bite by humans during ingestion of food. Seven attributes, including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience, are measured by plotting a graph of force versus time (Figure 8). Each attribute is characterized by calculation as shown in Table 11.

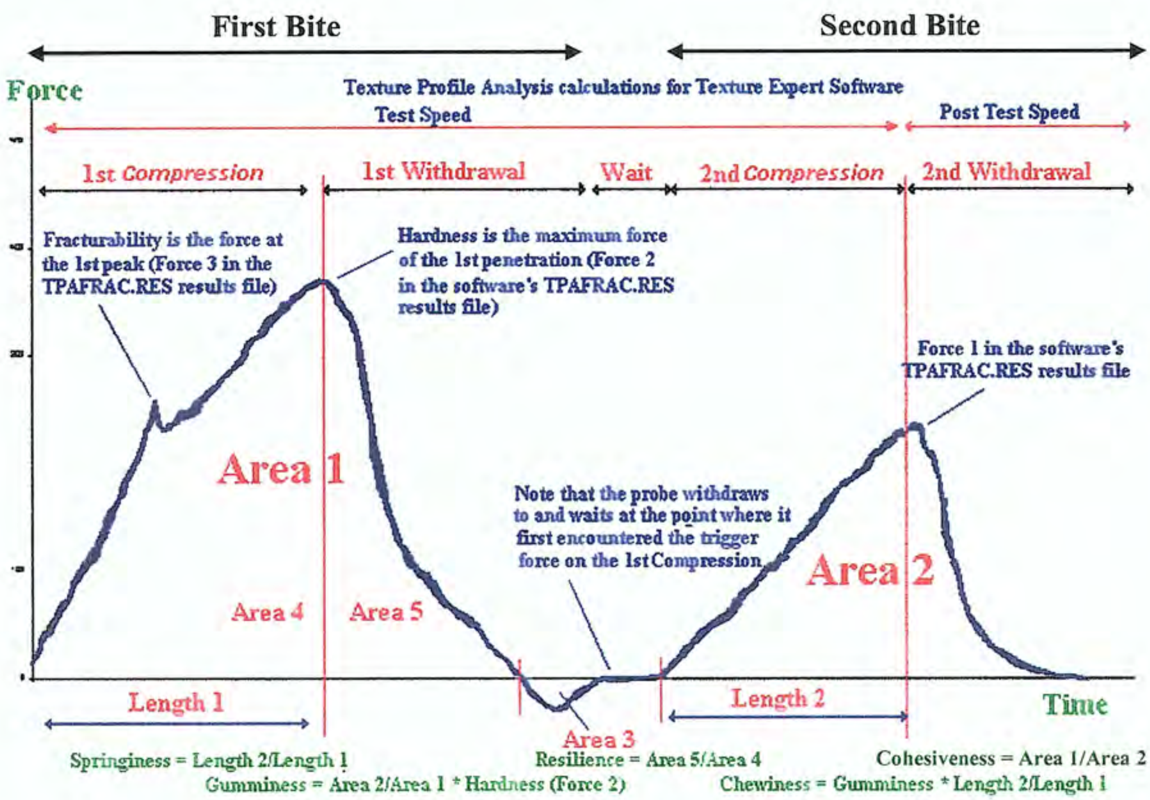


Figure 8. Texture profile analysis (TPA) (Source: Texture Technologies Corp. 2005.)

Table 11. Determination of Texture Profile Analysis (TPA) attributes.

Textural Attribute	Determination of Attribute
Hardness	Peak force of the first compression of the product.
Adhesiveness	Area 3
Fracturability	The first significant peak during the probe's first compression of the product.
Cohesiveness	Area of work during the 2nd compression / Area of work during the first compression (Area 2/Area 1)
Springiness	2nd compression distance / original compression distance (Length 2/Length 1)
Chewiness	Gumminess * Springiness (Length 1/Length 2)
Gumminess	Hardness * Cohesiveness (Area 2/Area 1)
Resilience	Area during the 1st withdrawal / Area of the 1st compression (Area 4/Area 5)

Source: Texture Technologies Corp. 2005.

Hardness of the tofu can be measured by taking the peak force of the first compression using the equipment such as Wilson® / Shore® Hardness Testers (Instron Corp., Norwood, MA) and TA-XT2*i* texture analyzer with Texture Expert Software (Texture Technologies Corp., Scarsdale, NY). Fracturability of a product occurs at the first significant peak of the plot during the first compression. However, fracturability has not been measured in analysis of tofu texture. Cohesiveness measures the ability of the product to withstand a second deformation relative to its behavior during the first deformation. Springiness determines how well a product springs back after it has been deformed during the first compression. Chewiness only applies to solid products while gumminess only applies to semi-solid products. Resilience measures how well the product "fights to regain its original position" (Texture Technologies Corp. 2005).

In conclusion, a number of studies have been published on the effects of radiation on quality and functionality of different types of food. However, studies on soybeans quality and functionality as influenced by radiation are still limited. Only a few studies showed that gamma irradiation changes functional properties of soy proteins. Therefore, studies on the effects of electron beam irradiation on the quality and functional properties of soybeans may solve more questions of radiation effects on food quality as well as concerns of both Homeland Security and NASA, which leads to two hypotheses of this study:

Hypothesis 1:

Radiation and storage conditions, during transit to Mars, interact to decrease the amount of natural antioxidants in the soybean, induce lipid oxidation and decrease the yield and quality of soymilk and tofu.

Hypothesis 2:

Radiation at 1, 5, 10kGy will decrease the germination rate of the soybeans.

OBJECTIVES

The objective of this research is to establish whether electron beam irradiation at 1, 5 and 10 kGy of soybean cultivars would have an influence on microbial load, seed germination, antioxidant potential, aroma of soymilk, and the yield and texture of tofu.

CHAPTER 3

MATERIALS AND METHODS

Two non-GMO soybean cultivars were selected based upon the results of Wilson and others (2004): Vinton 81 (Pattison Bros. Mississippi River Terminal Inc., Clayton, IA) and IA2032LS (Stonebridge Ltd., Cedar Falls, IA). Both cultivars are high in protein and large seeded. Vinton 81 is considered to be the gold standard cultivar of the soyfoods industry for soymilk and tofu production. IA2032LS lacks three lipoxygenase isozymes and thus has a milder aroma and flavor (Wilson and others 2004b, 2005). It is used for soymilk, tofu and edamame soybean production. Soybeans harvested in two different crop years (2003, 2004) were used in this study: 2003 and 2004.

One pound of soybeans was weighed into Ziploc® bags (10 x 12.002 seal top), air forced out, sealed and labeled prior to radiation treatment. The amount of soybeans in the bag allowed a single layer of seeds to be exposed to the radiation treatment. The bagged soybeans were irradiated with electron beam radiation at Texas A&M Univeristy (College Station, TX) Electron Beam Facility at three radiation dose levels: 1, 5, 10 kGy. As a control (0 kGy), one set of each cultivar accompanied the treated soybeans to the irradiation site but was not treated. Four 1-lb bags (four replicates) were used for each dose level for each cultivar. A second set of soybeans harvested in 2004 was irradiated at the same irradiator site the following year as a crop year treatment for the study. Dosimeters accompanied each bag to verify radiation doses the soybeans received. All soybeans were stored in the dark at 20 °C prior to and after irradiation.

The electron beam-treated dry whole soybeans were evaluated for proximate composition, vitamin E content, antioxidative capacity, thiobarbituric acid reactive substances (TBARS), peroxide value (PV), free fatty acids (FFA), microbial load, and germination rate. Functionality changes in soybeans were evaluated by manufacturing the beans into soyfood products (soymilk and tofu) and determining yield, color, texture and aroma.

PROXIMATE ANALYSIS

Proximate compositions of the soybeans were analyzed using a Near Infrared (NIR) spectrometer Infratec 1229 Whole Grain Analyzer (Foss North America, Inc., Eden Prairie, MN). Approximately 500 g of samples were poured into the hopper and analyzed at 8 nm intervals from 810.5-1075nm (Hardy and others 1996). Percentage of moisture, protein, lipid and fiber are reported on a 13% moisture basis. The control and treated soybeans for each cultivar were analyzed in 4 replicates.

VITAMIN E ANALYSIS

Two hundreds grams (± 0.05 g) of soybeans for each treatment were sent to Medallion Labs (Minneapolis, MN) for vitamin E analysis. Approved methods of the American Association of Cereal Chemist (AACC) Method 86-06 were used to determine the vitamin E (α-tocopherol) contents in the samples by using high performance liquid chromatography (HPLC) (AACC 2001). The percentage moisture of each sample was analyzed by using the vacuum oven method at 70 °C for 16 hours. The vitamin E content of each sample was reported as IU/100g. Only samples harvested in 2003 were analyzed for vitamin E content. All analyses were done in duplicate.

ISOFLAVONE ANALYSIS

The total isoflavone content as well as the amount of each isomer (daidzein, genistein, glycitein, daidzin, genistin, glycitin, malonyl daidzin, malonyl genistin, malonyl glycitin, acetyl daidzin, acetyl genistin, acetyl glycitin) were determined by Dr. Murphy's lab at Iowa State Univeristy using a Beckman System Gold High-Performance Liquid Chromatograph (HPLC) (Beckman, Fullerton, CA) with the Beckman System Gold HPLC data processing software (version 8, 1993) (Song 1998). The isoflavone content was reported as μg/g. Only samples harvested in 2003 were analyzed for vitamin E content. All analyses were done in duplicate.

ANTIOXIDANT CAPACITY

One gram (± 0.05 g) of each soybean sample was finely ground and mixed with 10 mL of HPLC grade methanol in a water bath shaker at room temperature for 5 minutes. The extracted solution was filtered through a syringe with a 0.45 μm cellulose acetate filter (Sartorius AG, Goettingen, Germany). A 30-μL aliquot was used to determine the antioxidant capacity of lipid soluble compounds with the Photochem® instrument using chemiluminesence following the ACL-Kit protocol (AnalytikjenaAG, Woodlands, TX). At least two blank measurements with less than 5% deviation were done and a calibration curve was constructed at a range of 0.5, 1.0, 2.0 and 2.5 nmol Trolox standards prior to sample measurements. Each sample was run in triplicates. Antioxidant capacity was calculated based on the equation below and reported as μg/mg:

$$\text{Concentration } (\mu g/mg) = \frac{\text{Quantity*Dilution*M*Volume}}{\text{Pipetted Volume*Weighted Sample}}$$

Quantity:	Trolox equivalents in nmol
M:	250.3 ng/nmol (Molar mass of Trolox)
Pipetted volume:	30 μL
Weighted sample:	1000 mg
Volume:	10 mL
Dilution:	10 (at 1:10 dilution factor)

THIOBARBITURIC ACID (TBA), PEROXIDE AND FREE FATTY ACID (FFA) ANALYSES

The SāfTest® System (SāfTest®, Inc., Tempe, AZ), AOAC certified method (certificate number: 030405) (AOAC International, Gaithersburg, MD), was used to measure the thiobarbituric acid reactive substances (TBARS), peroxide value and percent free fatty acid of dry whole soybeans. Two grams of ground soybeans (± 0.05 g) were weighed into the bottom of 50 mL-conical tube and 4.0 mL of SāfTest® Preparation Reagent (isopropanol, >99%) were added to make a 1:3 dilution. Ten glass beads were added to each conical tube. The sample was screw-capped and vortexed at the dial speed 10 for one minute followed by 15 minutes of heating on a Type 17600 Single Block Modular Dri-Bath (Barnstead International, Dubuque, IA). Samples were filtered through the membrane on the SāfTest®

Filtration Unit at vacuum pressure of 5-10 in. Hg. The samples were kept at 40 °C in the heat block until tested. An aliquot of sample was reacted with standardized reagents and analyzed by the SāfTest® Analyzer with 570/690 or 550/690 filter (Table 12).

Table 12. Summary of each SāfTest® Kit-STD Assays

SāfTest® Kit-STD Assays	Amount of sample used (μl)	Reaction time (min)	Filter*
AldeSafe™ Kit-STD Assays	150	90	550/690
PeroxySafe™ Kit-STD Assays	50	15	570/690
FASafe™ Kit-STD Assays	100	10	570/690

* Primary assay wavelength: 550nm or 570nm; reference wavelength: 690nm.

AldeSafe™ Kit-STD Assays test measured the malonaldehyde content as μmol of malonaldehyde/kg of sample. PeroxySafe™ Kit-STD Assays measured the lipid peroxide content as meq of peroxides/kg of sample. FASafe™ Kit-STD Assays measured the acid contents as percent oleic acid in the sample. The final results were obtained by adjusting the instrumental results based on the dilution factor:

Final result = SāfTest™ result * Dilution

where as SāfTest™ result: Instrument measurement (μmol/kg or meq/kg or %)

Dilution: 3 (at 1:3 dilution factor)

The equipment was calibrated with the standard calibrators for each kit and low, medium and high controls were tested as references prior to further measurement of samples. Each sample was tested in 2 replicates and three measurements were taken for each replicate.

MICROBIAL ANALYSIS

Five grams (± 0.05 g) whole soybeans for each treatment were packed into 6.5 cm x 9.3 cm sterile sealable bags and tested for microbial load at NASA Johnson Space Center Microbiology Laboratory (NASA Johnson Space Center, Houston TX). Each sample was tested in 5 replicates for total aerobic count (CFU/g), coliform count (coliform/g), salmonellae count (CFU/25g) and yeast and mold count (yeast/mold/g) based on the specification SD-T-0251 for raw material at NASA (Table 13).

Table 13. SD-T-0251 Specification for microbiology analysis.

Microbial Analysis	Specification
Total Aerobic count	IF any exceeds 20,000 and more than one exceeds 10,000 = FAIL
Coliform count	IF any exceeds 100 or more than one exceeds 10 = FAIL
Salmonellae count	Zero tolerance
Yeast and Mold count	IF any exceeds 1000 or more than one exceeds 100 or more than one exceeds 10 *A. Flavus* = FAIL

GERMINATION STUDY

The standard Warm Germination Test (AOSA and ISTA official test) was conducted at the Iowa State University (ISU) Seed Testing Laboratory using a tray method for the germination study. Two layers of crepe cellulose papers (KimPak®) were placed on a tray, sprayed with water and soaked for at least 12 hours. Four replicate measures of 100 soybeans seeds for each treatment were randomly planted on each corner of eight trays loaded with moist KimPak® using a planting size 4 or 5 board. A press board was used to lightly press the seed into the KimPak® to ensure adequate contact between seed and substrata. Each corner of the tray was labelled and the trays of seeds were put in two different carts and allowed to germinate in two different bays at 25 °C, 95% RH for 8 days. Each tray was evaluated and the amount of normal, abnormal germinated and dead seeds were recorded and converted to percentage. Random sampling of the germinated seed at each dose level was taken for germinated height measurement (length from root tip to cotyledon).

SOYMILK AND TOFU MANUFACTURE

The functionality of the soybeans was evaluated by manufacturing soymilk and tofu. The standardized methods of Johnson and Wilson (1984), Wilson (1996), Moizuddin and others (1999a), Wilson and others (2004b, 2005) 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 (5 x 4.5 x 9 cm^3; NASA FTCSC Equipment Grant 2004), with press weights (Wilson 2004b) for 100 g (dry beans) batches were used. Percent soluble solids of soymilk was measured by a Bausch & Lomb Abbe-3L Refractometer (Fisher Scientific Research, Pittsburgh, PA)] 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 and others (1999b). 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.

YIELD CALCULATION

Yield of soymilk was calculated based on the dry beans weight (Formula 1). Yield of tofu was determined based on initial amount of soymilk used to manufacture tofu (Formula 2) and initial amount of dry beans weight used to produce both soymilk and tofu (Formula 3).

$$\text{\% soymilk yield} = \frac{\text{wt. of soymilk (g)}}{\text{wt. of dry beans (g)}} \times 100\% \quad \text{------------- (1)}$$

$$\text{\% tofu yield (soymilk basis)} = \frac{\text{wt. of tofu (g)}}{\text{wt. of soymilk used (g)}} \times 100\% \quad \text{------------- (2)}$$

$$\text{\% tofu yield (dry beans basis)} = \frac{\text{wt. of tofu (g)}}{\text{wt. of dry beans (g)}} \times 100\% \quad \text{------------- (3)}$$

COLOR ANALYSIS USING HUNTER LABSCAN XE

Color of samples (soymilk, tofu, okara and whey) was measured as L, a, b values using Hunter LabScan XE 0/45 Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA) in the port-down orientation with 1.75-inch viewing area, 2.0-inch port size set at illuminant D65 and 10° standard observer. The instrument was calibrated with a white tile (X = 79.43, Y = 84.32, Z = 90.39) and a black tile. The samples were filled into a 6.0cm diameter x 1.5cm depth plastic Petri dish with optical clarity (Fisher Scientific Research, Pittsburgh, PA), placed on the instrument port and measured from top of the dish. Each measurement was done in triplicate on three different spots of the sample. L = 100 indicates lightness and $L = 0$ indicates darkness, $+ a$ = red and $- a$ = green, and $+ b$ = yellow and $- b$ = blue.

VISCOSITY MEASUREMENT

Haake RheoStress RS150 (Thermo Electron Corporation, Waltham MA) was used to measure the viscosity of soymilk. 6.3 mL of soymilk sample was filled into the measuring cup DG41 and was measured by sensor Rotor DG41 at the setting of starting speed at 10.00 1/s, ending speed at 1500.00 1/s, gap distance at 5.1 mm, temperature at 23.0 °C. Each sample was analyzed in duplicate and three measurements were taken for each replication. Viscosity of soymilk was recorded at average shear rate of 600.00 1/s. *K* and *n* values of the soymilk were determined by the Haake Software RheoWin-RS150 based on a power law function.

TEXTURE ANALYSIS

Tofu samples were cut into 2 cm x 2 cm x 2 cm cubes for analysis. The TA-XT2*i* texture analyzer (Texture Technologies Corp., Scarsdale, NY) was warmed up for at least 30 minutes prior to use. A cylinder probe, TA-30 (2" diameter, 20 mm tall) was attached to the instrument and calibrated by inputting the initial distance of the probe to the base. The force was calibrated without weight and with a 5 kg weight monitored by the software Texture Expert. A non-fracture texture profile analysis (TPA) was used and the parameter settings as listed below were entered:

Load cell	5 kg
Distance (probe)	40 mm

Pre-test speed	2.0 mm/sec
Test speed	1.7 mm/sec
Post-speed speed	5.0 mm/sec
Rupture test distance	1%
Distance	50%
Force	0.98 N
Time	1 sec
Trigger	0.05 N

Samples were analyzed for hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience. Each sample was analyzed in 4 replicates.

FLAVOR ANALYSIS BY GAS CHROMATOGRAPHY (GC)

A 25-ml aliquot of soymilk prepared from each control and treatment soybeans were placed into a labeled 100ml flat bottom crimp top headspace vials (Supelco, Bellefonte, PA). The bottles were sealed with a standard seal (20 mm for vial 5 ml) containing a 20 mm rubber septa Teflon (Supelco, Bellefonte, PA) using a 11mm-hand crimper. Samples were stirred with a magnetic stirring bar while headspaces were sampled at 40 °C for 45 minutes with a solid-phase microextraction (SPME) fiber coated with 2cm-50/30μm Divinylbenzene(DVB)/Carboxen/ Polydimethylsiloxane (Boylston and others 2003). A Model 6890 gas chromatograph (Hewlett-Packard, Inc., Wilmington, DE) with a splitless injection port and flame ionization detector (FID) was used to separate the volatile compounds. The SPME fiber was removed from the sample headspace and thermally desorbed via the injection port for three minutes at set temperature of 220 °C while the column temperature was held at 30 °C. Volatiles were swept onto an SPB-5 fused-silica capillary 30 m x 0.25 mm x 0.25 µm film thickness column (Supelco, Inc.). Ramp 1 was set to increase oven temperature from 30 °C at a rate of 3.5 °C/min. Ramp 2 increased oven temperature from 110 °C at a rate of 10.0 °C/min until the final temperature 200 °C is reached. The total run time was 39.86 min. The detector temperature was set at 220 °C and the flow rates of the detector gases were air at 400 mL/min, hydrogen at 30 mL/min and constant nitrogen flow (make-up gas) at 25 mL/min. Concentration of the volatiles were determined based on the peak area using the GC program; areas below 5 were not recorded (Crook and Boylston 2004).

Major volatile compounds were further identified by using a gas chromatograph-mass spectrometer (Micromass GCT, Waters Corp., Milford, MA) with a time-of flight mass analyzer. The samples were thermally desorbed into the GC injection port in a split (100:1) mode. GC condition were set at an initial temperature of 30 °C for 3 min, 3.5 °C/min from 30 °C to 110 °C, and 10.0 °C/min from 110 °C to 200 °C. The mass spectrometer conditions were set as electron ionization positive (EI +) polarity, source electron energy at 70 eV, source electron current at 200 µA, ion source temperature at 180 °C, source ion repeller at 0.8 V, electron multiplier voltage at 2700 V, scan range from 41 to 400 m/s, at a frequency of scanning cycle every 0.75 seconds. Mass spectra of the volatile compounds were then compared to a spectral library (Wiley Library), Flavornet (Acree and Am 2004) and a flavor and fragrance database (FlavorWORKS, Flavometrics, Version 2.0, Anaheim Hills, CA) for identification (Crook and Boylston 2004).

STATISTICAL ANALYSIS

A three-way analysis of variance (ANOVA): radiation (0, 1, 5, 10 kGy), year (2003, 2004), cultivar (Vinton 81, IA2032LS), radiation:year, radiation:cultivar, year:cultivar, and radiation:year:cultivar interactions, was performed to analyze the results statistically using program R, a statistical computing and graphics language and environment (R Development Core Team 2006, Vienna, Austria). A blocking effect was done on replicate (total of 4 replicates for this study design). Significance of the results were determined based on $p<0.05$. All pair wise comparison among means was analyzed using Tukey Honestly Significantly Different (HSD) with $p<0.05$ to be significant. However, results and discussion will be focused on radiation effect and its interaction.

Data for vitamin E and isoflavone analysis were statistically analyzed using a two-way ANOVA: radiation (0, 1, 5, 10 kGy), cultivar (Vinton 81, IA2032LS) and radiation:cultivar due to unavailability data from crop year 2004. Significance of the results were also determined based on $p<0.05$. All pair wise comparison among means was analyzed using Tukey Honestly Significantly Different (HSD) with $p<0.05$ to be significant.

CHAPTER 4

RESULTS AND DISCUSSION

Since the effect of radiation on all attributes of soybeans is the major concern in this study, significant effect of radiation and its interactions with year and cultivar effects will be discussed. Effects due to interactions between crop year and cultivars is expected because different growing conditions including environmental factors (soil, weather, oxidation stress) and *in vivo* condition of the crop (enzymes activity, metabolism rate) may result in variation from year to year for each cultivar. Therefore, effects of crop year and cultivar may not be discussed in depth. Statistical results indicating the significance of the effects of radiation, year, cultivar and the interactions for each variable are listed in Appendix A (Table 23 - 38).

PROXIMATE ANALYSIS

Proximate composition of the two soybean cultivars: Vinton 81 and IA2032LS, from crop years 2003 and 2004 are shown in Table 14 on a 13% moisture basis. No significant of radiation effect on the moisture, protein, oil and fiber of the soybeans was found. The results indicate that the composition of soybeans was highly dependent upon the cultivar and crop year of the soybeans, which is to be expected as growing conditions vary from year to year. Studies have shown that there is an effect of variety, crop year and location on the isoflavone composition of soybeans (Wang and Murphy 1994). Both cultivars from 2003 (10.28% and 10.99% respectively) had higher moisture content than cultivars from 2004 (9.30% and 10.58% respectively), and IA2032LS had slightly higher moisture than Vinton 81 across both years. Both cultivars were considered high protein but variation within each cultivar can be observed depending on its harvesting year. Statistical analysis shows that the effect of crop year on the moisture, protein, oil and fiber contents in these soybeans was strongly dependent on the cultivar (year:variety $p<0.05$). Vinton 81 from 2003 (average of 38.88%) had higher protein content than IA2032LS from the same year (average of 36.45%) while in 2004, IA2032LS (average of 38.98%) had higher protein content than Vinton 81 (average of 38.16%). A similar case was found for oil content of both cultivars, IA2032LS had a higher oil content than Vinton 81 in both crop years but oil content in both cultivars from 2003 were generally higher than cultivars from 2004. The oil content for each cultivar also varied for both years where IA2032LS was higher in 2003 (average of 19.87%) than in 2004 (average of 17.74%). A long-term crop rotation study revealed that environmental factors and putative changes in soil ecology could affect seed protein and oil content (Bennett 2005). Variation within a cultivar from different year may also due to changes in enzyme activities (desaturase) in synthesizing fatty acids, influenced by both temperature and light quality (Cheesbrough 1989; Britz and Cavins 1993). There is no fiber content difference between cultivars in 2003 (average of 4.50% for both) but Vinton 81 (average of 4.70%) has higher fiber content than IA2032LS in 2004 (average of 4.59%).

Table 14. Soybean Compositions of Vinton 81 and IA2032LS

Crop Year	Cultivar	Moisture	Protein*	Oil*	Fiber*
2003	Vinton 81	10.28a	38.88a	17.79a	4.50a
	IA 2032 LS	10.99b	36.45b	19.87b	4.50a
2004	Vinton 81	9.30c	38.16c	17.41c	4.70b
	IA 2032 LS	10.58d	38.98a	17.74a	4.59c

* Data are reported as % on a 13% moisture basis.

**Means within a column with different letters (a,b,c) are significantly different at $p<0.05$ ($n = 16$).

VITAMIN E ANALYSIS

Only dry whole soybeans from crop year 2003 were analyzed for vitamin E content. The Vinton 81 cultivar (average of 6.17 IU/100g) had a slightly higher vitamin E level than the IA2032LS cultivar (average of 5.26 IU/100g). A similar trend of radiation effect was observed in both cultivars where vitamin E level slightly decreased after radiation at 1 kGy and remained fairly constant at higher doses (5 and 10 kGy). However, the differences due to the effect of radiation as well as within both cultivars are not significant ($p>0.05$). Simontacchi's study (1993) suggested that α-tocopherol content in soybeans is physiologically adjusted as a response to conditions of oxidative stress due to environment (Simontacchi and others 1993).

ISOFLAVONE ANALYSIS

Preliminary study on isoflavone content of e-beam treated soybeans was done on cultivars harvested in 2003. Since isoflavones have been shown to be strongly linked to antioxidant activity (Rice-Evans and others 1996), these data were compared to antioxidant capacity of soybeans influenced by radiation in this study. Radiation did not significantly affect the total isoflavone content as well as the total genistein of the soybeans but significantly affected the total daidzein and glycitein content (Figure 9). Total daidzein and glycitein were increased at 10 kGy (averages of 591.75 μg daidzein/g and 113.75 μg glycitein/g) compared to the non-irradiated soybeans (averages of 584.75 μg daidzein/g and 109.5 μg glycitein/g). The total isoflavone content of the soybeans was different depending on the cultivars (Table 15). IA2032LS had higher total isoflavone content than Vinton 81 cultivar (averages of 1443.5 μg/g and 1158.0 μg/g respectively). Also, IA2032LS cultivar had higher content of daidzein, genistein and glycitein than the Vinton 81 cultivar as shown in Table 15.

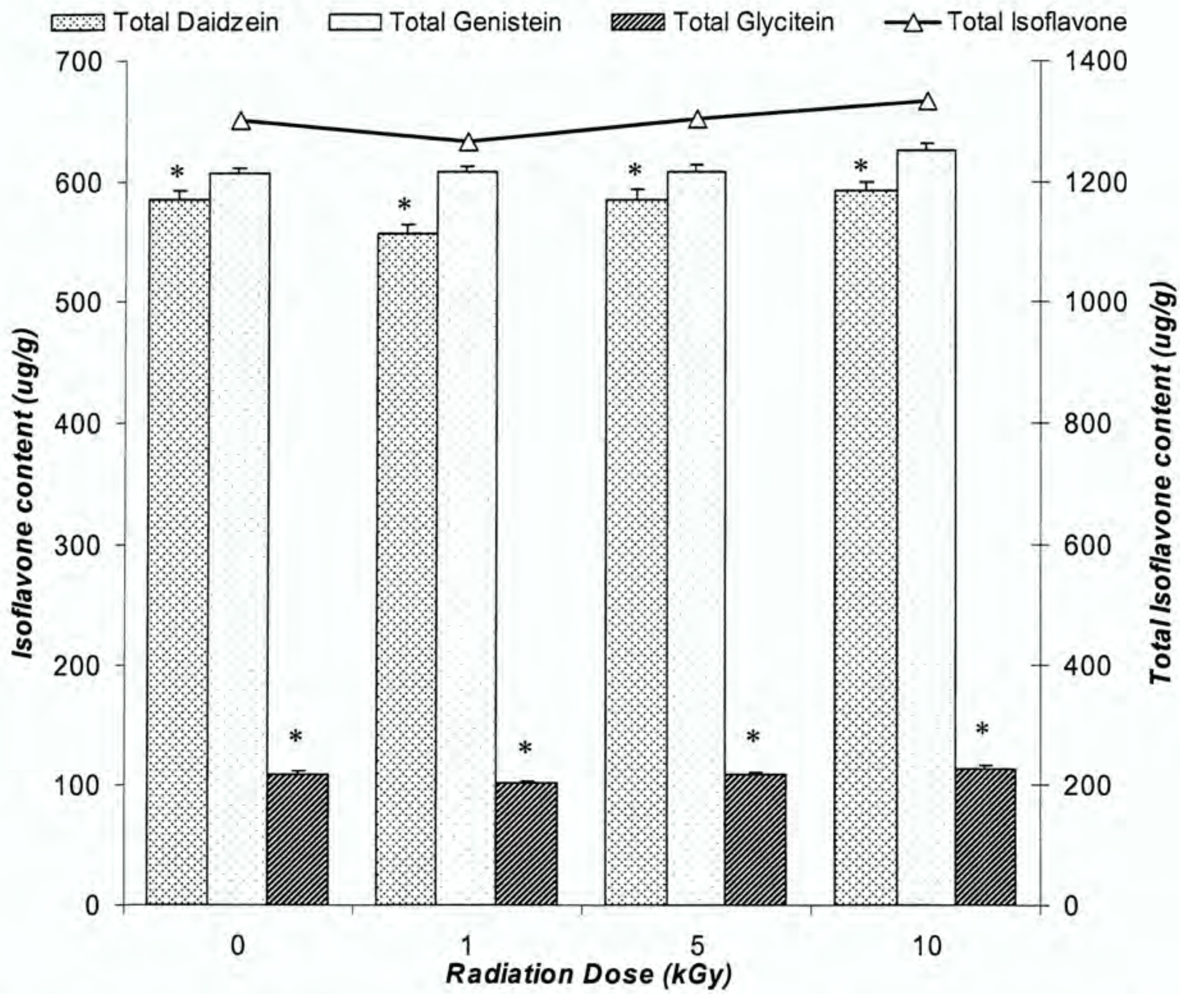


Figure 9. Influence of e-beam irradiation at 0, 1, 5, 10 kGy on total isoflavone and its isomers (daidzein, genistein, glycitein) (μg/g) of whole dry soybeans. *Note:* Error bar (⊤) indicates standard error for means of 2 replicates and 2 cultivars (n = 4); ' * ' indicates significant radiation effect ($p<0.05$).

Table 15. Isoflavone content of e-beam irradiated whole dry soybeans affected by cultivar (Vinton 81 and IA2032LS).

Cultivar	(μg isoflavone/g) *			
	Total Daidzein	Total Genistein	Total Glycitein	Total Isoflavone
IA2032LS	613.00 ± 30.57^a	717.00 ± 9.81^a	113.5 ± 10.30^a	1443.5 ± 46.46^a
Vinton 81	546.63 ± 10.78^b	508.13 ± 11.29^b	103.25 ± 3.48^b	1158.0 ± 24.58^b

* Means (± standard deviation) within a column with different letters (a, b) are significantly different at $p<0.05$ (n = 8).

ANTIOXIDANT CAPACITY

There was no significant effect of irradiation on the antioxidant capacity of the e-beam surface irradiated whole dry soybeans. However, there was a significant interaction between the crop year and cultivar effect (year:variety $p<0.001$), where the antioxidant capacity of the soybeans strongly depended on its cultivar and year harvested (Figure 10). There was no difference in antioxidant capacity for both cultivars in 2003 (average of 1.22 μg/mg). However, significant cultivar effect was observed in 2004: IA2032LS (average of 1.52 μg/mg) had higher antioxidant capacity than Vinton 81 (average of 0.92 μg/mg). This was comparable to results found in a recent study by Wilson and others (2004a) that antioxidant capacity is lower in Vinton 81 than IA2032LS. Different compounds including vitamin E, isoflavones and other natural phenolic compounds that are present in soybeans may serve as antioxidant. However, only vitamin E and isoflavones were analyzed in this study. As shown in both analyses, antioxidant compounds may vary from cultivar to cultivar as well as year to year. The antioxidant capacity highly depended on the variety of soybeans itself, the growing condition as well as storage conditions of the soybeans (Wang and Murphy 1994; Hoeck and others 2000; Lee and others 2003).

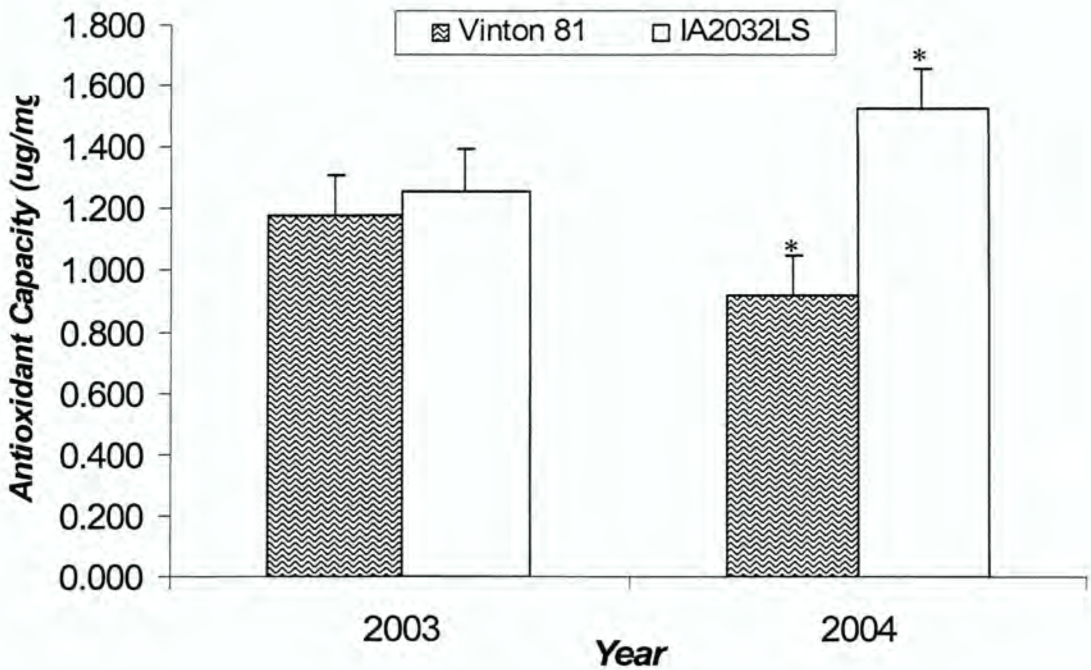


Figure 10. Antioxidant capacity in ug/mg of e-beam irradiated whole dry soybeans due to interaction of cultivar (Vinton 81, IA2032LS) and crop year (2003, 2004) effects. *Note:* Error bar (⊤) indicates standard error for means (n = 64); ' * ' indicates significant difference ($p<0.05$).

THIOBARBITURIC ACID (TBA), PEROXIDE AND FREE FATTY ACIDS (FFA) ANALYSES

Measurement of thiobarbituric acid reactive substances (TBARS) using AldeSafe™ Kit-STD Assays showed that irradiation did not significantly affect the malonaldehyde content of the dry whole soybeans but significant year and cultivar effects were observed (Figure 12). Data showed that average malonaldehyde content across both Vinton 81 and IA2032LS cultivars from 2003 (5.06 nmol/ml) was higher than the cultivars from 2004 (2.43 nmol/ml). The IA2032LS had significantly higher malonaldehyde content than the Vinton 81 cultivar (4.09 nmol/ml and 3.40 nmol/ml respectively). Although vitamin E and isoflavones are speculated to be responsible for the antioxidant capacity of the soybeans, both data cannot be correlated with the antioxidant capacity due to analyses being done at different period of study. Variation in malonaldehyde content of soybeans may due to the variation in oil content of the soybean, as shown in Table 14, that IA2032LS cultivar had the higher average oil content and higher average malonaldehyde content than the Vinton 81 cultivar (Figure 12). Therefore, both cultivar and crop year of soybeans played a significant role on soybean malonaldehyde content; the crops were grown at different time and in different condition. Priestley and Leopold (1979) found that unsaturation in whole soybean seeds did not change significantly during aging thus suggesting that oxidation of seed lipids may be unrelated to the process of seed aging (storage time). Storage conditions (time and temperature) also affected antioxidant capacity of soybean (Wilson and others 2004a).

There was no significant effect of irradiation, crop year or cultivar observed in lipid peroxide content of the e-beam irradiated whole dry soybeans. However, there was a significant interaction between crop year and cultivar ($p<0.05$) (Figure 13). The IA2032LS cultivar had higher peroxide content than Vinton cultivar in 2003, but the opposite case was observed in 2004. The IA2032LS cultivar from 2003 had the highest peroxide content compared to the Vinton 81 cultivar of 2003 and both cultivars in 2004 (Vinton 81: average of 0.041 meq/kg; IA2032LS: average of 0.019 meq/kg). Measurement of peroxide content of the soybeans was inconsistent in indicating levels of lipid oxidation due to the lack of sensitivity in this method where peroxide content of some samples were below detectable limit, which were report as 0 meq/kg.

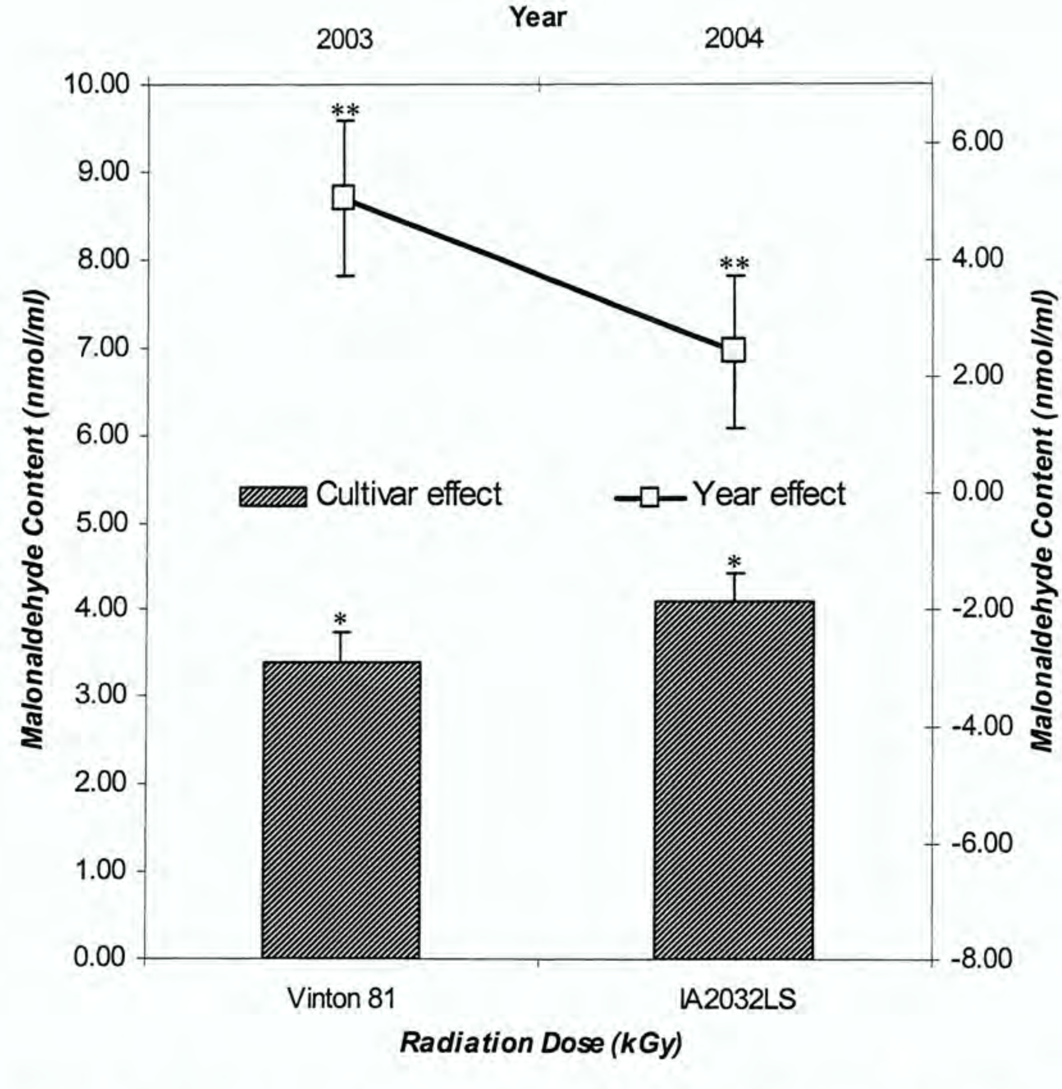


Figure 12. Influence of crop year (2003, 2004) and cultivar (Vinton 81, IA2032LS) on the malonaldehyde content (nmol/ml) of e-beam surface irradiated soybeans. *Note:* Error bar (┬) indicates standard error for means (n = 48); ' * ' indicates significant cultivar effect ($p<0.05$); ' ** ' indicates significant year effect ($p<0.05$).

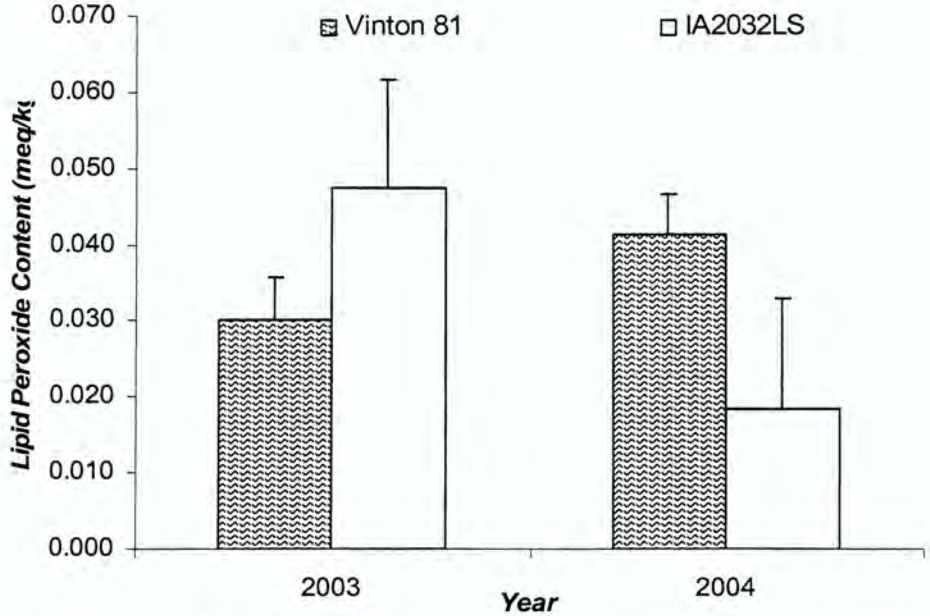


Figure 13. Interaction effects of crop year (2003, 2004) and cultivar (Vinton 81, IA2032LS) on the lipid peroxide content (meq/kg) of e-beam surface irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 24).

Fatty acid content was measured as percent oleic acid. There were significant effect of radiation, year and cultivar on the e-beam treated soybeans ($p<0.05$). However, significant interaction effects between radiation and cultivar as well as year and cultivar were observed. The effect of radiation on free fatty acid content of the dry whole soybeans was highly dependant on the cultivar (irradiation:year $p<0.05$) (Figure 14). An increase in percent oleic acid was observed for the 10 kGy e-beam irradiated Vinton 81 cultivar comparing the control and lower dose irradiated soybeans (0, 1, 5 kGy). However, a decrease was observed in the IA2032LS cultivar comparing the control soybeans and the e-beam treated soybeans (1, 5, 10 kGy). Variation of free fatty acid content also significantly depended on crop year and cultivars (year:cultivar $p<0.05$), which is expected due to the different growing condition and nutritional stress level of the crop during each crop year. The free fatty acid content in Vinton 81 cultivar from 2004 was significantly higher than the IA2032LS cultivar but no difference was observed in both cultivars from 2003 (data was not shown). It is also comparable to the malonaldehyde and peroxide content data in that IA2032LS has a significantly higher free fatty acid content than Vinton 81 ($p<0.05$). Higher amount of malonaldehyde, peroxides and free fatty acids that were found in IA2032LS could due to its higher oil content compared to Vinton 81 (Table 14). However, the majority of the samples had very low levels of free fatty acids that were at the detectable limit of this method, and therefore, reported as less than 0.120% oleic acid.

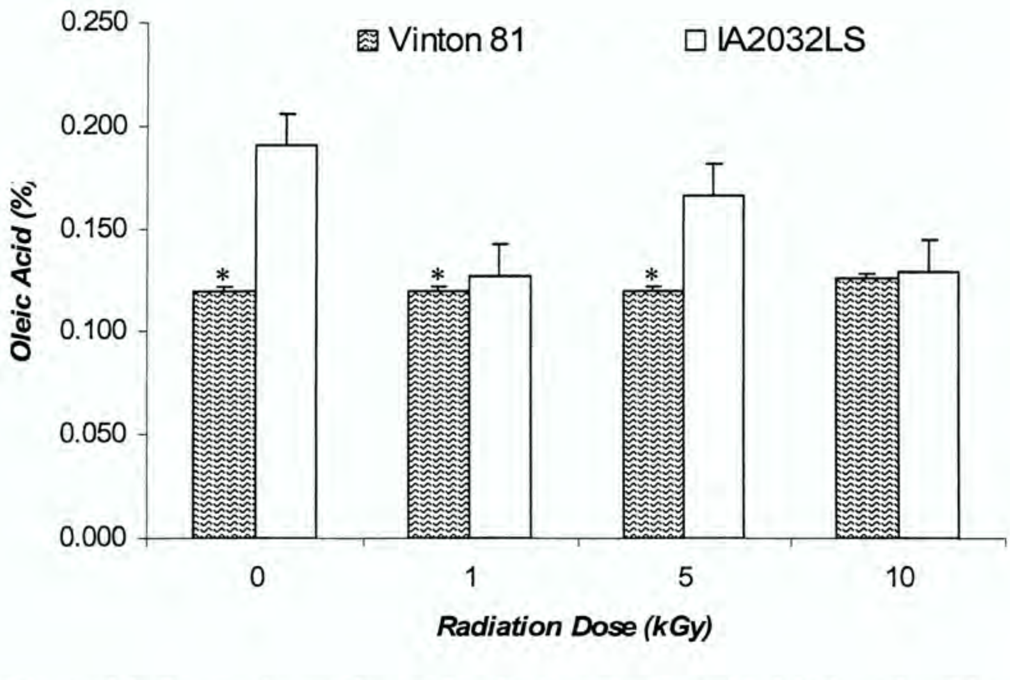


Figure 14. Interaction effects of radiation (0, 1, 5, 10 kGy) and cultivar (Vinton 81, IA2032LS) on the free fatty acid content measured as percent oleic acid (%) of e-beam surface irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 12). ' * ' indicates data below detectable limit and reported as less than 0.120%.

MICROBIAL ANALYSIS

The e-beam treated soybeans were analyzed for total aerobic, coliform, and salmonella bacteria and yeast and mold counts. No salmonella was found in any sample (Table 18). Coliform counts for Vinton 81 control (0 kGy) ranged from 0 to 8 CFU/g while no coliforms were found in the IA2032LS cultivar or in any of the irradiated soybeans. Results also show that e-beam surface irradiation did reduce the microbial load of the whole soybeans for both cultivars where the controls (0 kGy) had the highest total aerobic count/g (Vinton 81 ranged from 170 to 450 CFU/g and IA2032LS ranged from 50 to 170 CFU/g). Radiation reduced the total aerobic count to 0-280 CFU/g at 1 kGy; 0-20 CFU/g at 5 kGy and 0-10 CFU/g at 10 kGy (Table 18). Some yeasts and molds were isolated (0 to 18 CFU/g) and *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 (Table 13) (i.e. they could fly on Shuttle and International Space Station missions). No specific microbiological standard for dry whole soybeans from the field was found. In contrast to typical microbiological quantitative range of commercial "normal" cereal grains (total aerobic count is 10^2-10^6 /g, coliform count is 10^2-10^4 /g, yeasts and molds: 10^2-10^4 /g) (Deibel and Swanson 2001), NASA specification is 2 log tighter for the total aerobic count and coliform count, 1 log tighter for yeast and mold counts than the upper range of regular standard.

Table 18. Microbial study on e-beam irradiated soybeans at 0, 1, 5, 10kGy

	Vinton 81				IA2032LS			
Radiation Dose (kGy)	0	1	5	10	0	1	5	10
Total Aerobic count	170-450	0-280	0-10	0	50-170	20-30	0-20	0-10
Coliform	0-8	0	0	0	0	0	0	0
Salmonella	0	0	0	0	0	0	0	0
Yeast and Mold	0-20	0-36	0-10	0-14	8-32	0-2	0-6	0

** All counts are reported in CFU/gram.

* Ranges shown are crop year averages (n = 20).

GERMINATION STUDY

Germination ability of the control and treated soybeans was studied by evaluating the percent of normal, abnormal germination and dead seed. E-beam radiation significantly affected the growth of the soybean seed (Figure 15). The seeds were able to grow normally for the control (0 kGy), 1 kGy and 5 kGy but the height of the sprouts was affected. The non-irradiated soybeans were able to grow up to an estimated range of 15-17 cm; soybeans irradiated at 1 kGy were able to grow to an estimated height of 5-7 cm while seeds irradiated at 5 kGy grew to an estimated height of 2-3 cm. Most of the 10 kGy-irradiated soybeans appeared to be moldy dead seeds after 8 days of incubation. The influence of radiation and cultivar on the germination of soybeans are shown in Figures 16 and 17. Figure 16 shows the average of germination data across both years (2003 and 2004) for Vinton 81 while data for IA2032LS is shown in Figure 17. The germination ability of irradiated seeds was significantly decreased in both cultivars ($p<0.05$). Normal germination decreased significantly from 87.6% and 46.9% respectively for the control seeds (0 kGy) to 0 and 0.5% respectively for the 10 kGy irradiated seeds (Figures 16 and 17). As radiation dose increased, most of the seeds experienced abnormal germination or either loss of germination ability (dead seeds). Lesions in nucleic acids, due to radiation, played an important role in deterioration of seed (Roberts and Osborne 1973). The effect of radiation on the normal germination of soybeans strongly depended on the cultivar or crop year of the soybeans (radiation:year $p<0.0024$; radiation:cultivar $p<0.0001$). Radiation reduced normal germination of the e-beam treated soybeans at a higher rate in 2004 than in 2003 (Figure 18a) and for Vinton 81 cultivar (Figure 18b).

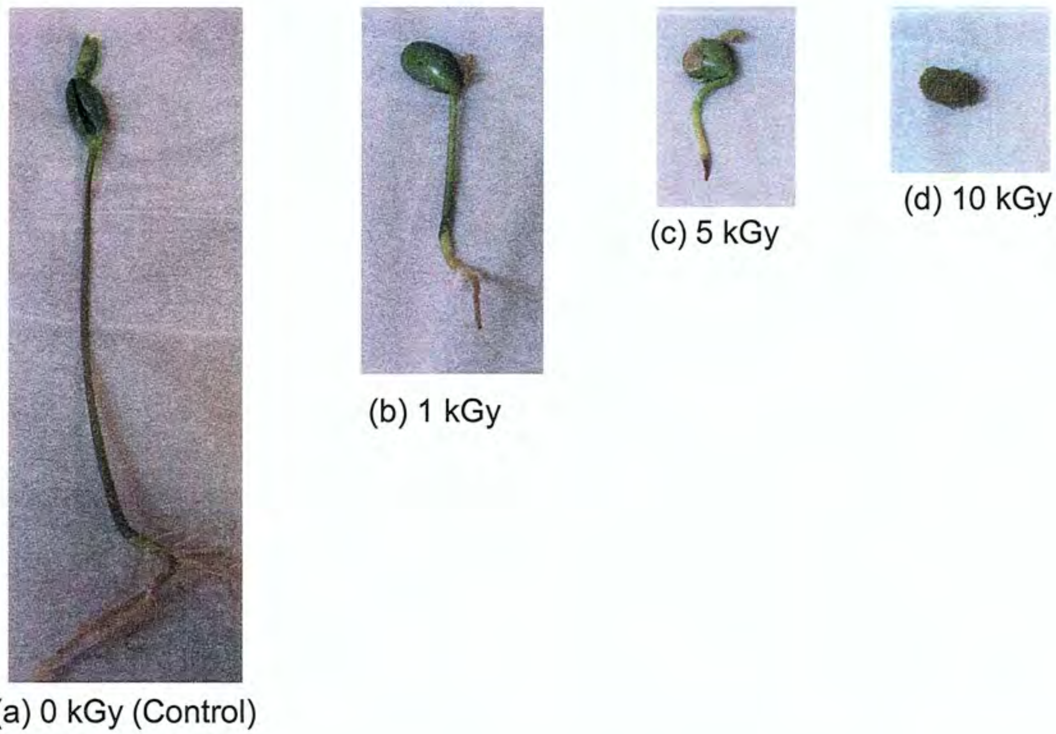


Figure 15. Influence of e-beam radiation at dose levels of 0, 1, 5, 10 kGy on seedling vigor of soybeans after 8 days germination at 25 °C, 95% humidity (a) control seed (0 kGy): 15 - 17 cm; (b) 1 kGy: 5 - 7 cm; (c) 5 kGy: 2 - 3 cm; (d) 10 kGy: 0 cm.

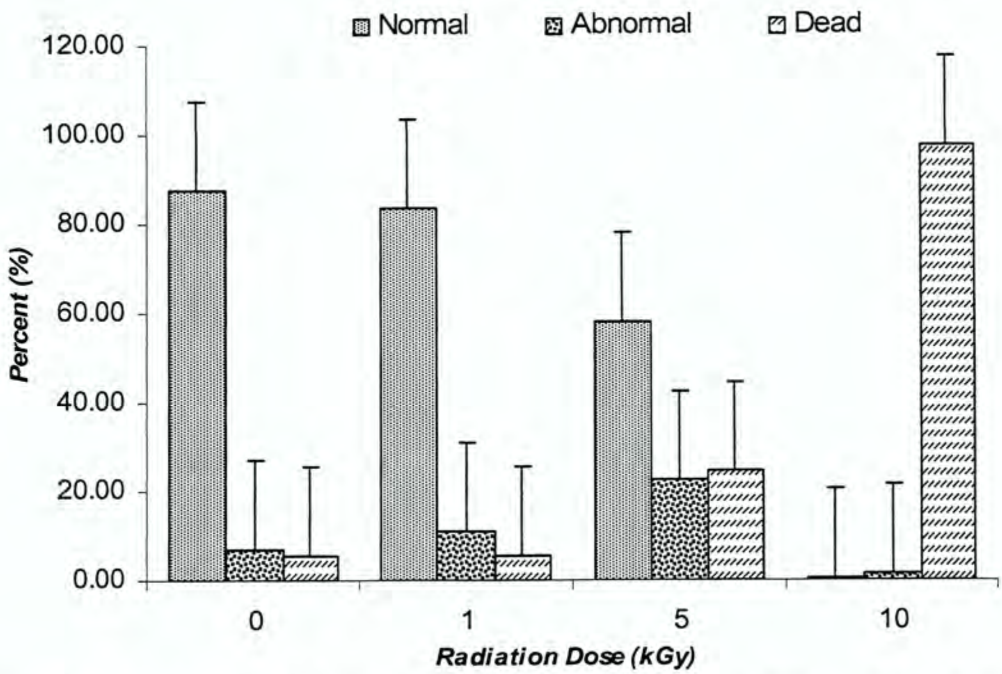


Figure 16. Germination study of e-beam irradiation soybean seeds (Vinton 81) at 0, 1, 5, 10 kGy. *Note:* Error bar (⊤) indicates standard error for means (n = 8).

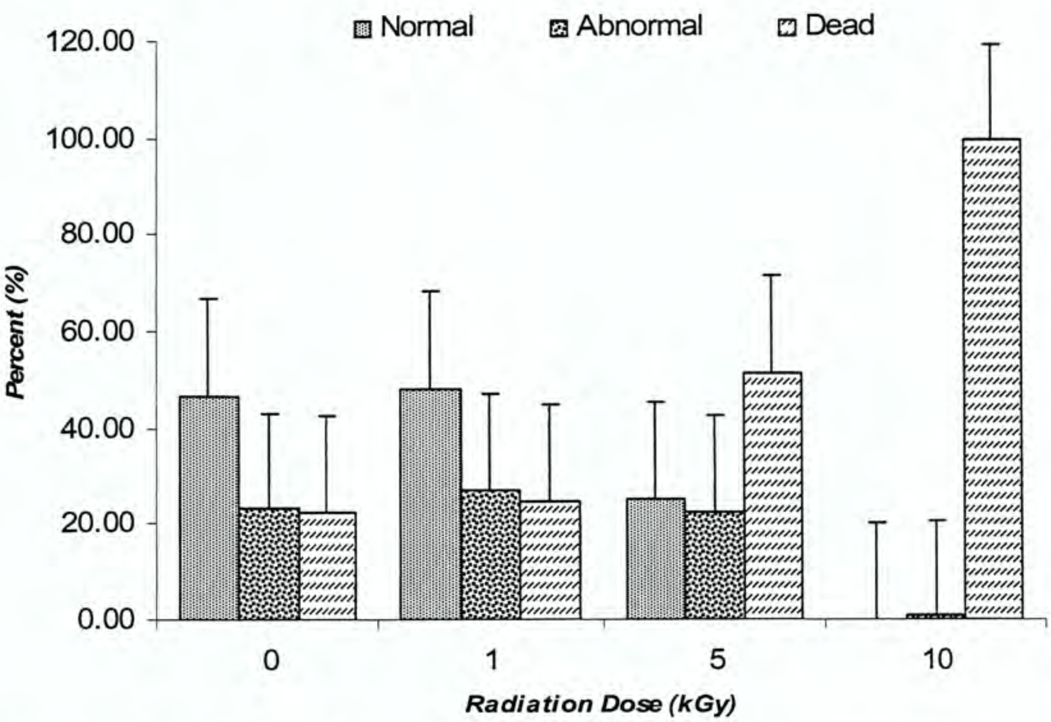
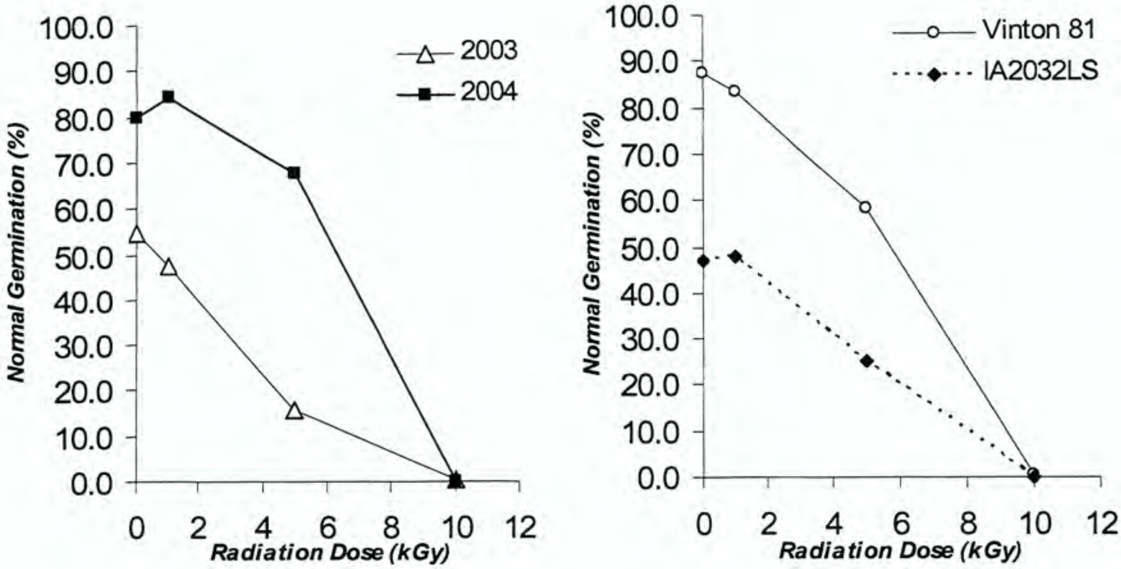


Figure 17. Germination study of e-beam irradiation soybean seeds (IA2032LS) at 0, 1, 5, 10 kGy. *Note:* Error bar (⊤) indicates standard error for means (n = 8).



(a) (b)

Figure 18. Interactions effects between (a) radiation and year, (b) radiation and cultivar on normal germination of e-beam irradiated soybean.

Age of the seeds also significantly affected germination ability in that seeds from 2004 (average of 97.8% and 62% for each cultivars) had higher percent of normal germination than seeds from 2003 (average of 77.5% and 31.8% for each cultivars) (Table 19). Comparing the two different cultivars, Vinton 81 (average of 87.65% normally germinated) had higher germination ability than IA2032LS (average of 46.90% normally germinated). These variations in germination ability may due to the genetic, composition (nutrient source) and growth condition differences between the two cultivars. One of the variations within a cultivar was the presence of stone beans (hard shell beans that do not absorb water) in the IA2032LS cultivar from 2003, with the lowest percentage of normal germination (31.8%) among all (Table 19).

Scientists also believe that aging of dried seeds in storage is accompanied by changes in membranes and nucleic acids (Heydecker 1972; Robert and Osborne 1973; Villiers 1973). Alterations in seed membranes during aging are due to peroxidation and autooxidation of polyunsaturated fatty acids that leads to free radical formation and possible peroxidative damage to lipids, proteins, and nucleic acids. Changes in the membranes of aged seeds may enhance leakage of solutes during seed imbibition, indicating inability of seeds to re-form coherent membranes during rehydration, thus resulting in loss of vigor and lack of germination (Ching and Schoolcraft 1968; Parrish and Leopold 1978; Priestley and Leopold 1979). Soybeans treated at 10 kGy were softer and had some broken cotyledons after soaking compared to the control soybeans. Wilson (2005) observed that soybeans treated at 10 and 30 kGy using gamma ray and e-beam were visually damaged after soaking. Soybeans irradiated at 30 kGy lost up to 4% solid into the soaking water. Harman and Mattick (1976) studied accelerated aging of pea seeds and found that the decrease in germination rate was paralled by a pronounced decline in linoleic (C18:2) and linolenic (C18:3) acids whereas the saturated and monoenoic fatty acids remained unchanged.

Percent of abnormal beans decreased drastically at 10 kGy (averages of 1.8% and 0.8% for each cultivar) as most of the seeds were dead due to the effect of irradiation (Figures 16 and 17). A slightly higher percentage of abnormal beans was found in IA2032LS. Hard seeds were classified as dead seeds after 8 days of germination. Percent dead seeds increased as the radiation dose increased, from averages of 5.38% and 22.38% for each cultivar at 0 kGy to the highest averages of 97.75% and 99.25% at 10 kGy. Wilson and others (2005) and Wilson (2004) also found that e-beam or gamma ray irradiation at 10 kGy or 30 kGy caused loss of ability of the seed to germinate. High levels of irradiation negatively affected the physiology of the seedlings, hence, reduced its germinating ability (Buck and others 2003). In contrast to the normal germination data, IA2032LS cultivar had higher percentage of dead seeds at control (0 kGy) (average of 22.38%) compared to the Vinton 81 cultivar (average of 5.38%) (Figures 16 and 17). Crop year of the soybeans also decreased its germination ability, where both cultivars (Vinton 81 and IA2032LS) had higher percent of dead seeds for harvest from 2003 (average of 10.50% and 41.25% respectively) than 2004 (average of 0.25% and 3.50% respectively) (Table 19). The higher percentage of dead seeds in IA2032LS cultivar from 2003 may also due to the presence of stone beans.

Table 19. Germination study of e-beam irradiated soybeans at 0, 1, 5, 10 kGy (Vinton 81, IA2032LS) for 2003 and 2004.

	Radiation Dose (kGy)	*Percent (%)*			
		Vinton 81		*IA2032LS*	
		2003	*2004*	*2003*	*2004*
Normal	0	77.50	97.80	31.80	62.00
	1	72.50	94.50	22.50	73.80
	5	31.00	85.30	0.00	50.30
	10	1.00	0.00	0.00	0.00
Abnormal	0	12.00	2.00	27.00	19.50
	1	18.00	3.75	29.75	24.50
	5	33.00	12.00	10.50	34.25
	10	3.50	0.00	0.00	1.50
Dead	0	10.50	0.25	41.25	3.5
	1	9.50	1.75	47.75	1.75
	5	46.00	2.75	89.50	13.00
	10	95.50	100.00	100.00	98.50

* Each value is the mean of four replicates of 100 seeds.

WATER ABSORPTION

Water absorption of the soybeans after 10 hours of soaking is shown in Figures 19 and 20. Considering solely the radiation effect, water absorption of the beans significantly increased as the radiation dose level increased especially at 10 kGy for both cultivar ($p<0.01$) (Figure 19). The water absorption ranged from 1% to 7% for the control beans (0 kGy) and 10 kGy e-beam treated soybeans respectively. This maybe due to seed coat and cellulose damage caused by radiation treatment that allowed more water absorption. Considering the individual effects (cultivar and crop year), Vinton 81 cultivar had higher percent of water absorption than IA 2032LS cultivar ($p<0.001$) after soaking while soybeans from 2004 had higher percentage of water absorption than soybeans from 2003 ($p<0.001$) (Figure 20). Vinton 81 had higher water absorption in 2003 due to the presence of stone beans in IA2032LS cultivar, which failed to absorb water. This problem could be improved by removing the seed coat, allowing water permeability of the seed. Each cultivar also behaved differently in each year (year: cultivar $p<0.0001$) where IA2032LS from 2004 had higher water absorption than IA2302LS from 2003 due to the absense of the 'hard coat' seeds. Different growing conditions during each year may have affected the water absorption ability of the crop seeds. Mullin and Xu (2001) showed that water uptake ratio of the seeds as well as occurrence of hard seeds correlated to the differences in hemicellulose content of the seed coat fractions. It also had been shown that resistance to water absorption was consistent with the calcium content in the seed coat (Saoi 1976). Fluctuation of environment temperature during development of the seeds may increase the creation of stone beans.

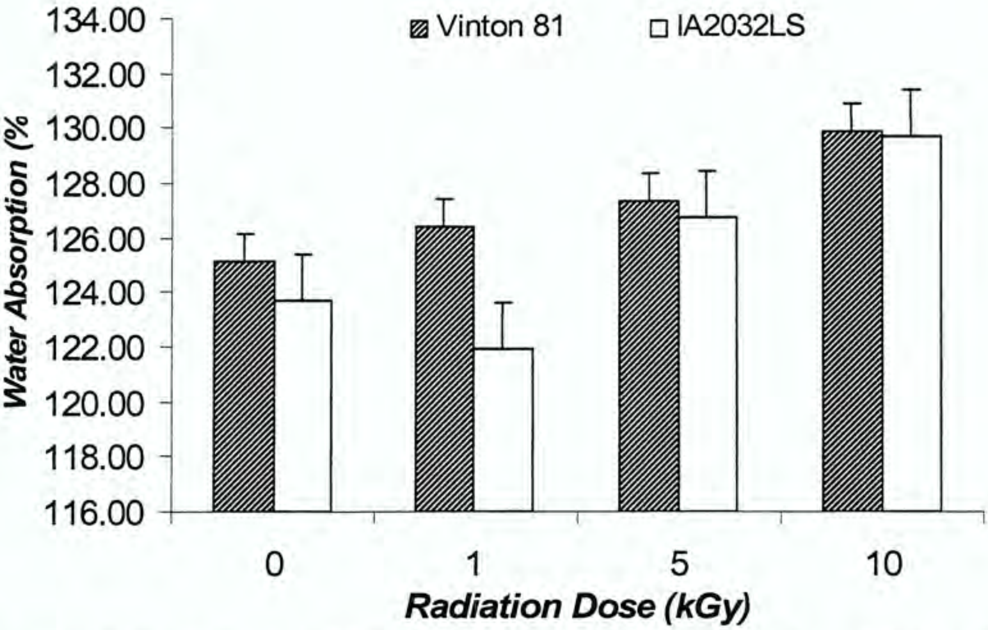


Figure 19. Water absorption in percent of e-beam irradiated soybeans (Vinton 81, IA 2032LS) at radiation doses of 0, 1, 5, 10 kGy. *Note:* Error bar (⊤) indicates standard error for means (n = 8).

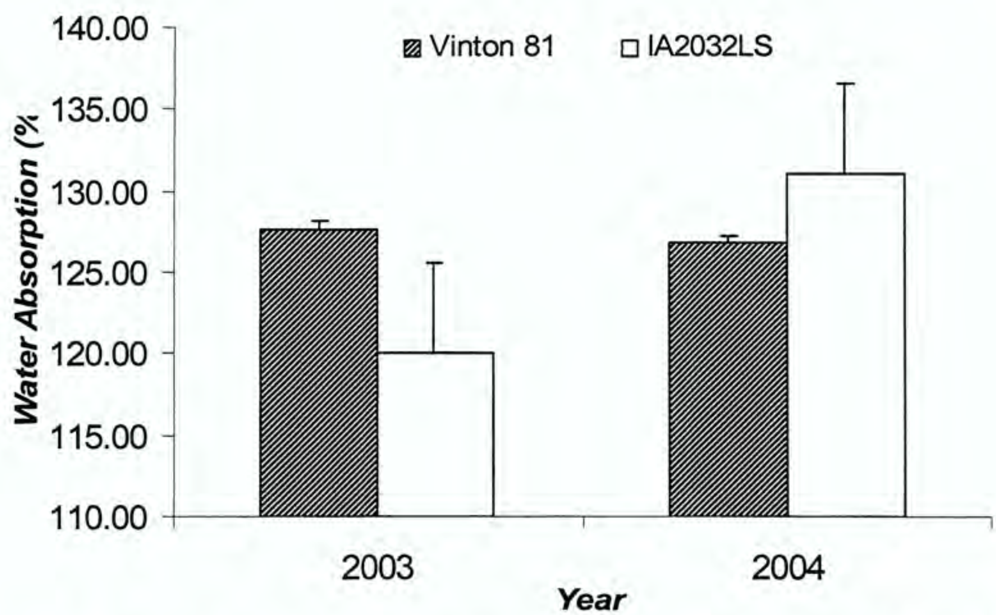


Figure 20. Interaction effects between year (2003, 2004) and cultivar (Vinton 81, IA2032LS) on the water absorption of the e-beam irradiated soybeans after 10 hours soaking. *Note:* Error bar (⊤) indicates standard error for means (n = 16).

SOYMILK AND TOFU YIELD

Percent yield of soymilk was calculated based on the initial weight of the dry beans. Overall, soybeans from 2004 (977.49%) had a higher percent yield of soymilk as compared to soybeans from 2003 (957.42%) (Figure 21) ($p<0.05$). No significant effect of radiation and cultivars on the percent yield of soymilk was observed. As suggested by Gayler and Sykes (1985), nutritional stress during seed development of the soybeans affects the storage proteins. Environmental factors may have played a significant role in affecting the storage protein of the seeds thus resulting in soymilk yield variation.

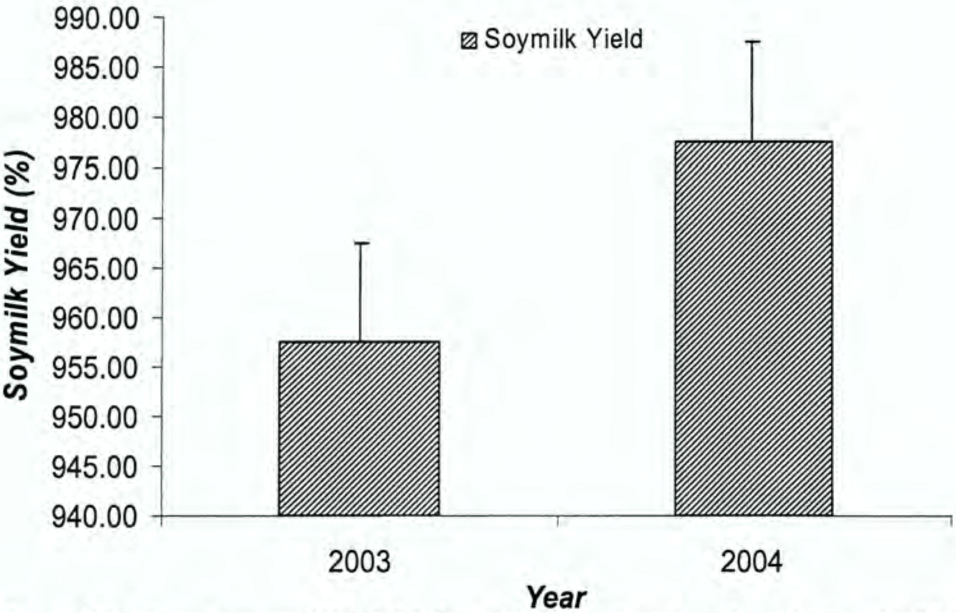


Figure 21. Percent soymilk yield from E-beam irradiated soybeans in 2003 and 2004. *Note:* Error bar (⊤) indicates standard error for means (n = 32).

Percent tofu yield was calculated two ways: 1) based on the initial starting amount of soymilk by weight, and 2) based on of the initial starting amount of dry soybeans by weight (Table 20). Both calculations demonstrated similar results - the soy proteins were extracted into the soymilk and in turn were able to be coagulated by the calcium sulfate dihydrate (coagulant). Irradiation did not affect °Brix of soymilk or tofu yield at radiation doses as high as 10 kGy, but lower tofu yield was found in soybeans irradiated at 30 kGy (Wilson and others 2005). Significant cultivar effect was observed - Vinton 81 (average of 189.50% on dry beans basis) had a higher percent tofu yield than IA2032LS (average of 169.20% on dry beans basis) (Table 20) ($p<0.05$). This may be due to Vinton 81 cultivars yielded a higher °Brix in soymilk. On a dry beans basis, the higher protein content of Vinton 81 contributed to the higher tofu yield. No significant difference in tofu yield was found when calculated on the soymilk used basis. However, there was no crop year effect on the tofu yield for both calculations. Textural changes due to cultivar and irradiation are noted later in this report and previously by Wilson and others (2005).

Table 20. Percent tofu yield of e-beam irradiation soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

Radiation Dose (kGy)	Tofu Yield (%)[1]			
	Vinton 81		*IA2032LS*	
	2003	2004	2003	2004
0	24.01 ± 1.19	25.49 ± 1.06	22.40 ± 2.30	22.02 ± 0.83
1	23.69 ± 1.51	24.07 ± 1.01	22.36 ± 1.26	22.10 ± 0.94
5	23.04 ± 2.31	24.71 ± 0.42	21.64 ± 1.38	22.17 ± 0.52
10	24.76 ± 0.46	25.17 ± 2.68	22.09 ± 0.89	22.56 ± 1.35
Average	24.37 ± 0.57		22.17 ± 0.18	
	Tofu Yield (%)[2]			
0	187.12 ± 11.06	196.82 ± 10.74	175.10 ± 23.05	170.13 ± 6.84
1	184.51 ± 8.15	189.02 ± 9.99	153.82 ± 21.42	172.90 ± 9.57
5	183.50 ± 11.66	191.86 ± 6.69	165.96 ± 11.41	171.56 ± 5.10
10	188.86 ± 3.33	194.29 ± 26.21	171.63 ± 4.53	172.46 ± 10.18
Average	189.50 ± 11.97 *		169.20 ± 13.32 *	

[1]Percent tofu yield determined based on amount of soymilk used.

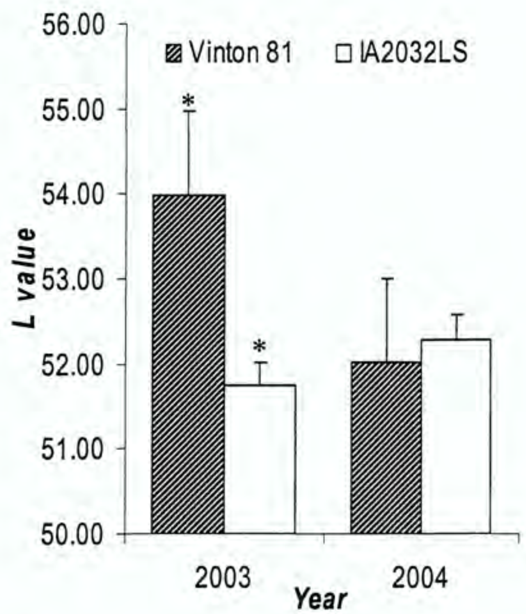
[2]Percent tofu yield determined based on amount of dry soybeans.

Note: All data shown are mean ± standard deviation. ' * ' indicates significant cultivar effect ($p<0.05$).

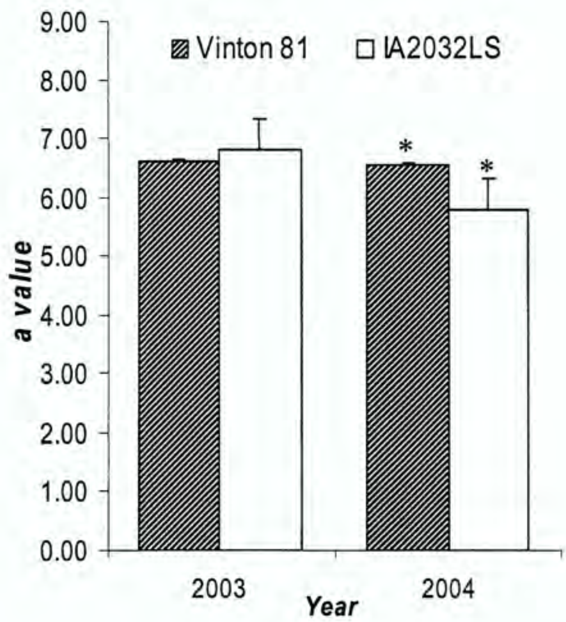
COLOR ANALYSIS OF
WHOLE SOYBEANS, SOYMILK, TOFU, OKARA AND WHEY

Color of Whole Soybeans

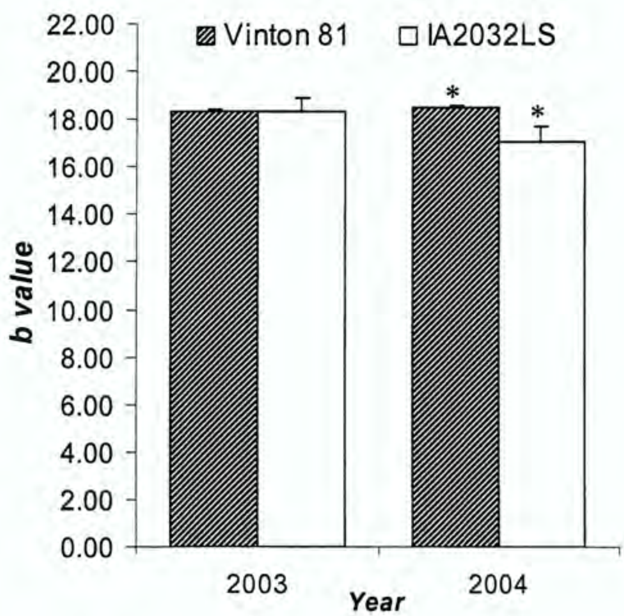
Color of irradiated whole soybeans was analyzed and reported as Hunter L, a, b values in Figures 22 (a), (b), (c). Radiation at 1, 5, 10 kGy did not significantly affect the physical appearance of the whole dry soybeans. There was a significant cultivar effect on lightness (L value) and significant year effect on the redness (+ a value) and yellowness (+ b value) of the whole dry soybeans ($p<0.05$). Vinton 81 cultivar generally had a lighter color than the IA2032LS cultivar. However, soybeans harvested in 2003 had more redness (+ a value) and yellowness (+ b value) than soybeans harvested in 2004. However, interaction effects between crop year and cultivar can be seen in lightness (L value), redness (+ a value) and yellowness (+ b value) of the soybeans ($p<0.001$). Vinton 81 cultivar had higher lightness (L value) than IA2032LS cultivar in 2003 but no difference in lightness (L value) in 2004 for two cultivars (Figure 22 a). IA2032LS cultivar harvested in 2004 had the lower redness (+ a value) and yellowness (+ b value) values than Vinton 81 cultivar but no difference in redness (+ a value) and yellowness (+ b value) of the whole soybeans in the 2003's harvest (Figures 22 b and 22 c).



(a)



(b)



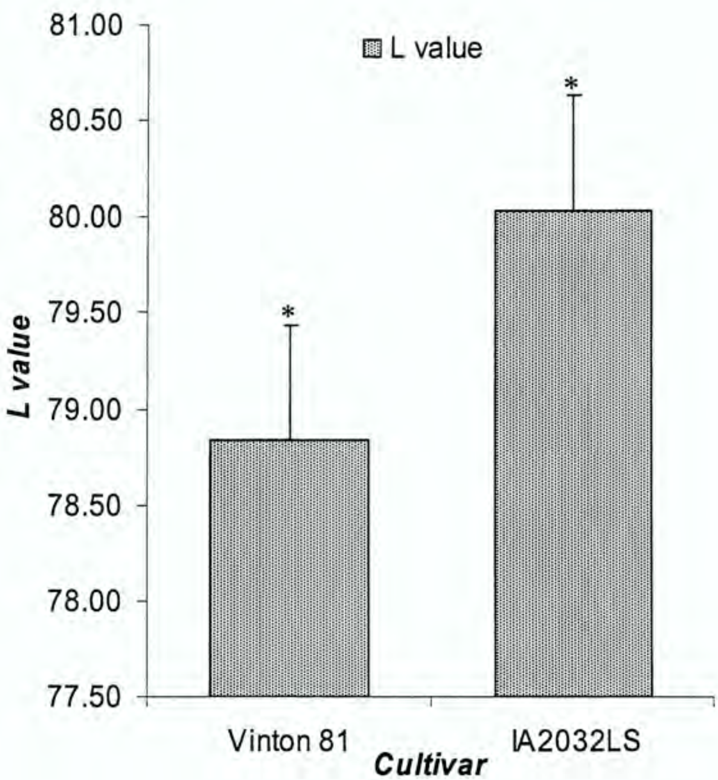
(c)

Figure 22. Interactions of year (2003, 2004) and cultivar (Vinton 81, IA2032LS) effects on (a) lightness (L value); (b) redness (+ a value); (c) yellowness (+ b value) of e-beam surface irradiated whole dry soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 48). ' * ' indicates significant difference between two means.

Color of Soymilk

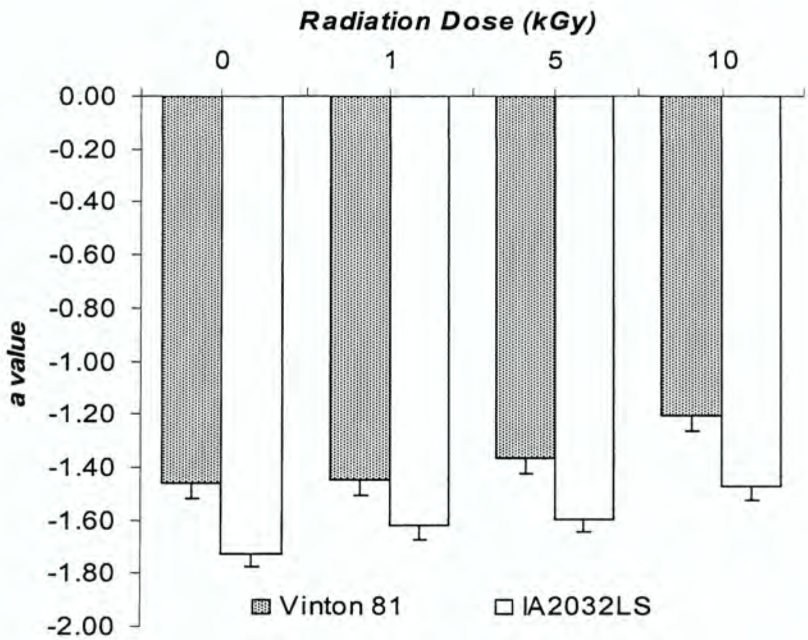
Color analysis of soymilk produced from E-beam irradiated soybeans is shown in Figures 23 (a), (b), (c). There was no significant effect of irradiation and crop year on the lightness (L value) of the soymilk. However, soymilk produced from the IA2032LS cultivar had higher L value than Vinton 81 cultivar (Figure 23 a). Significant color changes due to radiation effect were observed in the greenness (- a value) and yellowness (+ b value) of the soymilk (Figure 23 b & c). Radiation decreased the greenness (- a value) and yellowness (+ b value) of the soymilk. This may be due to the effect of radiation on the structure (double bonds) of pigments (such as chlorophyll) and polyunsaturated fatty acids in the soymilk. Unsaturated structures were being oxidized resulting in less greenness. There was significant cultivar effect on the greenness (- a value) and yellowness (+ b value) of soymilk, in that soymilk from Vinton 81 had less greenness (-a value) and less yellowness (+ b value) than soymilk from IA2032LS at all dose levels (Figures 23 a and 23 b). This may be due to the "oxidative" reaction of lipoxygenase on color pigments [e.g. bleaching of β-carotene and use of colorimetric method for lipoxygenase activity determination (Cha and others 2000; Anthon and Barrett 2001)] in Vinton 81 that reduced the greenness (- a value) and yellowness (+ b value) in comparison to the soymilk from the lipoxygenase-free IA2032LS. Soymilk produced from soybeans harvested in 2003 had more yellowness (+ b value) than soymilk produced from soybeans harvested in 2004 but it is expected to be different from year to year due to different environmental stresses (nutrient, oxygen, weather etc.) (Data was not shown).

Statistical analysis shows that there is interaction effects between radiation and year as well as year and cultivar on the yellowness (+ b value) of the soymilk. Interaction effects of radiation and year is shown in Figure 23 c but interaction between year and cultivar is not a major concern (data not shown). Consistent decrease in yellowness (+ b value) of soymilk can be seen in the IA2032LS cultivar. However, yellowness (+ b value) of the soymilk was increased at 1 kGy but decreased at higher dose level (5 and 10 kGy) (Figure 23 c). This could due to the environmental effects (nutrient source, weather) during the growing of soybeans in different years that caused inconsistent metabolism rate and enzyme activities, resulting in color differences of soybeans and later in the soymilk.

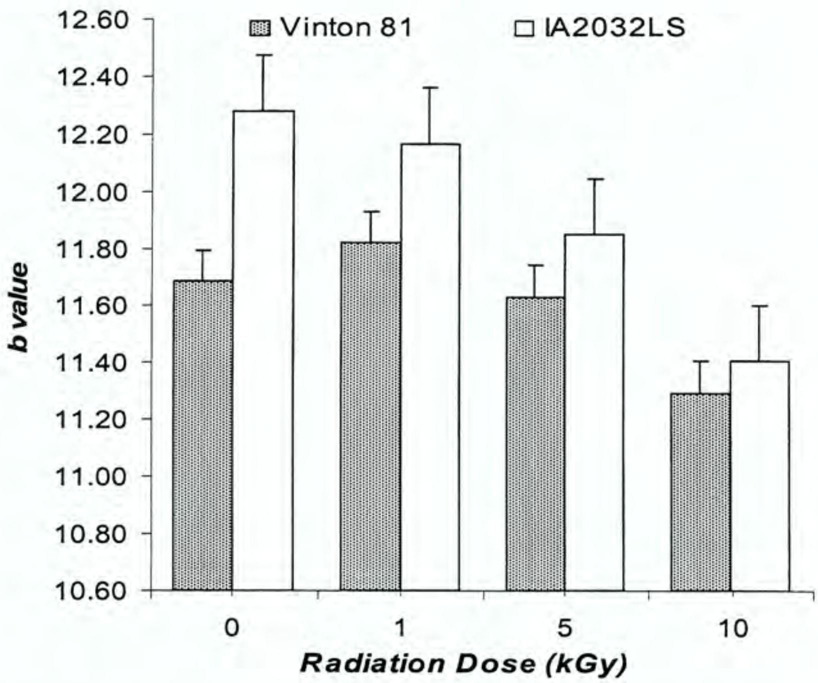


(a)

Figure 23 (a). Cultivar effect on the lightness (L value) of soymilk produced from e-beam irradiated soybeans (Vinton 81, IA2032LS). *Note:* Error bar (⊤) indicates standard error for means (n = 48). ‘ * ’ indicates significant difference between two means.



(b)

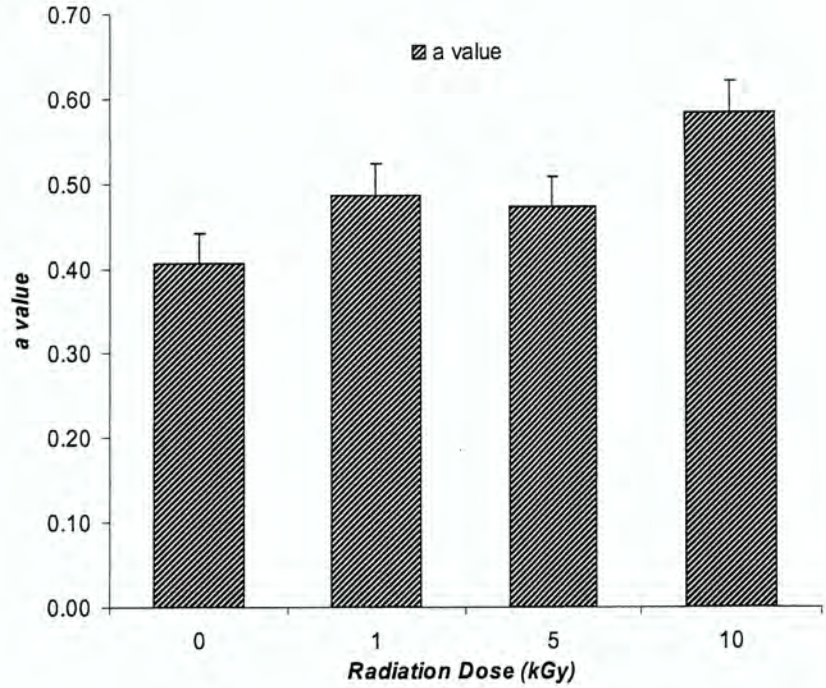


(c)

Figure 23 (b) and (c). Radiation and cultivar effects on greenness (- a value) (c) Interaction effects of radiation and cultivar on yellowness (+ b value) of soymilk from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy. *Note:* Error bar (⊤) indicates standard error for means (n = 24).

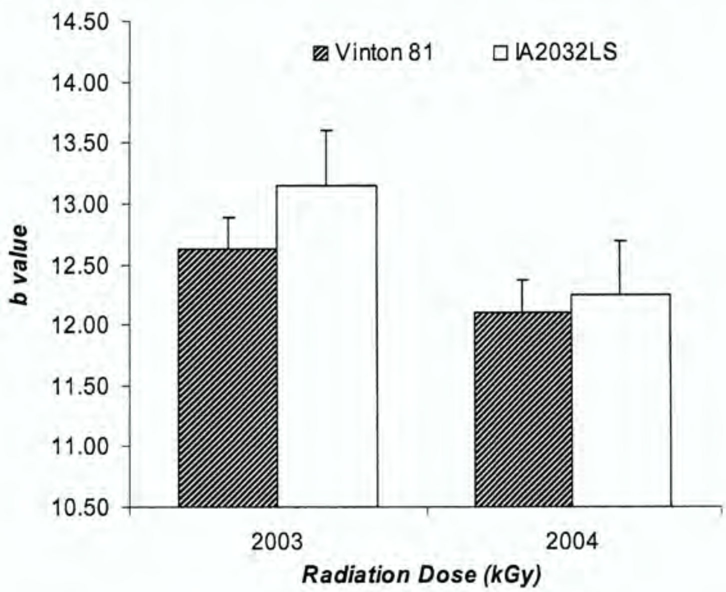
Color of Tofu

Figures 24 (a), (b) and (c) show the results for color of tofus made from e-beam irradiated soybeans at dose levels of 0, 1, 5, 10 kGy for both Vinton 81 and IA2032LS cultivars. Irradiation did not affect the lightness (L value) of the tofus but did affect its redness (+ a value) and yellowness (+ b value). Soybeans harvested in 2004 produced tofus with more lightness (L value) than tofus from soybeans harvested in 2003. Tofus from IA2032LS had more lightness (L value) than tofus from Vinton 81. Soybean cultivars harvested in 2003 produced tofus with higher redness value (+ a value) than cultivars harvested in 2004 (data not shown since year and cultivar effect are expected). As shown in Figure 24 a, radiation increased the redness (+ a value) of tofus where the 10 kGy e-beam treated soybeans produced tofus with the highest redness (+ a value) compared to lower doses and control beans. The radiation treatment also significantly decreased the yellowness (+ b value) of the (Figure 24 b). This is similar to the finding of Wilson and others that e-beam or gamma irradiation of soybeans at 10 and 30 kGy decreased the lightness (L value) and yellowness (+ b value) but increased redness (+ a value) of tofus (Wilson and others 2005). Tofus produced from IA2032LS had more yellowness (+ b value) than Vinton 81 in general. Tofus made from soybeans harvested in 2003 had higher + b value (yellowness) than tofus made from soybeans harvested in 2004 (data not shown). Considering the interaction effects of cultivar and year, yellowness (+ b value) of the tofus from each year was affected differently depend on each cultivar. This is comparable to the color data of soymilk where soymilk with more yellowness produced tofus with more yellowness.



(a)

Figure 24 (a). Influence of radiation on redness (+ a value) of tofu made from e-beam irradiated soybeans at radiation doses of 0, 1, 5, 10 kGy. *Note:* Error bar (⊤) indicates standard error for means (n = 48).

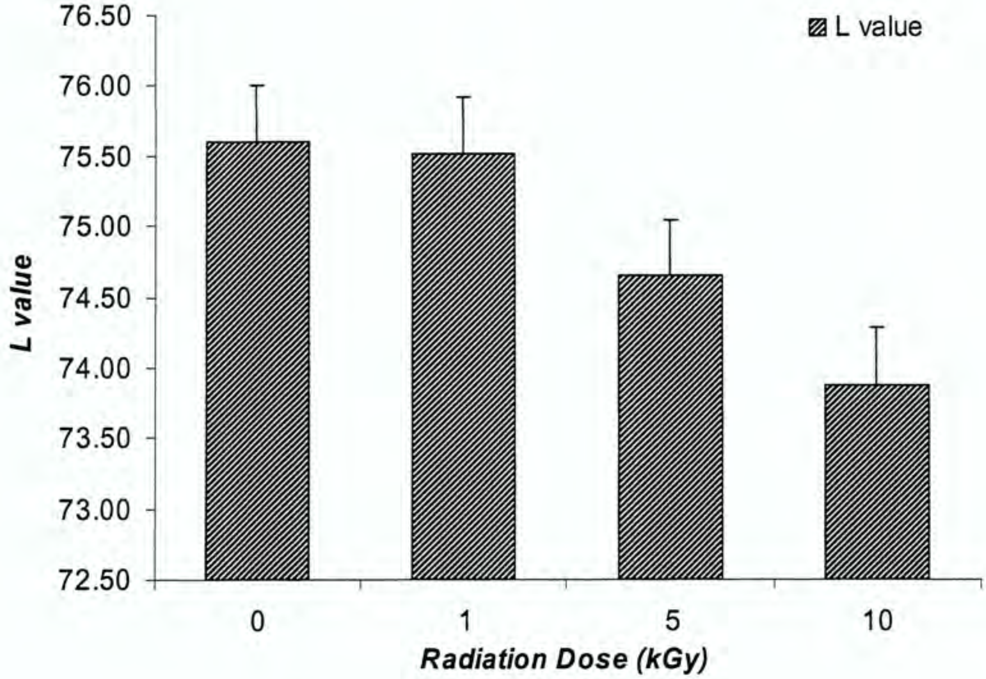


(b)

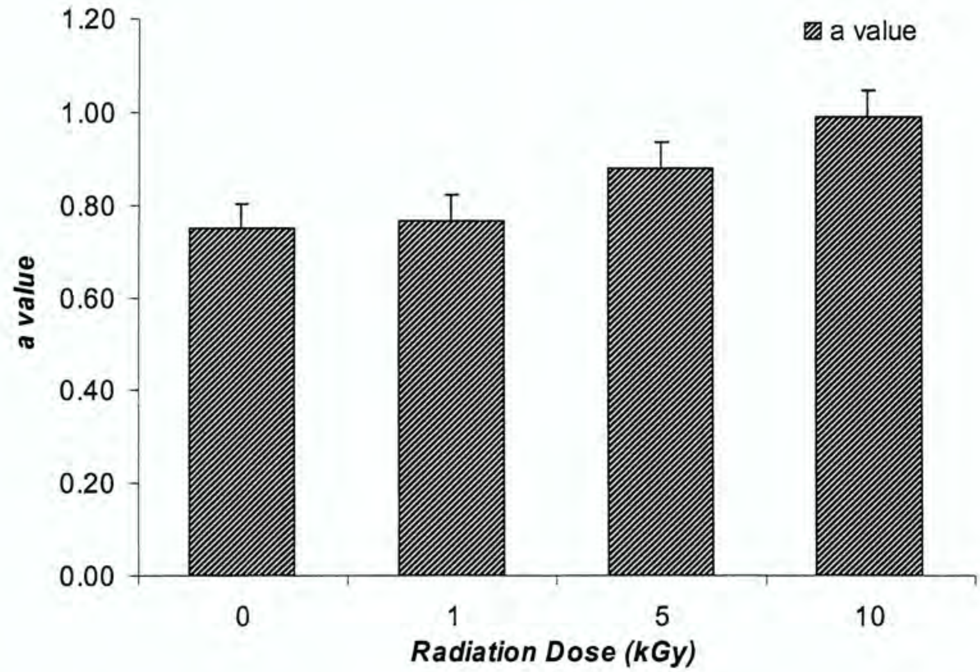
Figure 24 (b). Interaction effects of cultivar (Vinton 81, IA2032LS) and year (2003, 2004) on yellowness (+ b value) of tofu made from e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 48).

Color of Okara

Okara, a by product of soybean production, is used as ingredient in food products to increase the fiber content. Hence, the color of okara may affect the color or appearance of the food products. Figures 25 (a), (b), (c) show the color analysis of okara. Radiation at 1 - 10 kGy decreased the lightness (L value) of the okara and increased the redness (+ a value) of the okara as the radiation doses increased (Figures 25 a and 25 b). However, it had no effect on the yellowness (+ b value) of the okara. There was significant year effect on the lightness (L value), redness (+ a value) and yellowness (+ b value) of the okara ($p<0.0001$). Okara from soybeans harvested in 2003 has lower lightness (L value), higher redness (+ a value) and higher yellowness (+ b value) than okara from soybeans harvested in 2004. There was no difference in lightness (L value) and yellowness (+ b value) of the okara in both cultivars except okara from Vinton 81 had lower redness (+ a value) than IA2032LS in general. Crop year effect on redness (+a value) and yellowness (+ b value) of okara strongly depended on the cultivar where okara from Vinton 81 cultivar had more redness (+ a value) than okara from IA2032LS in 2004 but less redness (+ a value) in 2003 (data not shown). Significant interaction effects between year and cultivar was observed on yellowness (+ b value) of okara. Yellowness (+ b value) of the okara was higher for Vinton 81 cultivar than IA2032LS in crop year 2004 but no difference in 2003 (Figure 25 c). Color changes in okara were also observed due to cultivar (Wilson and others 2004) and due to high dose irradiation at 10 kGy and 30 kGy (Wilson and others 2005).

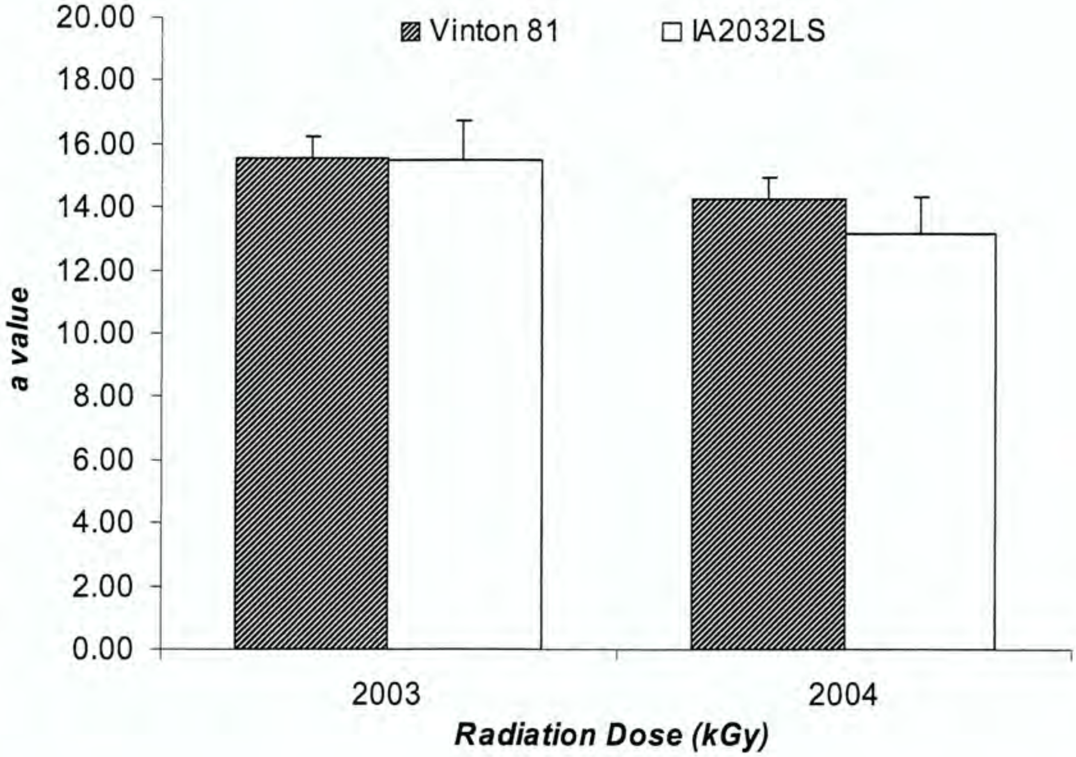


(a)



(b)

Figure 25 (a) and (b). Influence of radiation on (a) L value; (b) a value of okara from soymilk processing of e-beam irradiated soybeans at radiation doses of 0, 1, 5, 10 kGy. *Note:* Error bar (⊤) indicates standard error for means (n = 48).

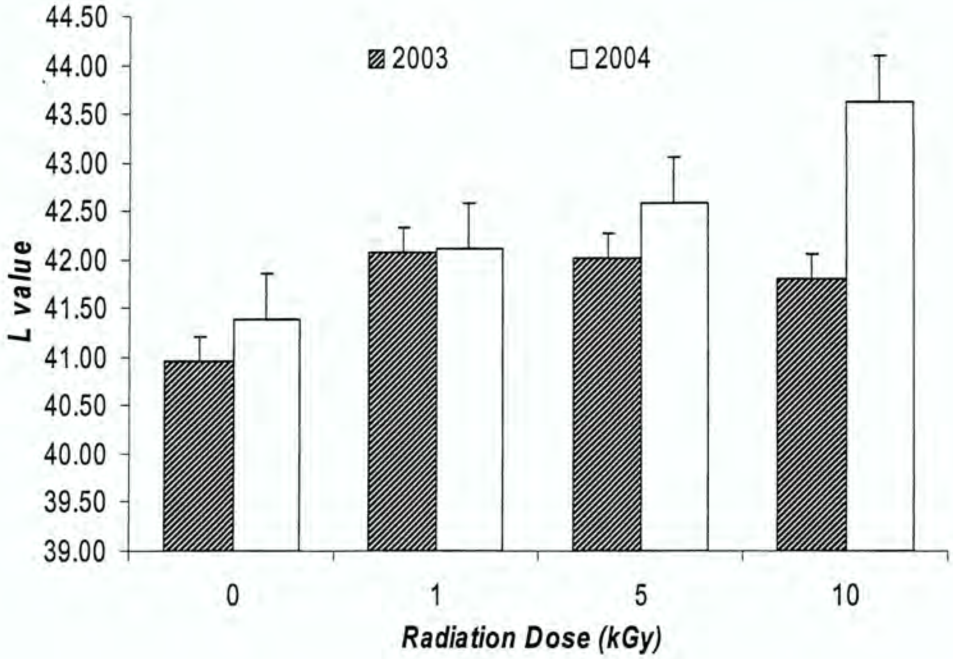


(c)

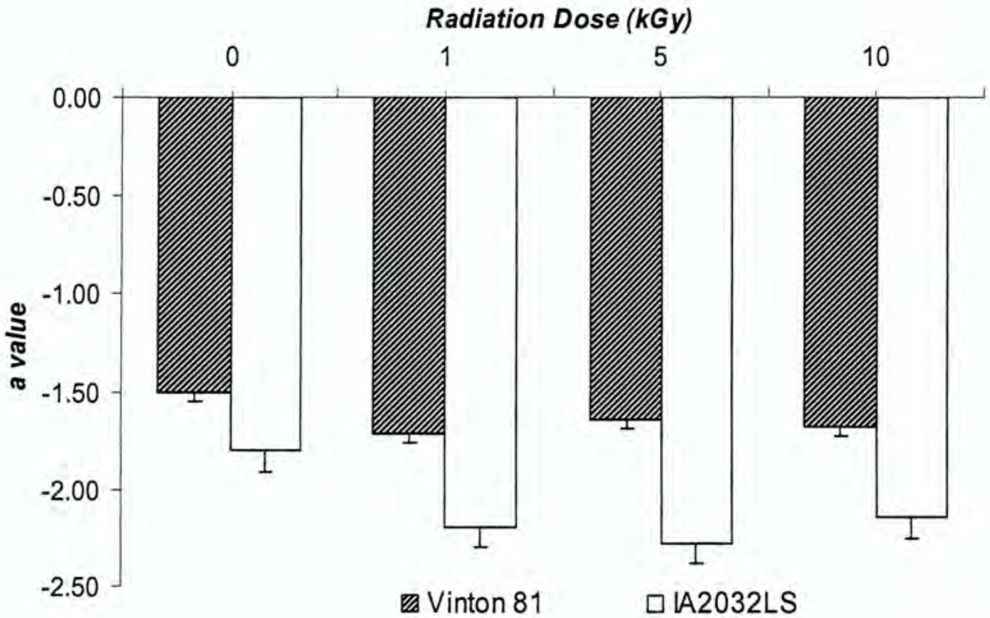
Figure 25 (c). Interaction effects of cultivar (Vinton 81, IA2032LS) and year (2004, 2004) on the yellowness (+ b value) of okara from soymilk processing using e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 48).

Color of Whey

Color analysis of whey from E-beam irradiated soybeans is shown in Figures 26 (a), (b) and (c). While other effects remain constant, radiation significantly affected the greenness (- a value) and the yellowness (+ b value) of the whey, but did not affect the lightness (L value) of the whey. Radiation generally increased the greenness (- a value) and decreased the yellowness (+ b value) of the whey produced. Both year and cultivar significantly affected the lightness (L value), greenness (- a value) and yellowness (+ b value) of the whey. Whey from IA2032LS had more lightness (L value), greenness (- a value) and yellowness (+ b value) than whey from Vinton 81. Soybeans from 2003 produced whey with slightly higher lightness (L value), greenness (- a value) and yellowness (+ b value) than whey from soybeans harvested in 2004. Data discussed above was not shown. Statistical analysis indicated the significant interactions between radiation and year effects as well as year and cultivar effects on the lightness (L value), greenness (- a value) and yellowness (+ b value) of the whey ($p<0.001$). Only interaction effects between radiation and year on lightness (L value) of whey is shown (Figure 26 a). Radiation increased the lightness (L value) of whey produced from 2004 soybeans as radiation dose increased, but lightness (L value) of whey from 2003 soybeans was increased at 1 kGy and remain after 1 kGy (Figure 26 a). Significant interaction between radiation and cultivar effects was observed on the greenness (- a value) and yellowness (+ b value) ($p<0.001$). Radiation increased the greenness (- a value) of the whey from the IA2032LS cultivar but did not significantly increase the greenness (- a value) of whey from Vinton 81 cultivar after 1 kGy (Figure 26 b). Increase of greenness (- a value) in the whey may be due to some residual chlorophyll pigments being extracted into the whey due to radiation treatment, hence, increased the redness (+ a value) in the tofu (Figure 24 a). Radiation also increased the yellowness (+ b value) of whey from Vinton 81 at 1 kGy but increased yellowness (+ b value) of whey from IA2032LS at 1 and 5 kGy then decreased at 10 kGy (Figure 26 c). As a result, color of soy products and by-products was affected by environment and condition of the soybeans, cultivars and sometimes the effect of radiation.

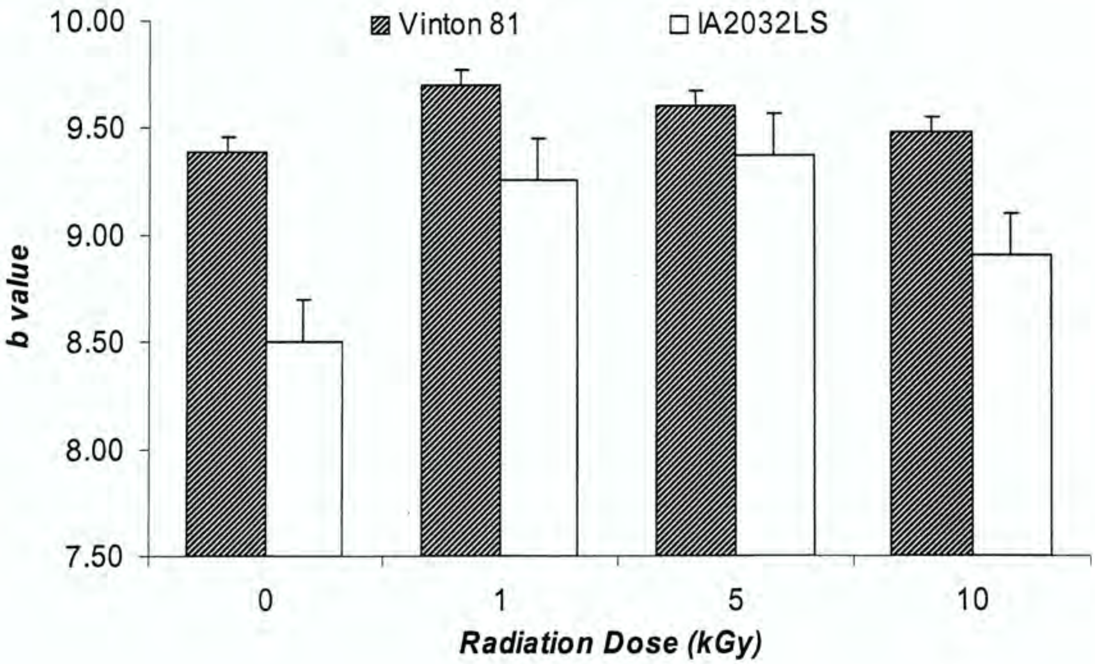


(a)



(b)

Figure 26 (a) and (b). (a) Interaction effects of radiation (0, 1, 5, 10 kGy) and year (2003, 2004) on the lightness (L value); (b) Interaction effects of radiation (0, 1, 5, 10 kGy) and cultivar (Vinton 81, IA2032LS) on the greenness (- a value) of whey from tofu processing using e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 24).



(c)

Figure 26 (c). Interaction effects of radiation (0, 1, 5, 10 kGy) and cultivar (Vinton 81, IA2032LS) on the yellowness (+ b value) of whey from tofu processing using e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 24).

RHEOLOGICAL EFFECTS IN SOYMILK AND TEXTURAL CHANGES IN TOFU

Effect of Irradiation on Viscosity of Soymilk

All soymilk samples were found to be newtonian fluids because the shear stress was directly proportional to shear rate without yield stress (Figure 27). Flow consistent coefficient (*K*) and flow behavior index (*n*) of the soymilk were determined based on Power's law. Averages of flow consistent coefficient (*K*), flow behavior index (n) and viscosity for soymilk of Vinton 81 and IA2032LS cultivars are shown in Table 21. There was significant cultivar effects and interaction effects of year and cultivar on the *K* value, *n* value and viscosity of soymilk produced from e-beam treated soybeans ($p<0.0001$). Soymilk produced from Vinton 81 cultivar had higher *K* value and viscosity but lower *n* value than soymilk produced from IA2032LS cultivar (Table 21). The soymilk produced from Vinton 81 had a higher °Brix (most likely higher protein) in comparison to the soymilk from IA2032LS. Even though the variation of the flow consistent coefficient (*K*) and the flow behavior index (*n*) was very small, but it was significant enough to indicate the variability between each cultivars and crop year. Overall, the flow consistent coefficient (*K*) of soymilk was 0.0026 Pa·s^n on average, and the average flow behavior index (*n*) was 1.09, which is very close to 1 (indicating properties of newtonian fluid) (Table 21). Significant year effect was also observed in the flow behavior index (*n*) of the soymilk ($p<0.05$).

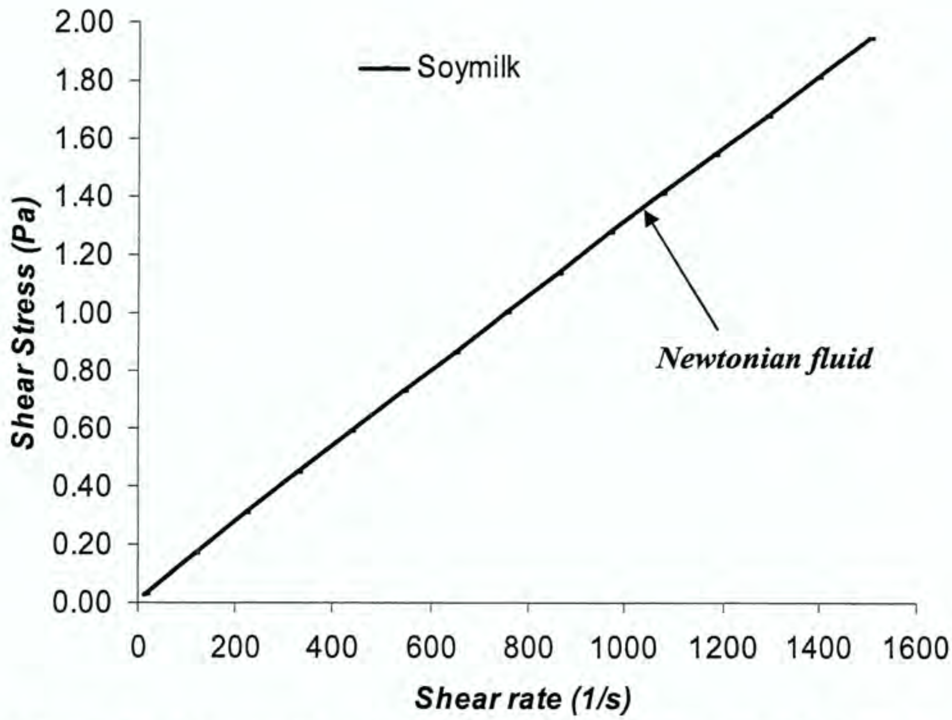


Figure 27. Rheological characteristics of soymilk produced from e-beam treated soybeans. Direct proportional of shear stress to shear rate indicates the Newtonian property of the soymilk.

Table 21. Summarized rheological properties (*K*, *n*, viscosity) of soymilk produced from soybeans irradiated at 0, 1, 5, 10 kGy.

Cultivar	*°Brix*	*K*	*n*	*Viscosity (cP)***
Vinton 81	7.2[a]	0.0029[a]	1.09[a]	1.56[a]
IA2032LS	6.8[b]	0.0023[b]	1.11[b]	1.39[b]
Average	7.0	0.0026	1.10	1.49

* Means within a column with different letters (a, b, c) are significantly different at $p<0.05$ ($n = 48$).
** Viscosity (cP) is reported at the shear rate of 600.00 1/s.

Radiation of whole soybeans significantly affected viscosity of soymilk produced but not the flow consistent coefficient (*K*) and flow behavior index (*n*). Figure 28 shows the interaction effects of radiation and year on the viscosity of the soymilk produced from e-beam irradiated soybeans. Radiation had increased viscosity of soymilk produced from 2004 soybeans, especially at 10 kGy, but did not significantly increased the viscosity of soymilk produced from 2003 soybeans (Figure 28). The same interaction effect of radiation and year can be seen for the flow consistent coefficient (*K*) of the soymilk (Figure 29). Radiation affected the flow consistent coefficient (*K*) of soymilk differently in both years. The *K* value of soymilk produced from 2004 soybeans was decreased at 1 kGy but increased to the highest value at 10 kGy. In comparison to the soymilk produced from 2003 soybeans, radiation increased the *K* value at 1 kGy but remained consistently higher than control (0 kGy) at 5 kGy and 10 kGy. Increase in viscosity and flow consistent coefficient (*K*) of the soymilk due to radiation effect may be due to interaction of radiolysis products (free radicals) on the cellulose of soybeans forming shorter fragments that flow through the filter cloth during separation of okara from the soymilk. This was also observed by Wilson and others

(2005) in that the okara from soybeans irradiated at 10 and 30 kGy were mushy and hard to be separated from the milk.

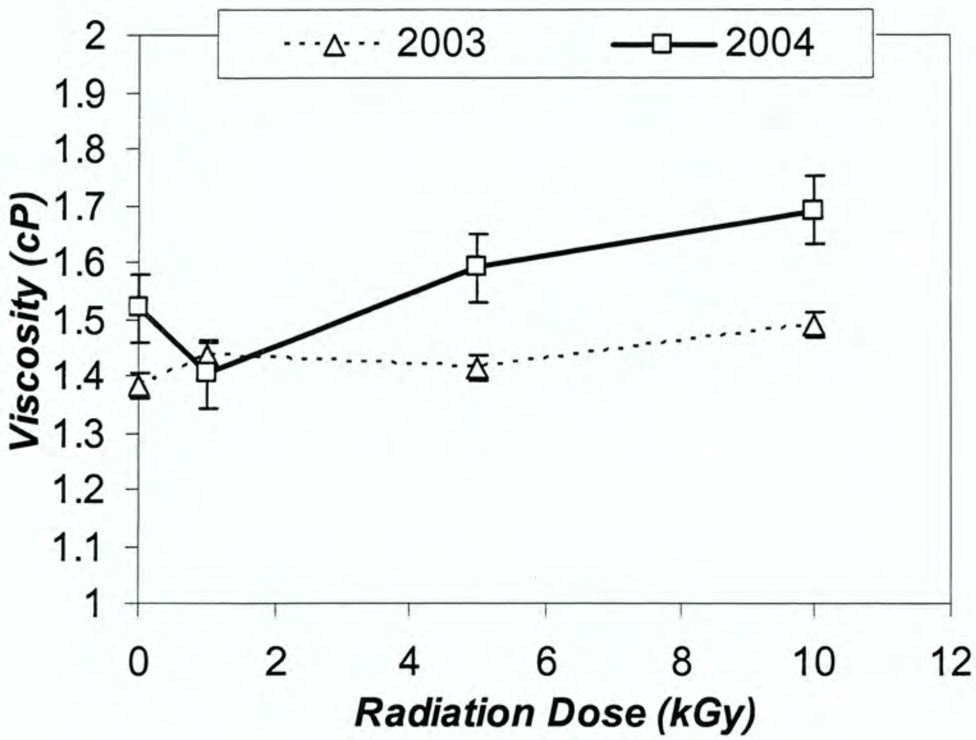


Figure 28. Interaction of radiation (0, 1, 5, 10 kGy) and year (2003, 2004) effects on the viscosity (cP) of soymilk made from e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 12).

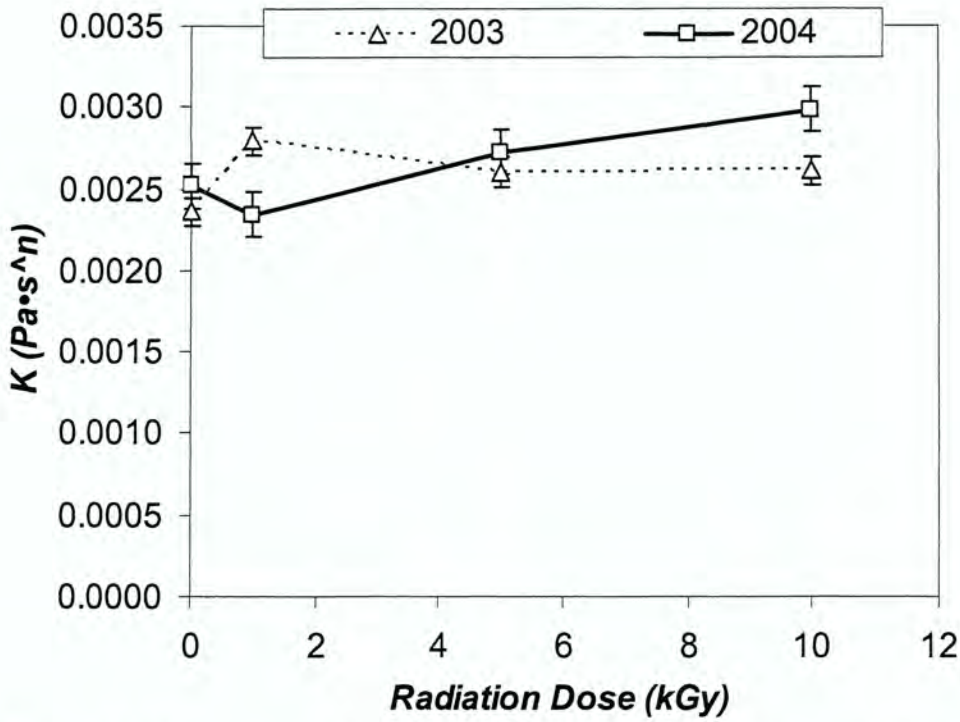


Figure 29. Interaction of radiation (0, 1, 5, 10 kGy) and year (2003, 2004) effects on the flow consistent coefficient (*K*) of soymilk made from e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 12).

Rheological properties of the soymilk depend on the protein composition of the soybeans: its glycinin(11S)/β-conglycinin(7S) ratio. Kim and Wicker (2005) found that soybeans with higher 11S/7S ratio produced more non-newtonian soymilk (lower flow behavior index, *n*). Soymilk produced from the Vinton 81 cultivar had lower flow behavior index (n = 1.089), indicating that it is more non-newtonian than the soymilk produced from the IA2032LS cultivar (n = 1.105). This data is similar to protein analysis of high dose study (0 - 30 kGy) that Vinton 81 had higher 11S/7S ratio than the IA2032LS (2.09 vs 1.94) (Table 22 in Appendix B).

<u>Effect of Irradiation on Texture of Tofu</u>

The texture of tofu produced from E-beam irradiated soybeans was analyzed for hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. There was significant year effect on the hardness, gumminess, chewiness and resilience of the tofus. Cohesiveness, gumminess and chewiness were significantly affected by the cultivar effect. However, effects of year, cultivar and their interactions on texture of tofu were not discussed in detailed because it is expected that the protein composition of soybeans and its subunits is different in each cultivar and may vary from year to year within each cultivar depending on the growing conditions. Significance of each effect on the textural attributes of the tofu is shown in Appendix A (Table 37).

Radiation decreased the cohesiveness and resilience of the tofus. However, there were significant interaction between radiation and cultivar on the cohesiveness and resilience of the tofu (Figures 30 and 31). Cohesiveness of the tofu made from Vinton 81 was decreased consistently but cohesiveness of tofu made from IA2032LS was slightly increased at 1 kGy and further decreased at 5 kGy and 10 kGy (Figure 30). Radiation did not decreased the resilience of the tofu made from Vinton 81 at 1 kGy and 5 kGy but significantly decreased the resilience at 10 kGy. In comparison to tofu made from IA2032LS, resilience of tofu was decreased at 5 kGy (Figure 31). Wilson's study also found that irradiated soybeans at high levels (10 kGy and 30 kGy) produced tofu with softer texture, less adhesive, less springy, less cohesive, and less resilient (Wilson and others 2005). Modification of the coagulation and pressing process of the tofu could possibly be modified to mediate some of these textural changes (Wilson and others 2004b).

Various studies had shown that gelation properties of the soy globulins played significant role in hardness of the gels. Depending on proportion of the protein subunits in each cultivar, gels made from glycinin was harder than gels made from β-conglycinin (Renkema and others 2001; Rickert and others 2004; Saio and others 1969; Watanabe 1997; Yagasaki and others 2000). Differential gelling properties of the specific subunits within glycinin and β-conglycinin may also contribute to the variation in hardness of the tofu (Tezuka and others 2000; Mujoo and others 2003; Yagasaki and others 2000; Mohamad Ramlan and others 2004).

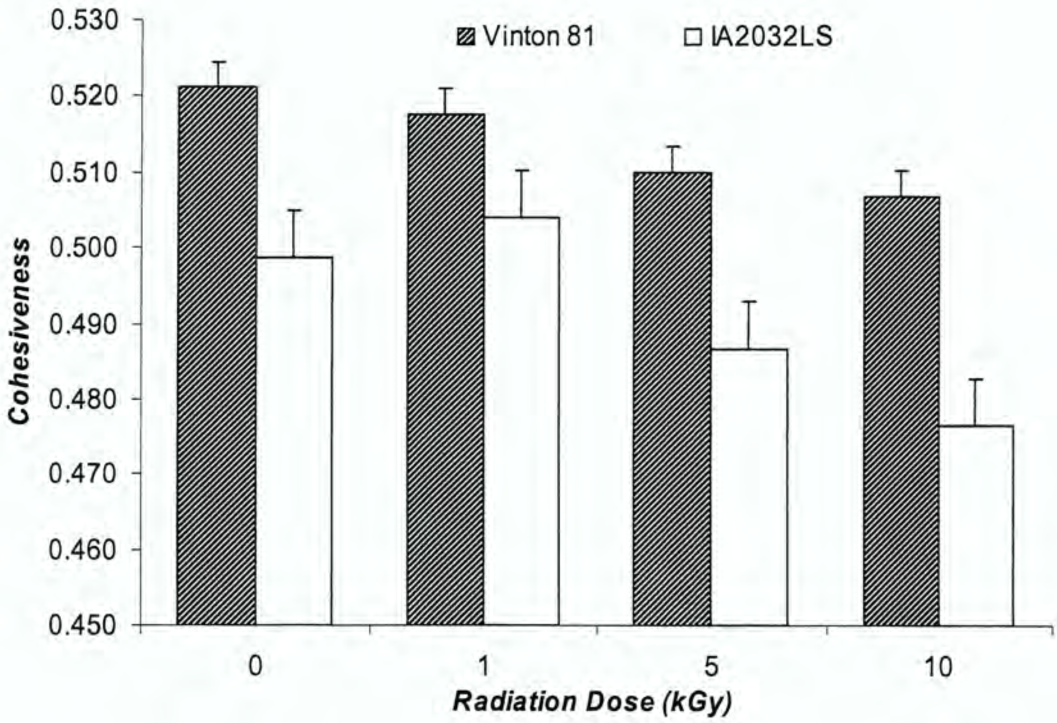


Figure 30. Interaction effects of radiation (0, 1, 5, 10 kGy) and cultivar (Vinton 81, IA2032LS) on cohesiveness of tofu made from e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 24).

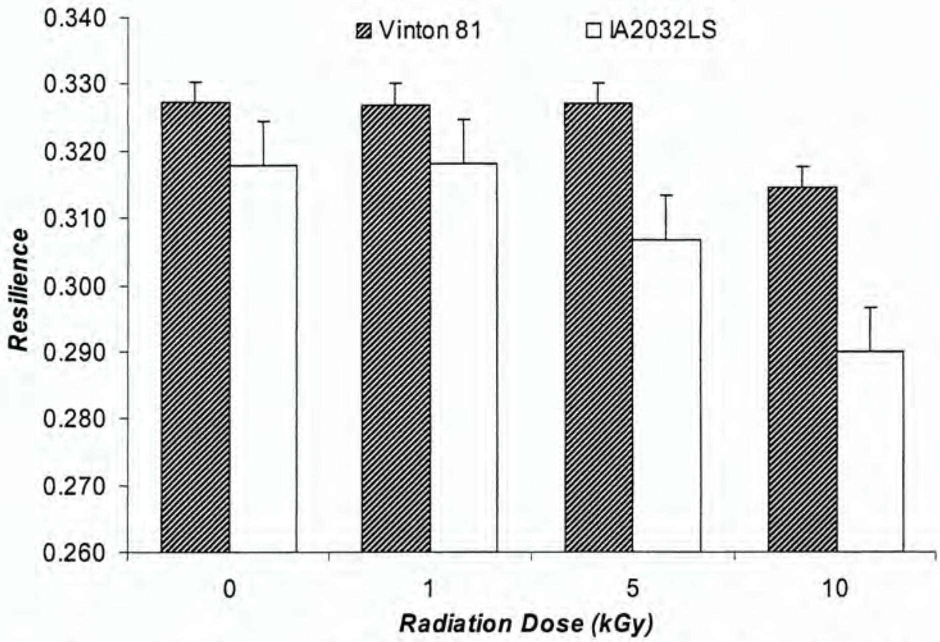


Figure 31. Interaction effects of radiation (0, 1, 5, 10 kGy) and cultivar (Vinton 81, IA2032LS) on resilience of tofu made from e-beam irradiated soybeans. *Note:* Error bar (⊤) indicates standard error for means (n = 24).

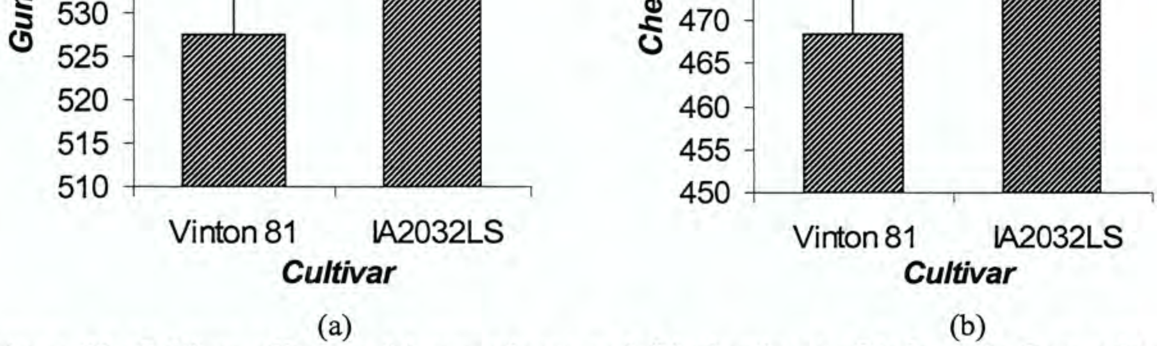


Figure 32. Cultivar effect on (a) gumminess and (b) chewiness of tofu made from e-beam irradiated soybeans (Vinton 81, IA2032LS). *Note:* Error bar (⊤) indicates standard error for means (n = 48).

Storage protein subunits in the soybeans contributed significantly to the textural and rheological properties of soy food products. Differences in protein content and protein ratio may affect the viscosity, textural properties and color of soymilk, indicating changes in quality and functionality. Kim and Wicker (2005) had shown that soybeans with higher ratio of 11S subunit produced harder, chewier and gummier tofu. Tofu produced from the IA2032LS cultivar is gummier and chewier than tofus produced from the Vinton 81 cultivar (Figures 32 a and 32 b), which is inconsistent to Kim and Wicker's finding in that the IA2032LS cultivar had slightly lower 11S/7S ratio than the Vinton 81 cultivar (Table 39 in Appendix B). Variation of the protein subunits composition may be affected due to nutritional stress during development of seeds as shown by Gayler and Skyes (1985) that sulfur deficiency in developing seeds caused a 40% decrease in glycinin and an increase in β-conglycinin.

FLAVOR AND AROMA ANALYSIS BY GAS CHROMATOGRAPHY

During manufacturing of soymilk and tofu, some observations on flavor and aroma changes were noticeable. Pleasant fresh soymilk aroma was detected during the heating step of the soy slurry produced from the control soybeans (0 kGy) but slight off-odors were detected during the processing of 1 kGy-treated soybeans. The off-aromas were increasingly strong at 5 kGy and became the worst at 10 kGy. Wilson and others (2005) found strong off-aromas in the soymilk made from high dose irradiation (10 kGy and 30 kGy) irradiated soybeans. A "wet dog" aroma of other irradiated meat products (e.g. pork, chicken, beef) was detected. A sensory study to determine the type of off-aroma and consumer acceptability of the off-aroma in the soymilk from irradiated soybeans is suggested.

The flavor profile of both normal soybeans and lipoxygenase-free soybeans were studied. With the presence of lipoxygenase, flavor compounds in soymilk made from Vinton 81 was more complicated, as more peaks were detected by gas chromatography, than soymilk from IA2032LS (lipoxygenase-free) (Figure 52 in Appendix C). Seventeen volatile compounds in the soymilk were identified including hexanal, *trans*-2-hexenal, *cis*-2-hexenal, N heptanal, *trans*-2-heptanal, 1-octenal-3-ol, 2-pentyl-furan, octanal, 2,4-heptadienal, 2-octenal, nonanal, *trans*-2-nonenal, *trans, trans*-2,4-nonadienal, decanal, *trans*-2-decenal, 2-hexyl furan, and *trans, trans*-2,4-decadienal (Figures 35 - 51 in Appendix C). Most volatiles were detected in both soybeans except that *trans*-2-nonenal was not found in soymilk from IA2032LS (Figure 46 in Appendix C). Most of the compounds determined have grassy, green, fat and rancid flavor. Table 22 shows the description of each flavor compounds as described in Flavornet (Acree & Am 2004). Amount of these different volatile compounds identified in the soymilk varied depending on the cultivars and crop year of the soybeans used.

As the radiation dose increased, the amount of flavor compounds (peak area) in soymilk produced from the lipoxygenase-free beans (IA2032LS) increased, especially hexanal, which is the major contributor to the grassy and beany flavor of the soymilk (Figure 33 in Appendix C). With the absence of lipoxygenase in soybeans, oxidative rancidity still occurs slowly which contributes to the low level of grassy, beany flavor in soymilk (peak area range 102-482) (Figure 33). Other volatile compounds such as trans-2-hexenal, trans-2-heptanal, 1-octen-3-ol and decanal were increased in soymilk from IA2032LS as radiation dose increased. Compared to soymilk produced from the lipoxygenase-present soybeans (Vinton 81), the hexanal level was higher (peak area range 438-958) as were most of other volatile compounds than lipoxygenase-free soybeans (Figure 34). However, irradiation seemed to decrease the amount of some volatiles determined in soymilk from Vinton 81, including hexanal, *trans-2*-heptanal and 1-octen-3-ol, which showed more change as radiation dose increased. This could due to the binding of these volatiles to proteins and other components in the soymilk as well as non-covalent interactions through hydrophobic, hydrophilic, van der Waals forces (Aspelund and Wilson 1983; O'keefe and others 1991). These lipid oxidation products may have interacted with lysine residues via Michael addition reactions or alkoxyl radicals to form protein carbonyls (Refsgaard and others 2000). Meanwhile, consistent formation of minor compounds with higher polarity was observed in both soybean cultivars as the radiation dose increased. These compounds formed are speculated to be some nitrogen and sulfur containing compounds from proteins breakdown. In comparison to the dry beans lipid oxidation analysis (SāfTest®), higher level of oxidation was observed in soymilk (higher production of hexanal). This is due to activation of enzymes through soaking of the soybeans and release of enzymes via disruption (grinding) of the natural compartmentalization (which protects the lipids from enzymes reactions) thus resulting in higher levels of lipid oxidation products in soymilk. In addition, Vitamin E (antioxidant) itself was being destroyed once the beans were ground and exposed to oxygen, hence, low level of antioxidative capacity was expected in the soymilk that results in high level of oxidation products. Loss of vitamin E was also observed in processing of soybeans into tofu (Guzman and Murphy 1986).

Table 22. Flavor description of volatile compounds determined in soymilk.

Volatile compound	Retention Time	Description
hexanal	7.600	Grass, tallow, fat
trans-2-hexenal	10.100	Apple, green
cis-2-hexenal	10.900	-
N heptanal	12.400	Fat, citrus, rancid
trans-2-heptanal	15.200	Green
1-Octenal-3-ol	16.200	Mushroom
2-pentyl-furan	16.600	Green bean, butter
octanal	17.369	Fat, soap, lemon, green
2,4-heptadienal	17.835	Nut, fat
2-octenal	19.900	Green
nonanal	22.000	Soap, plastic
trans-2-nonenal	24.452	Cucumber, fat, green
trans, trans-2,4-nonadienal	25.936	Watermelon
decanal	26.361	Soap, orange peel, tallow
trans-2-decenal	28.286	Tallow
2-hexyl furan	29.153	-
trans, trans-2,4-decadienal	29.700	Fried, wax, fat

Source: Acree T, Am H. 2004. Flavornet. Geneva, NY: DATU Inc.

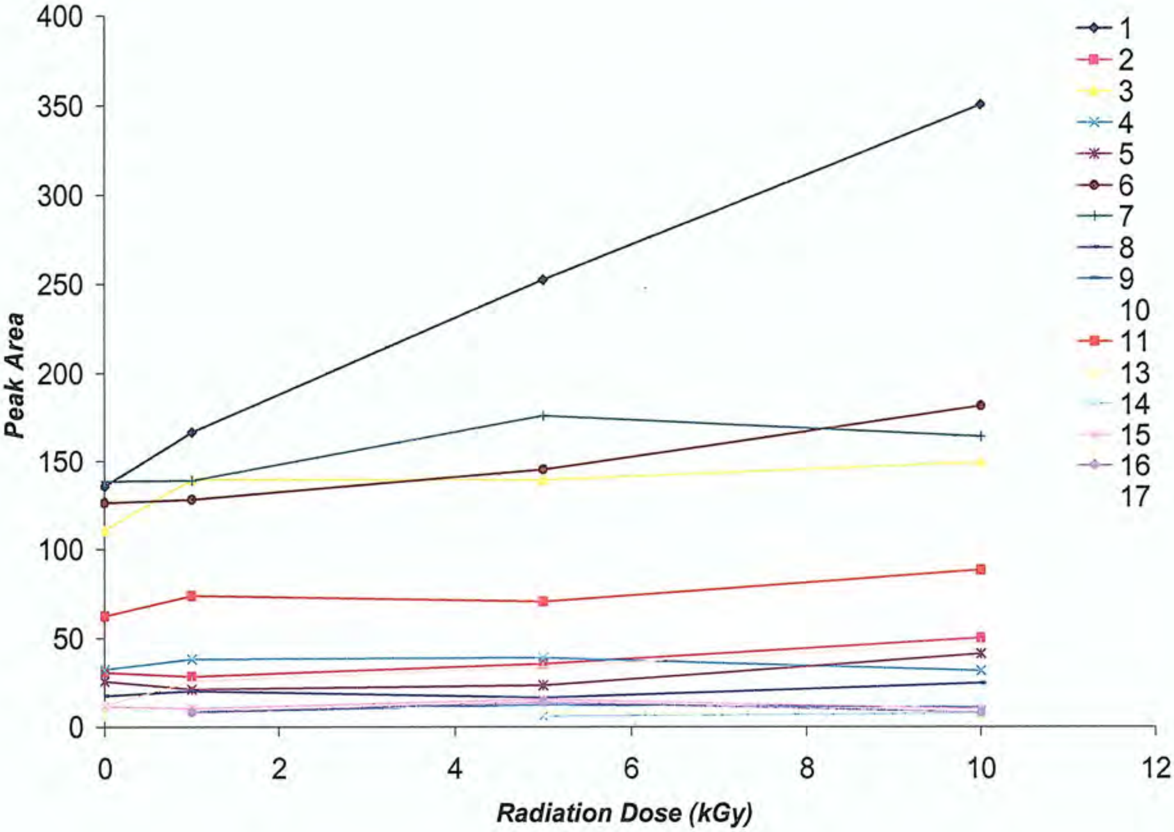


Figure 33. Flavor compounds determined in soymilk produced from E-beam irradiated IA2032LS cultivar (lipoxygenase-free cultivar). 1: Hexanal; 2: *Trans*-2-hexenal; 3: *Cis*-2-hexenal; 4: N heptanal; 5: *Trans*-2-heptanal; 6: 1-0ctenal-3-ol; 7: 2-pentyl-furan; 8: Octanal; 9: 2,4-heptadienal; 10: 2-octenal; 11: Nonanal; 12: *Trans*-2-nonenal; 13: *Trans,trans*-2,4-nonadienal; 14: Decanal; 15: *Trans*-2-decenal; 16: 2-hexyl furan; 17: *Trans,trans*-2,4-decadienal.

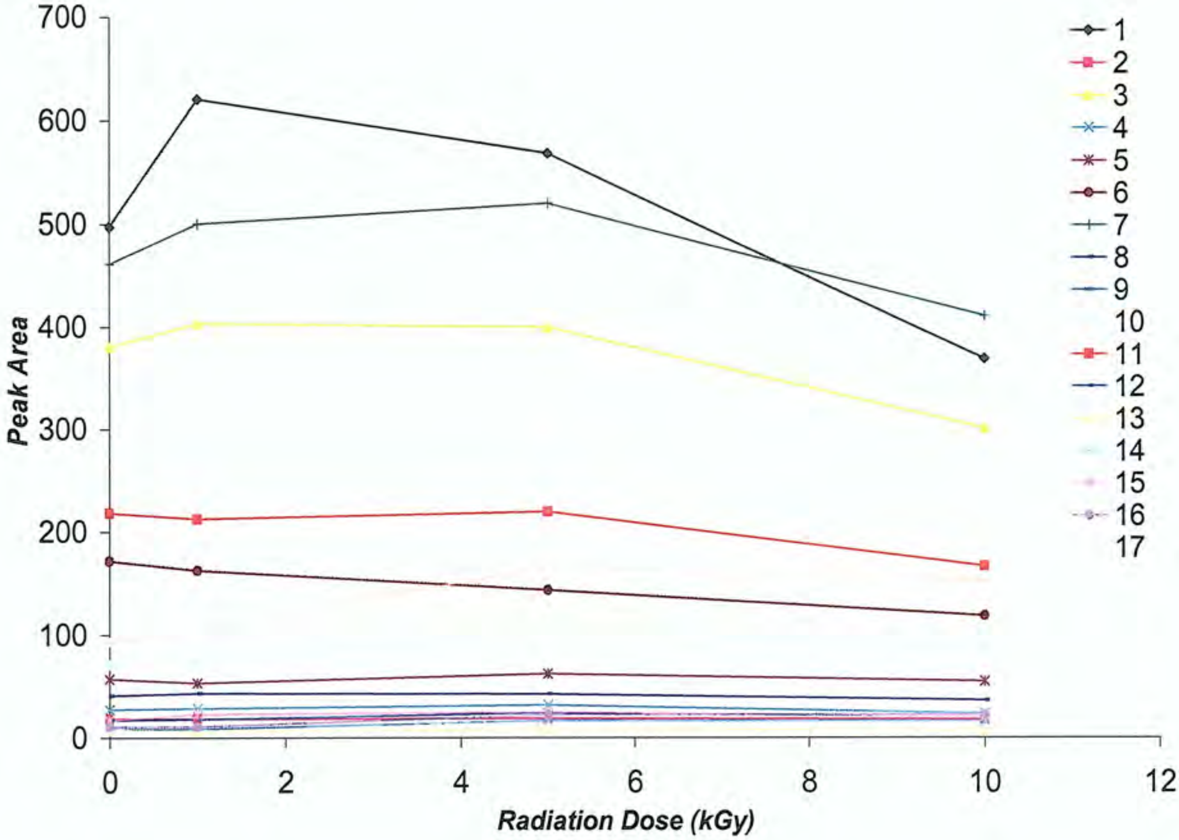


Figure 34. Volatile compounds determined in soymilk produced from e-beam irradiated Vinton 81 cultivar (lipoxygenase-present cultivar). 1: Hexanal; 2: *Trans*-2-hexenal; 3: *Cis*-2-hexenal; 4: N heptanal; 5: *Trans*-2-heptanal; 6: 1-0ctenal-3-ol; 7: 2-pentyl-furan; 8: Octanal; 9: 2,4-heptadienal; 10: 2-octenal; 11: Nonanal; 12: *Trans*-2-nonenal; 13: *Trans,trans*-2,4-nonadienal; 14: Decanal; 15: *Trans*-2-decenal; 16: 2-hexyl furan; 17: *Trans,trans*-2,4-decadienal.

CHAPTER 5

SUMMARY AND CONCLUSIONS

SUMMARY

- Proximate composition of soybeans was different depending on its cultivar and harvested year.
- A similar vitamin E content was observed in the dry whole soybeans of both Vinton 81 and IA2032LS cultivars, while surface irradiation of soybeans did not significantly affect the Vitamin E content in whole soybeans at doses of 1-10 kGy.
- A higher isoflavone content was observed in the IA2032LS cultivar compared to Vinton 81 cultivar. E-beam radiation at 1-10 kGy did not significantly affect the isoflavone content of the dry whole soybeans.
- No significant effect of radiation (1-10 kGy) was observed on the antioxidative capacity in soybeans, but cultivar and harvest year affected the antioxidative capacity of the soybeans.
- Radiation treatment of dry whole soybeans at 1, 5, 10 kGy decreased the malonaldehyde content, but the malonaldehyde content was not correlated well to the rancid off-flavor (hexanal) in the soymilk product. After grinding, radiation-induced off-odor was detected in soymilk from irradiated soybeans. Lipid oxidation was shown to be affected by the soybean cultivars and crop year as well as its storage duration or shelf life of the soybeans.
- E-beam irradiation of soybeans at dose levels of 1, 5, 10 kGy did provide microbial safety for the astronauts but surviving yeasts and molds were found at 1 kGy.

- The germination ability of the soybeans was significantly affected by irradiation. The normal germination percentage decreased as the radiation dose increased. Initial seed quality of the soybean cultivars also played a significant role in seed germinating ability (stone bean content).
- Radiation increased the water absorption of the soybeans. IA2032LS soybeans from crop year 2003 had the lowest water absorption ability due to the presence of 'stone beans'.
- Surface irradiation of the soybeans did not significantly change the yield of soymilk and tofu. Soymilk yield varied due to the harvest year but not cultivar. Cultivar played a significant role in tofu yield even though it was not significant in soymilk yield.
- Radiation at 1-5 kGy did not affect the color of whole dry soybeans, but it induced color changes in soymilk, tofu, okara and whey. Irradiation of whole dry soybeans was shown to decrease lightness (L value) of okara, but it had no effect on lightness of soymilk, tofus and whey. Redness (a value) of the tofus and okara increased due to radiation where as greenness (-a value) of soymilk decreased and greenness of whey increased. Yellowness of soymilk, tofus and whey was reduced due to radiation effect. The color of the soyfood products was highly dependent on cultivar and harvest year.
- No significant radiation effect was observed on the viscosity and flow consistent coefficient of the soymilk produced from the soybeans treated at 1 and 5 kGy, but a significant increase in both factors was observed at the radiation dose of 10kGy.
- Surface irradiation of whole soybeans decreased the cohesiveness and resilience of tofu, but did not affect other attributes (hardness, adhesiveness, springiness,

gumminess and chewiness). The radiation effect on the textural properties of the tofus varied depending on the cultivar and year harvested. Vinton 81 cultivar produced tofus with higher cohesiveness, gumminess and chewiness than tofus from the IA203LS cultivar.

- Identification of volatile compounds by GC-MS confirmed the presence of lipid oxidation compounds in the soymilk produced from irradiated soybeans. More volatile compounds were produced in the lipoxygenase-present soybeans (Vinton 81) than in the lipoxygenase-free soybeans (IA2302LS). Hexanal concentration was lower in soymilk from lipoxygenase-free soybeans (IA2032LS) than soymilk from lipoxygenase-presence soybeans (Vinton 81). Radiation increased the generation of hexanal in the lipoxygenase-free soybeans, but even with the observed reduction of hexanal due to flavor binding in the lipoxygenase-present soybeans, the hexanal level of Vinton 81 soymilk remained higher than the hexanal in soymilk made from the lipoxygenase-free soybeans.

CONCLUSIONS

E-beam irradiation of bulk soybeans at 1, 5, 10kGy affected the quality of the soybeans and its products by darkening soymilk and tofu color, reducing the seed germination ability, and increasing off-aromas. Radiation at 1, 5, 10 kGy also increased the water absorption of the seeds and altered the texture of the soymilk and tofus. Irradiation increased the oxidative rancidity compounds in the lipoxygenase-free soybeans but reduced the hexanal level (grassy and beany flavor) in soymilk produced from regular soybeans, however, hexanal was still higher than in the lipoxygenase-free soybeans. This leads to answers to the hypotheses of this study:

(1) Radiation and storage conditions, prior to and during transit to Mars, interact but did not decrease the amount of natural antioxidants in the soybeans. No lipid oxidation was induced in the dry whole soybeans but the oxidation process was accelerated after hydration and grinding of the treated soybeans, which resulted in oxidative rancidity compounds in its products (soymilk and tofu). The yield of soybeans was not affected by radiation but quality was affected.

(2) Radiation at 1-10kGy altered the germination rate of the soybeans.

Different types of radiation (x-ray, gamma ray, protons, etc.) may cause different effects on the functionality and quality of soyfood products. Soybeans cultivars and their storage conditions should be considered prior to shipping to Mars so that the highest quality cultivars can be selected and then be stored under optimum conditions (dry, low temperature storage and protected from light). Appropriate shielding for the soybeans as well as the astronauts during Mars missions needs to be developed to protect them from radiation damage. A high protein lipoxygenase-free cultivar similar to IA2032LS should be selected for long term missions to give maximum yield and desirable sensory quality. A sensory evaluation panel needs to be conducted to determine the detectable limits and acceptability of the off-flavor in the soyfood products. Flavor masking maybe needed for soyfoods produced from soybeans treated at higher doses (5kGy and 10kGy). While irradiation to provide microbial safety for the astronauts can be accomplished by applying this HACCP step as a critical control point (CCP) (irradiation), the sensory properties of products made from these soybeans has been compromised. Likewise, this CCP will prevent the soybeans from being successfully grown on Lunar and Mars missions. Counter measures could include vacuum packaging, nitrogen flushing, adding antioxidants, and radiating under freezing conditions. Doses below 1kGy need to be further investigated to determine the influence of the radiation encountered during Mars missions.

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APPENDIX A: STATISTICAL ANALYSIS (P-VALUE)

Table 23. Radiation, year and cultivar effects on composition (moisture, protein, oil, fiber) of e-beam irradiated whole dry soybeans.

	Pr > F*			
Effect	**Moisture**	**Protein**	**Oil**	**Fiber**
Radiation	0.3655	0.6089	0.6235	1.0000
Year	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar	<0.0001	<0.0001	<0.0001	1.0000
Radiation: Year	0.2859	0.9181	0.5977	0.1811
Radiation: Cultivar	0.2440	0.5657	0.6308	1.0000
Year: Cultivar	0.0202	<0.0001	<0.0001	<0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 24. Radiation, year and cultivar effects on vitamin E content of e-beam irradiated whole dry soybeans.

Effect	**Pr > F***
Radiation	0.3405
Cultivar	0.3059
Radiation: Cultivar	0.9693

* P<0.05 indicates the significance of each effect and its interactions.

Table 25. Radiation, year and cultivar effects on isoflavone content (total daidzein, total glycitein, total genistein, total isoflavone) of e-beam irradiated whole dry soybeans.

	Pr > F*			
Effect	**Total Daidzein**	**Total Glycitein**	**Total Genistein**	**Total Isoflavone**
Radiation	0.0279	0.0011	0.1858	0.0667
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001
Radiation: Cultivar	0.0186	0.0005	0.6392	0.0761

* P<0.05 indicates the significance of each effect and its interactions.

Table 26. Radiation, year and cultivar effects on antioxidant capacity of e-beam irradiated whole dry soybeans.

Effect	**Pr > F***
Radiation	0.5996
Year	<0.0001
Cultivar	0.1236
Radiation: Year	0.5040
Radiation: Cultivar	0.8367
Year: Cultivar	<0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 27. Radiation, year and cultivar effects on lipid oxidation (malonaldehyde content, peroxide value, free fatty acid) of e-beam irradiated whole dry soybeans.

	Pr > F*		
Effect	**Malonaldehyde content**	**Peroxide Value**	**Free Fatty Acid**
Radiation	0.0892	0.3866	0.0085
Year	<0.0001	0.1513	<0.0001
Cultivar	0.0204	0.2575	<0.0001
Radiation: Year	0.9614	0.3549	0.0603
Radiation: Cultivar	0.0968	0.8741	0.0260
Year: Cultivar	0.7200	0.0350	0.0008

* P<0.05 indicates the significance of each effect and its interactions.

Table 28. Radiation, year and cultivar effects on germination ability of e-beam irradiated whole dry soybeans.

	Pr > F*		
Effect	**Normal**	**Abnormal**	**Dead**
Radiation	0.0003	<0.0001	<0.0001
Year	<0.0001	0.6942	<0.0001
Cultivar	<0.0001	0.0679	<0.0001
Radiation: Year	0.0024	0.1532	0.0193
Radiation: Cultivar	0.0001	0.0133	0.0525
Year: Cultivar	0.0629	0.0689	0.0014

* P<0.05 indicates the significance of each effect and its interactions.

Table 29. Radiation, year and cultivar effects on water absorption of e-beam irradiated whole dry soybeans.

Effect	Pr > F*
Radiation	0.0024
Year	<0.0001
Cultivar	<0.0001
Radiation: Year	0.4268
Radiation: Cultivar	0.0510
Year: Cultivar	<0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 30. Radiation, year and cultivar effects on the yield of soymilk and tofu (soymilk basis, dry bean basis) produced from e-beam irradiated whole dry soybeans.

Effect	Pr > F*		
	Soymilk Yield	Tofu Yield[a]	Tofu Yield[b]
Radiation	0.1726	0.6273	0.4906
Year	0.0156	0.6781	0.2765
Cultivar	0.0853	0.0553	0.0017
Radiation: Year	0.2317	0.4421	0.6996
Radiation: Cultivar	0.1679	0.3432	0.7813
Year: Cultivar	0.2609	0.1296	0.9225

* P<0.05 indicates the significance of each effect and its interactions.
[a]Tofu yield based on initial amount of soymilk.
[b]Tofu yield based on initial amount of dry beans.

Table 31. Radiation, year and cultivar effects on color (Hunter L, a, b) of e-beam irradiated whole dry soybeans.

Effect	Pr > F*		
	L	a	b
Radiation	0.5449	0.0732	0.4547
Year	0.1148	<0.0001	<0.0001
Cultivar	<0.0001	0.4113	0.2271
Radiation: Year	0.6636	0.9369	0.1874
Radiation: Cultivar	0.2965	0.2465	0.1781
Year: Cultivar	0.0082	0.0001	0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 32. Radiation, year and cultivar effects on color (Hunter L, a, b) of soymilk made from e-beam irradiated whole dry soybeans.

Effect	**Pr > F***		
	L	**a**	**b**
Radiation	0.5006	0.0007	<0.0001
Year	0.7590	0.1277	<0.0001
Cultivar	0.0003	0.0211	<0.0001
Radiation: Year	0.0677	0.1331	0.0745
Radiation: Cultivar	0.7541	0.5627	0.0030
Year: Cultivar	0.0983	0.3556	0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 33. Radiation, year and cultivar effects on color (Hunter L, a, b) of tofu made from e-beam irradiated whole dry soybeans.

Effect	**Pr > F***		
	L	**a**	**b**
Radiation	0.9761	0.0037	<0.0001
Year	<0.0001	<0.0001	<0.0001
Cultivar	0.0048	0.9857	<0.0001
Radiation: Year	0.5339	0.0719	0.1679
Radiation: Cultivar	0.4343	0.9539	0.1918
Year: Cultivar	0.2292	0.1181	0.0048

* P<0.05 indicates the significance of each effect and its interactions.

Table 34. Radiation, year and cultivar effects on color (Hunter L, a, b) of okara produced from e-beam irradiated whole dry soybeans.

Effect	**Pr > F***		
	L	**a**	**b**
Radiation	0.0025	0.0004	0.6042
Year	<0.0001	<0.0001	<0.0001
Cultivar	0.0696	0.0011	0.9735
Radiation: Year	0.3785	0.0812	0.3571
Radiation: Cultivar	0.1341	0.7289	0.6517
Year: Cultivar	0.1622	<0.0001	<0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 35. Radiation, year and cultivar effects on color (Hunter L, a, b) of whey produced from e-beam irradiated whole dry soybeans.

Effect	**Pr > F***		
	L	**a**	**b**
Radiation	0.7433	0.0010	<0.0001
Year	0.0001	<0.0001	<0.0001
Cultivar	<0.0001	<0.0001	0.0002
Radiation: Year	<0.0001	<0.0001	<0.0001
Radiation: Cultivar	0.3447	0.0008	0.0035
Year: Cultivar	<0.0001	<0.0001	<0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 36. Radiation, year and cultivar effects on the rheological properties (*K*, *n*, viscosity) of soymilk made from e-beam irradiated whole dry soybeans.

Effect	**Pr > F***		
	K	**n**	**Viscosity**
Radiation	0.0582	0.1856	0.0015
Year	0.8225	0.0395	0.0018
Cultivar	<0.0001	<0.0001	<0.0001
Radiation: Year	0.0420	0.1437	0.0326
Radiation: Cultivar	0.7717	0.3532	0.9481
Year: Cultivar	<0.0001	<0.0001	<0.0001

* P<0.05 indicates the significance of each effect and its interactions.

Table 37. Radiation, year and cultivar effects on texture of tofus (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, resilience) made from e-beam irradiated whole dry soybeans.

	Pr > F*			
Effect	**Hardness**	**Adhesiveness**	**Springiness**	**Cohesiveness**
Radiation	0.4986	0.1701	0.4632	<0.0001
Year	<0.0001	0.3104	0.4122	0.4944
Cultivar	0.0894	0.9284	0.9599	0.0223
Radiation: Year	0.0767	0.2251	0.4725	0.0022
Radiation: Cultivar	0.1956	0.4502	0.1179	0.0116
Year: Cultivar	<0.0001	0.5300	0.0065	0.9607

Effect	**Gumminess**	**Chewiness**	**Resilience**
Radiation	0.1485	0.1545	<0.0001
Year	<0.0001	<0.0001	0.0545
Cultivar	0.0409	0.0436	0.1195
Radiation: Year	0.2558	0.2811	0.0006
Radiation: Cultivar	0.4187	0.3938	0.0010
Year: Cultivar	0.0001	0.0001	0.7203

* P<0.05 indicates the significance of each effect and its interactions.

Table 38. Radiation, year and cultivar effects on volatile compounds determined in soymilk produced from e-beam irradiated whole dry soybeans.

	Pr > F*				
Effect	**Hexanal**	***Trans*-2-hexenal**	***Cis*-2-hexenal**	**N-heptanal**	***Trans*-2-heptanal**
Radiation	0.0084	<0.0001	0.7171	0.5280	0.0422
Year	0.5186	0.0002	<0.0001	<0.0001	0.5575
Cultivar	0.0062	0.0002	0.0071	0.0546	<0.0001
Radiation: Year	0.1109	0.2328	0.0149	0.6505	0.0129
Radiation: Cultivar	0.0386	0.0012	0.9617	0.7306	0.6617
Year: Cultivar	0.0019	-	-	0.0078	-

Effect	**1-Octen-3-ol**	**2-Pentylfuran**	**Octanal**	**2,4-heptadienal**
Radiation	0.0445	0.4475	0.4853	0.9670
Year	0.0012	0.0660	0.0014	0.2263
Cultivar	0.0078	<0.0001	0.0026	0.9552
Radiation: Year	0.2940	0.6177	0.0402	0.6233
Radiation: Cultivar	0.2157	0.5183	0.8173	0.3446
Year: Cultivar	<0.0001	0.4464	-	-

Effect	**2-Octenal**	**Nonanal**	***Trans*-2-nonenal**	***Trans,trans*-2,4-nonadienal**
Radiation	0.7383	0.3842	0.5253	0.6008
Year	0.0516	0.5328	0.0218	0.7460
Cultivar	0.0027	<0.0001	<0.0001	0.7932
Radiation: Year	0.2456	0.4079	0.6913	-
Radiation: Cultivar	0.3912	0.3782	-	0.6849
Year: Cultivar	-	0.0163	-	-

Effect	**Decanal**	***Trans*-2-decenal**	**2-Hexylfuran**	***Trans,trans*-2.4-decadienal**
Radiation	0.0147	0.9392	0.7994	0.1289
Year	0.9509	0.0093	0.4904	0.0255
Cultivar	0.0500	0.0742	0.6598	<0.0001
Radiation: Year	0.8762	0.8280	0.2915	0.0318
Radiation: Cultivar	-	0.9260	0.0975	0.0312
Year: Cultivar	-	-	-	-

* P<0.05 indicates the significance of each effect and its interactions.

APPENDIX B: PROTEIN ANALYSIS

Table 39. Preliminary study on protein composition of soybeans (Vinton 81 and IA2032LS) treated with gamma ray at 0, 10 and 30 kGy.

Soybean cultivar		*Protein Fraction (%)*		*11S/7S ratio*
		Glycinin (11S)	*β-conglycinin (7S)*	
Vinton 81		45.06 ± 0.01*	21.56 ± 0.02*	2.09
Radiation dose (kGy)	0	46.82	23.14	2.02
	10	44.16	21.68	2.04
	30	44.18	19.84	2.23
IA2032LS		45.30 ± 0.03*	23.30 ± 0.03*	1.94
Radiation dose (kGy)	0	45.02	23.56	1.91
	10	43.63	26.10	1.67
	30	47.24	20.25	2.33

* Average protein fraction of 0, 10, 30 kGy (n = 6).

APPENDIX C: FLAVOR ANALYSIS

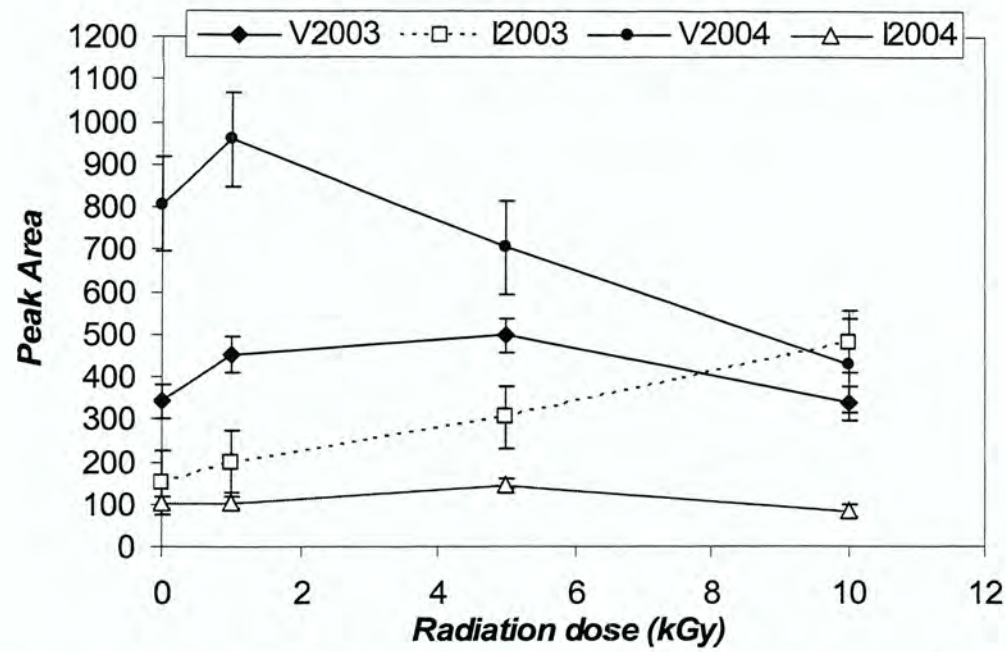


Figure 35. Hexanal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

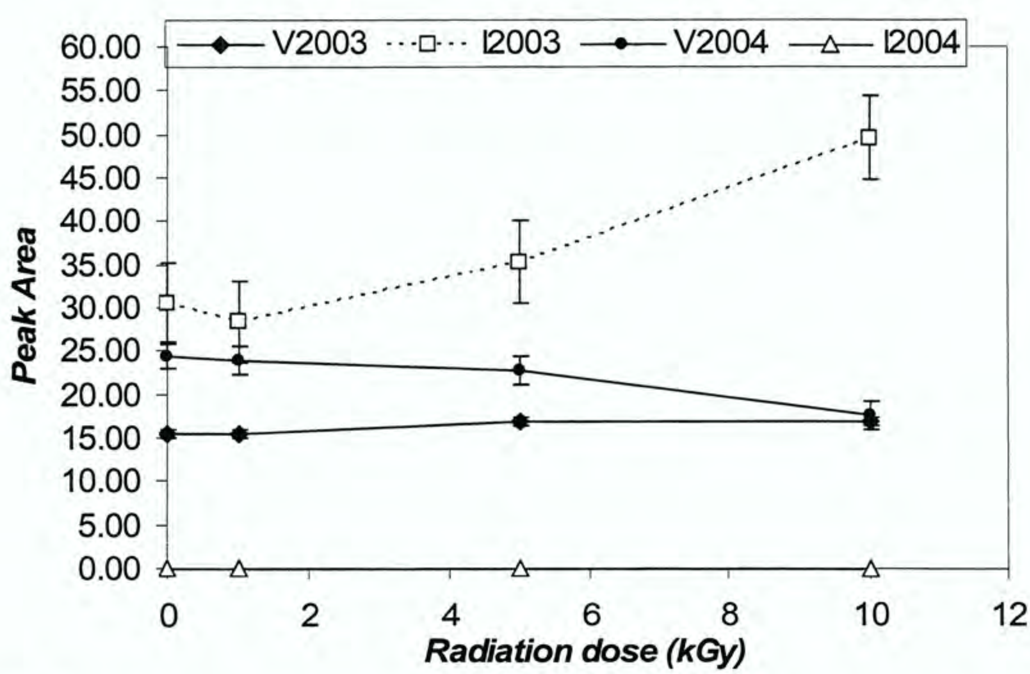


Figure 36. *Trans*-2-hexenal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

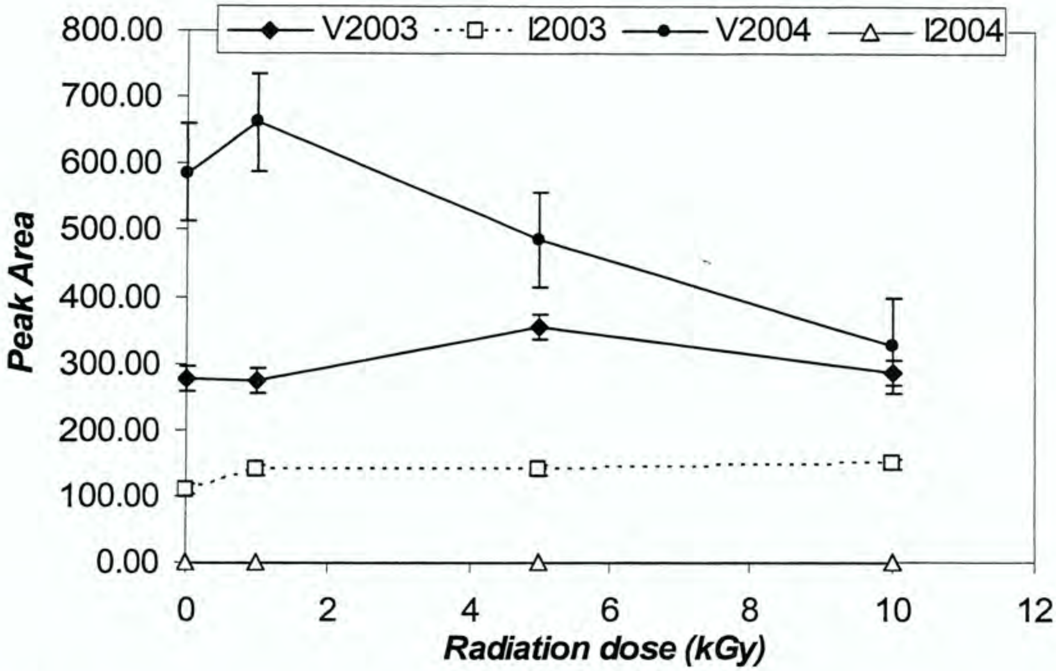


Figure 37. *Cis*-2-hexenal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

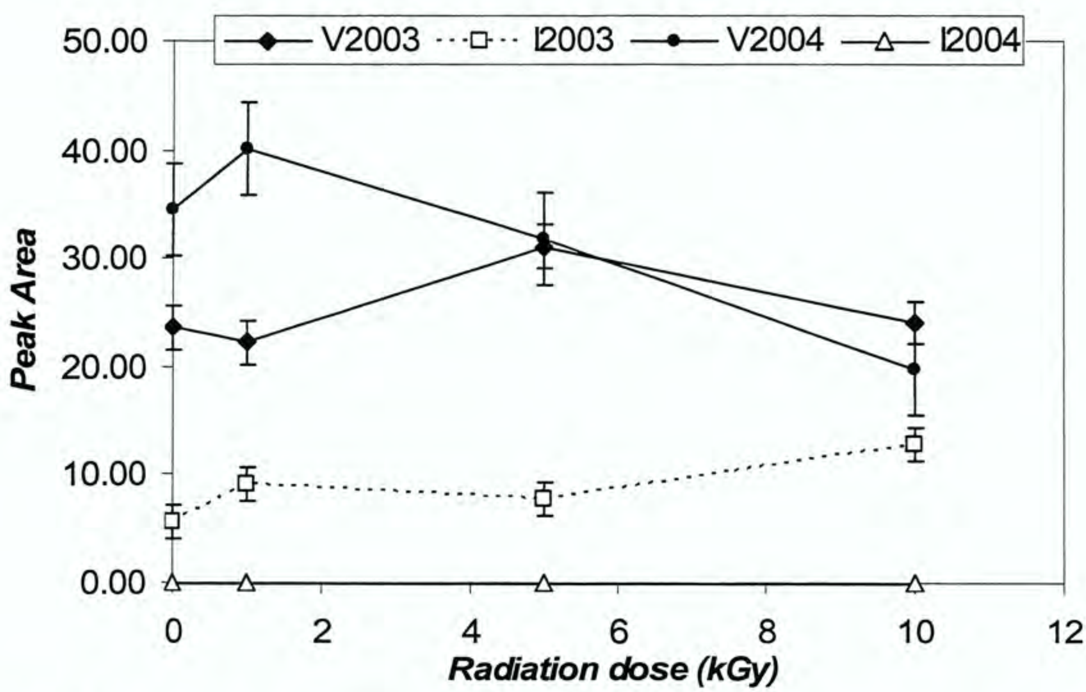


Figure 38. N heptanal detected in soymilk made from d-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

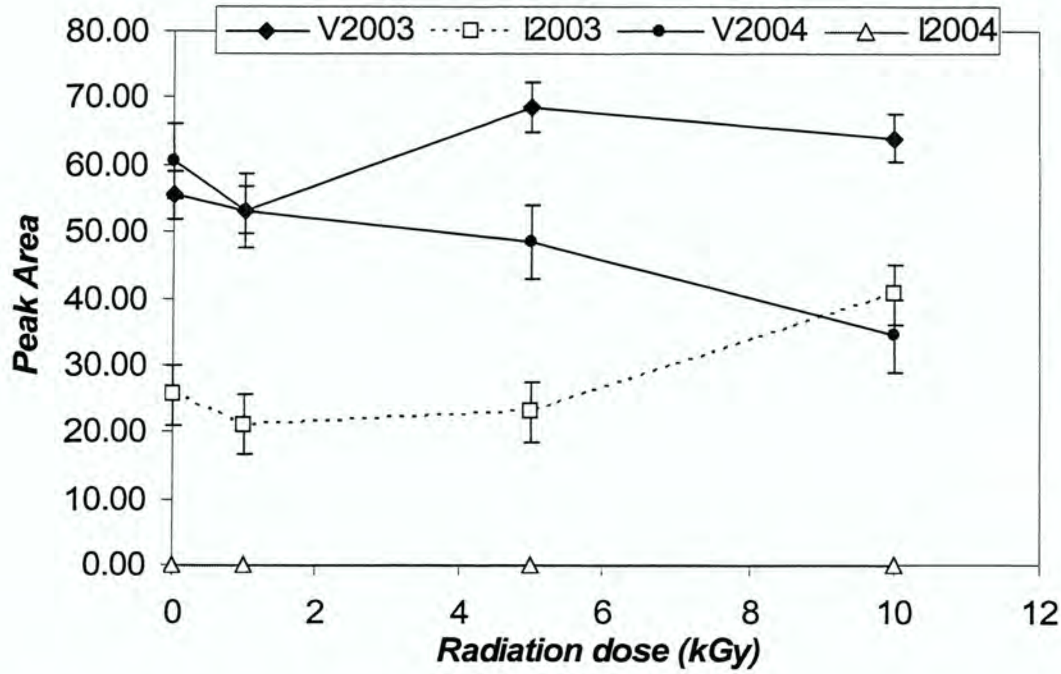


Figure 39. *Trans*-2-heptanal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

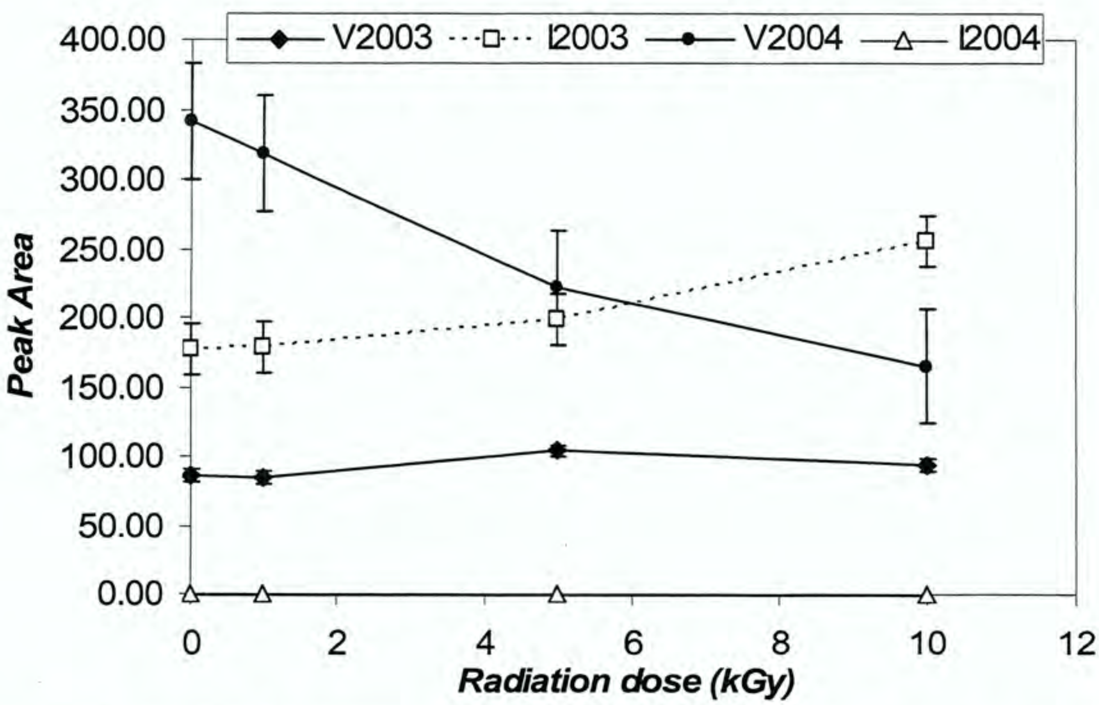


Figure 40. 1-Octen-3-ol detected in soymilk made from E-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10kGy.

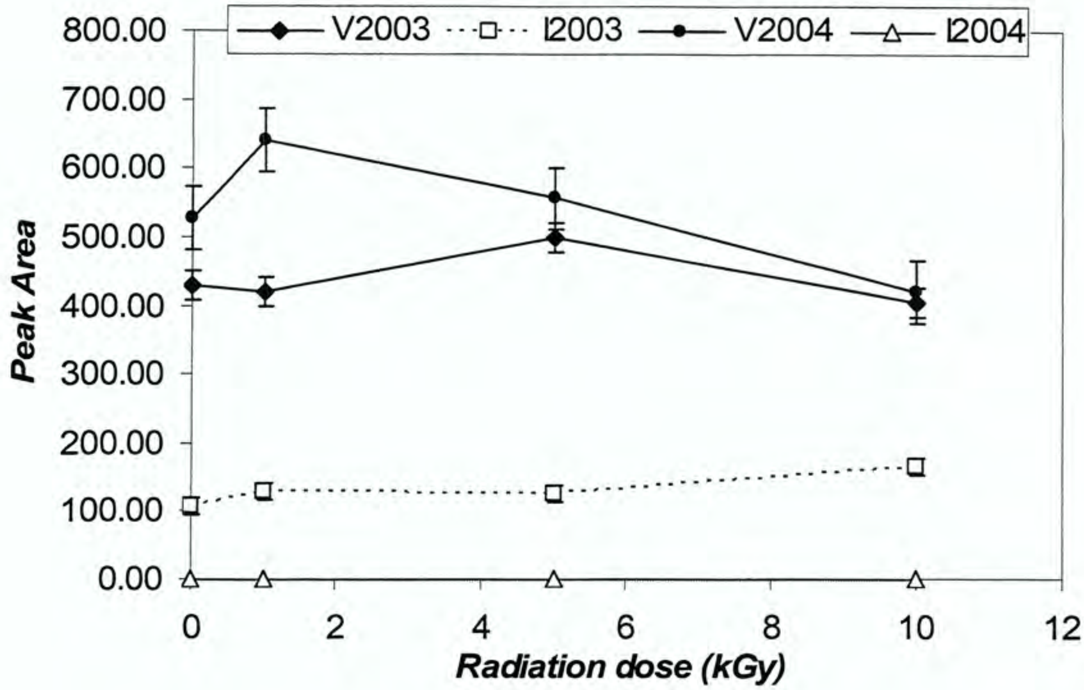


Figure 41. 2-Pentylfuran detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

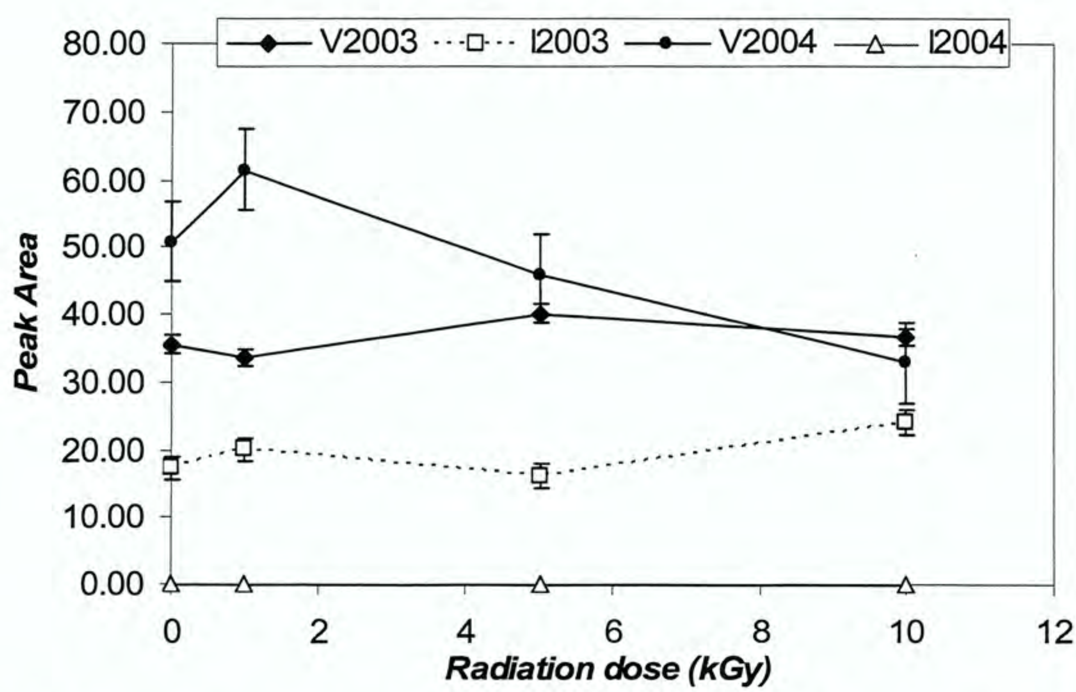


Figure 42. Octanal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

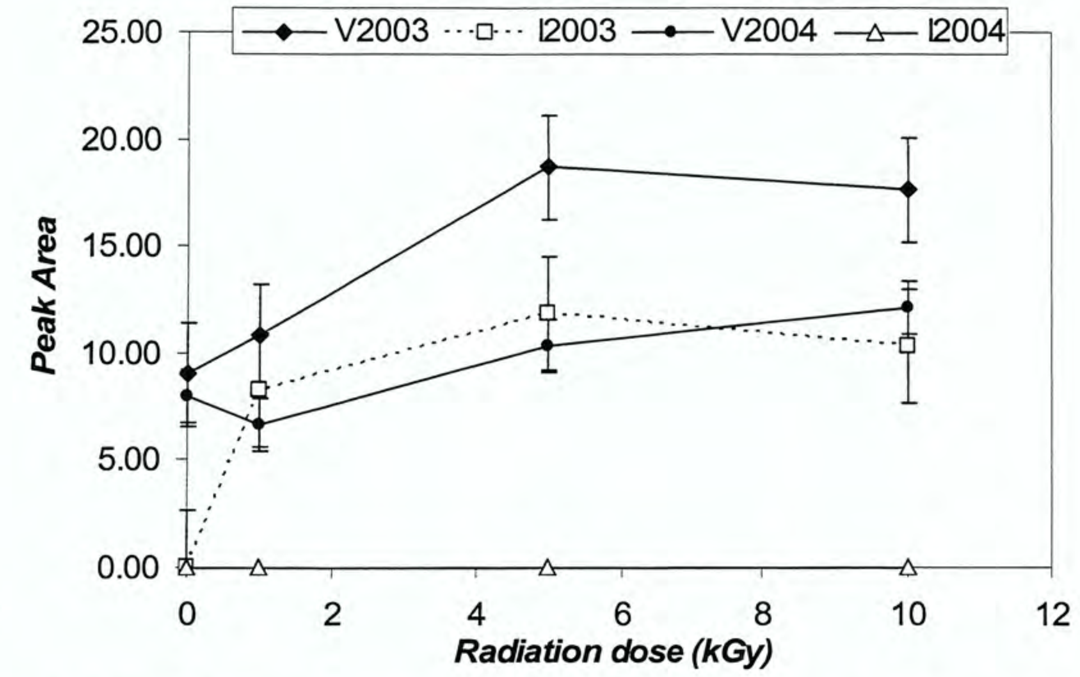


Figure 43. 2,4-Heptadienal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

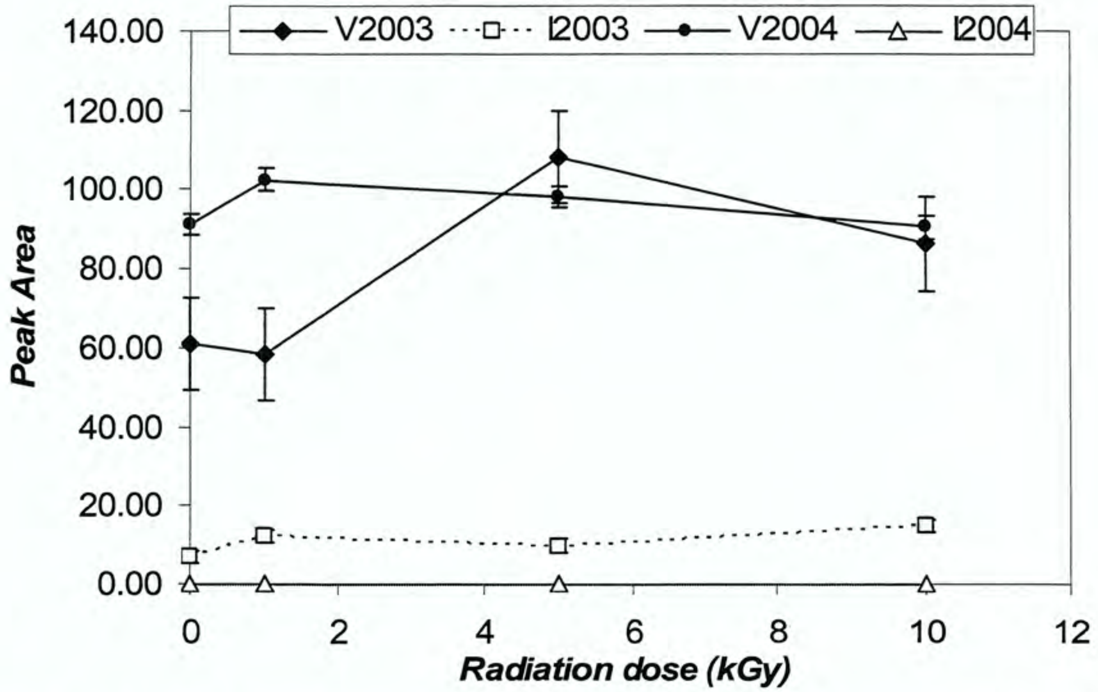


Figure 44. 2-Octenal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

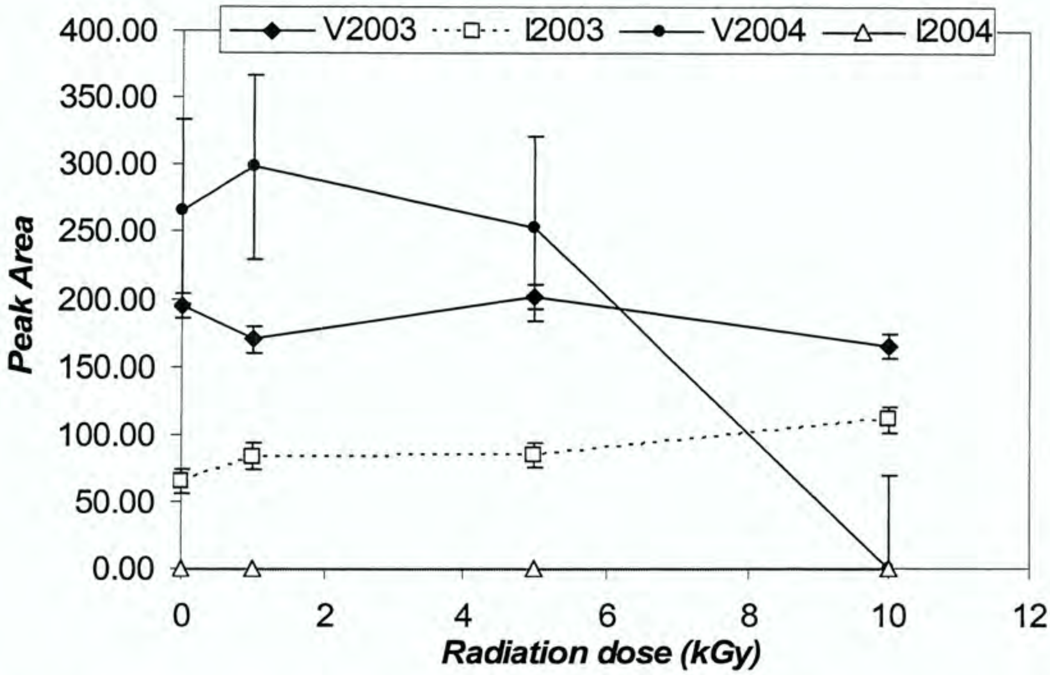


Figure 45. Nonanal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

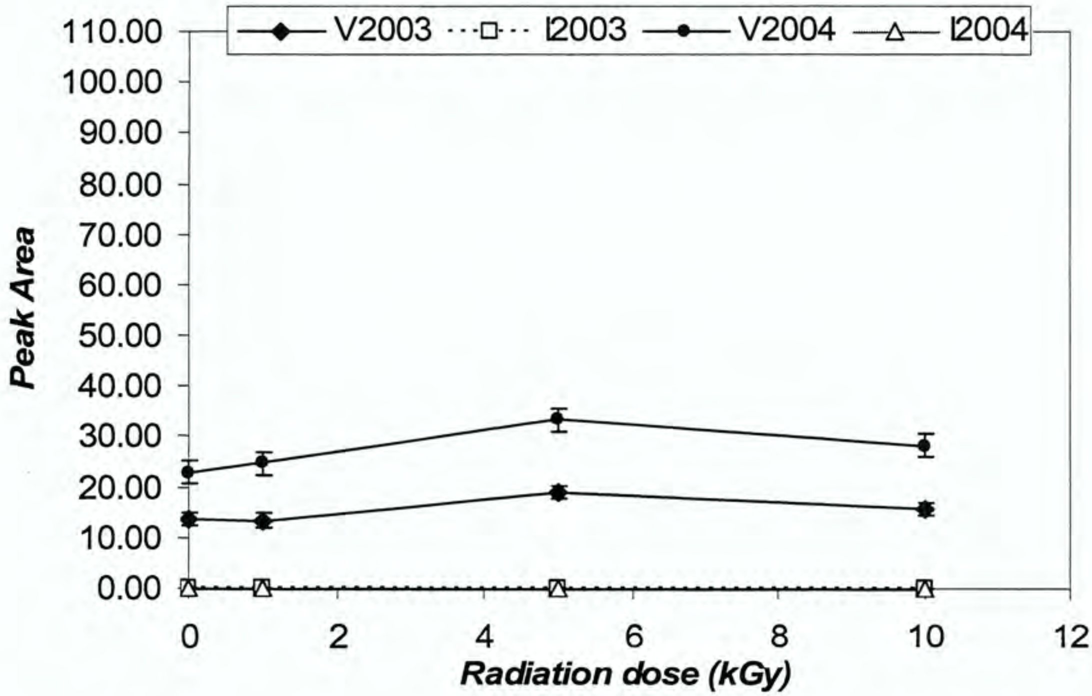


Figure 46. *Trans*-2-nonenal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

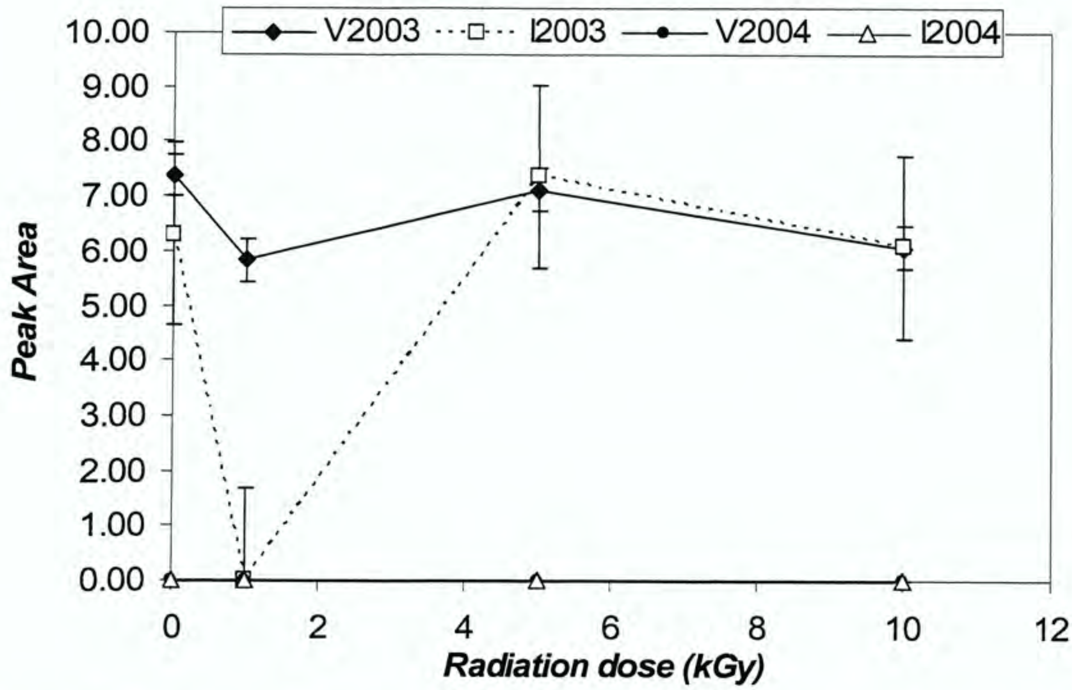


Figure 47. *Trans, trans*-2,4-nonadienal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

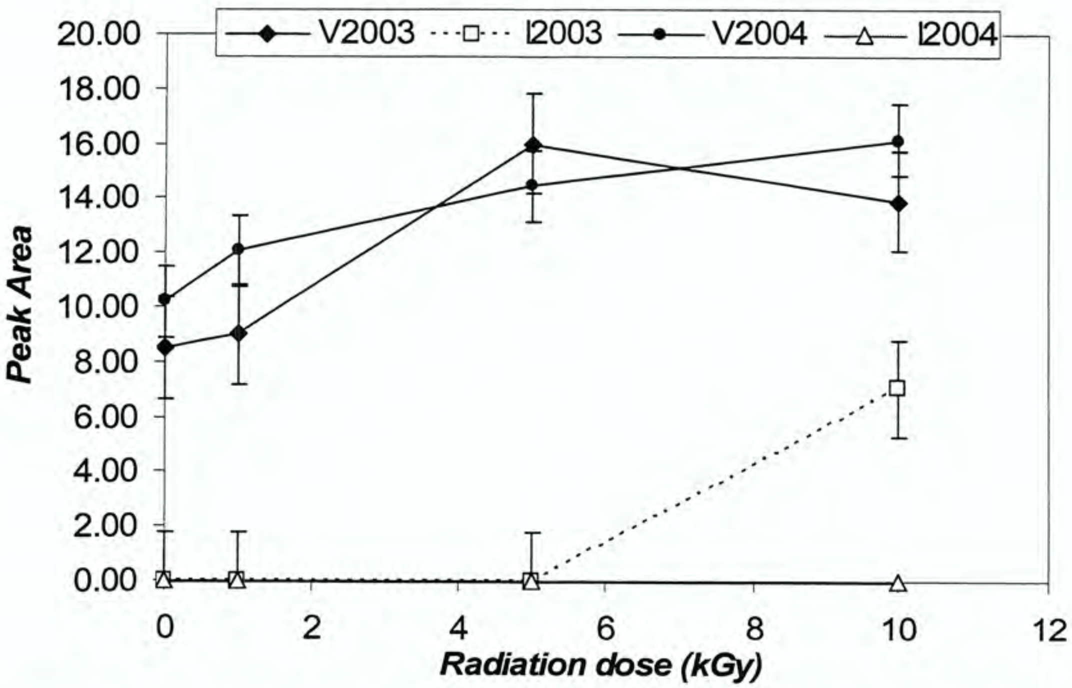


Figure 48. Decanal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

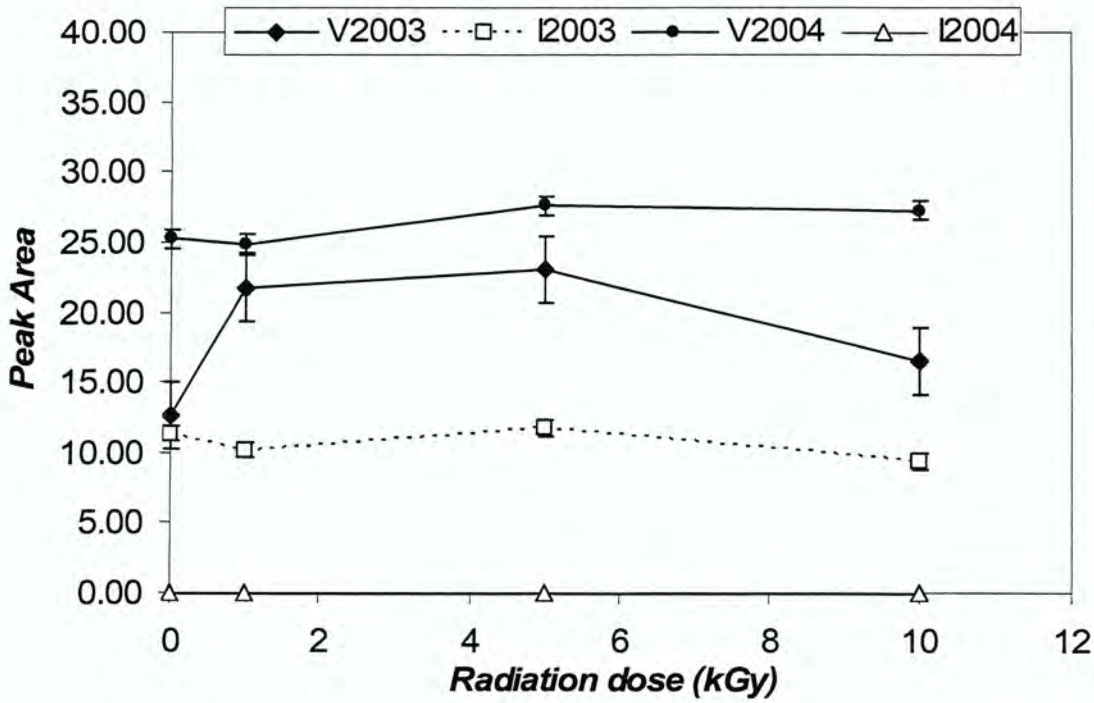


Figure 49. *Trans*-2-Decenal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

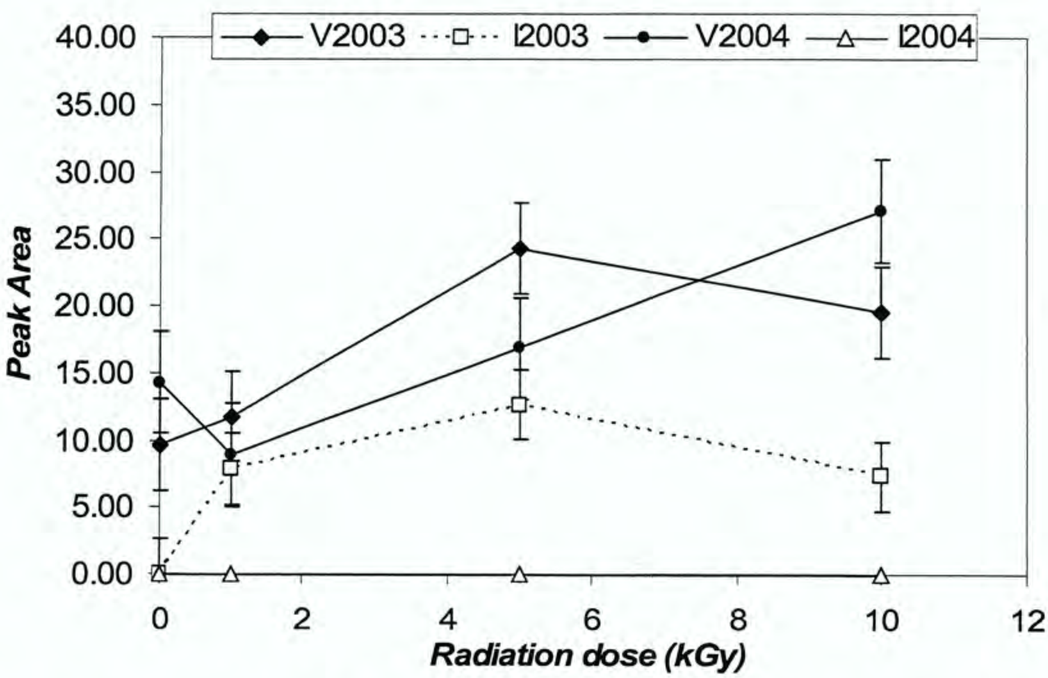


Figure 50. 2-Hexylfuran detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.

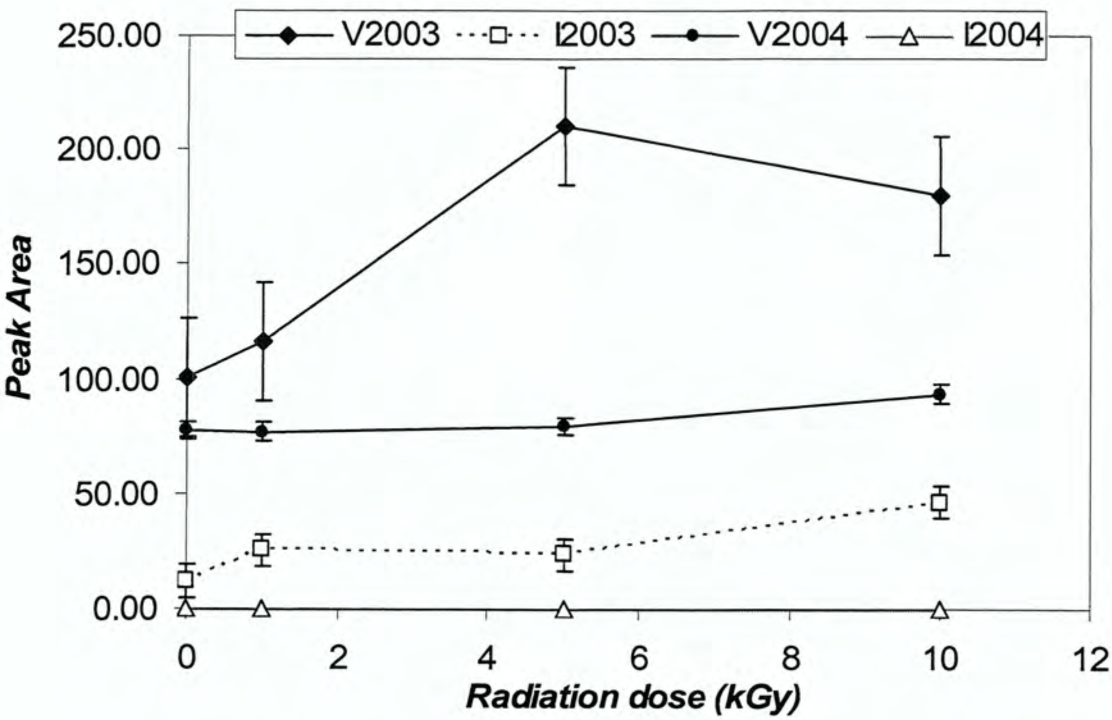
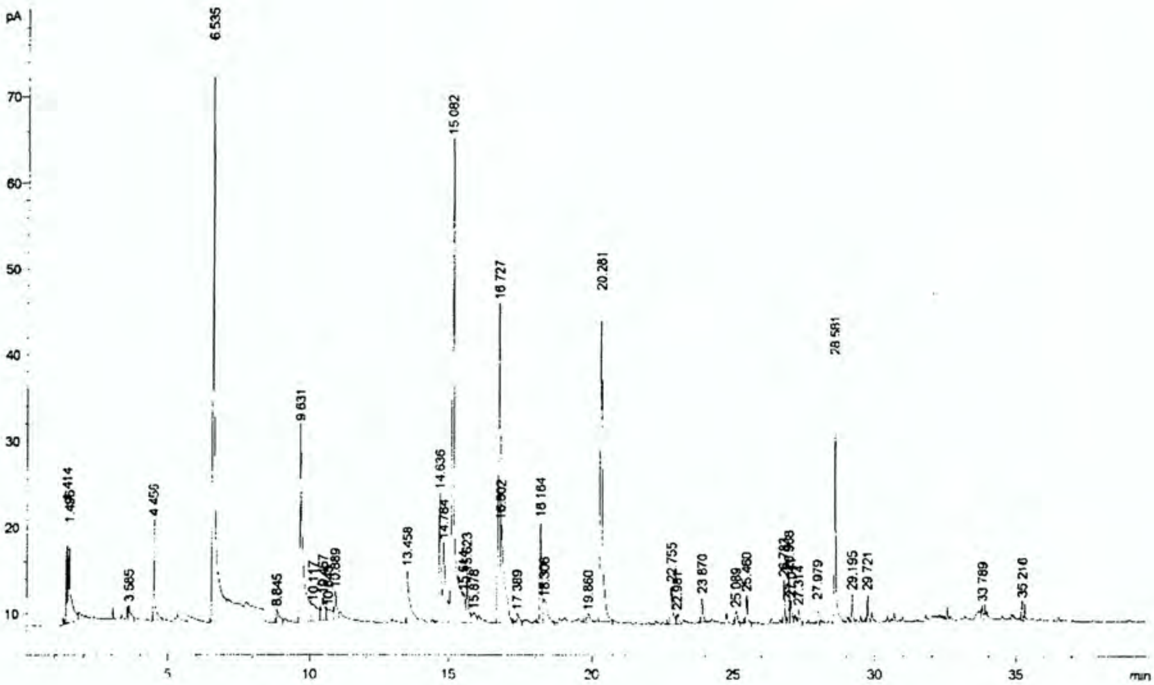
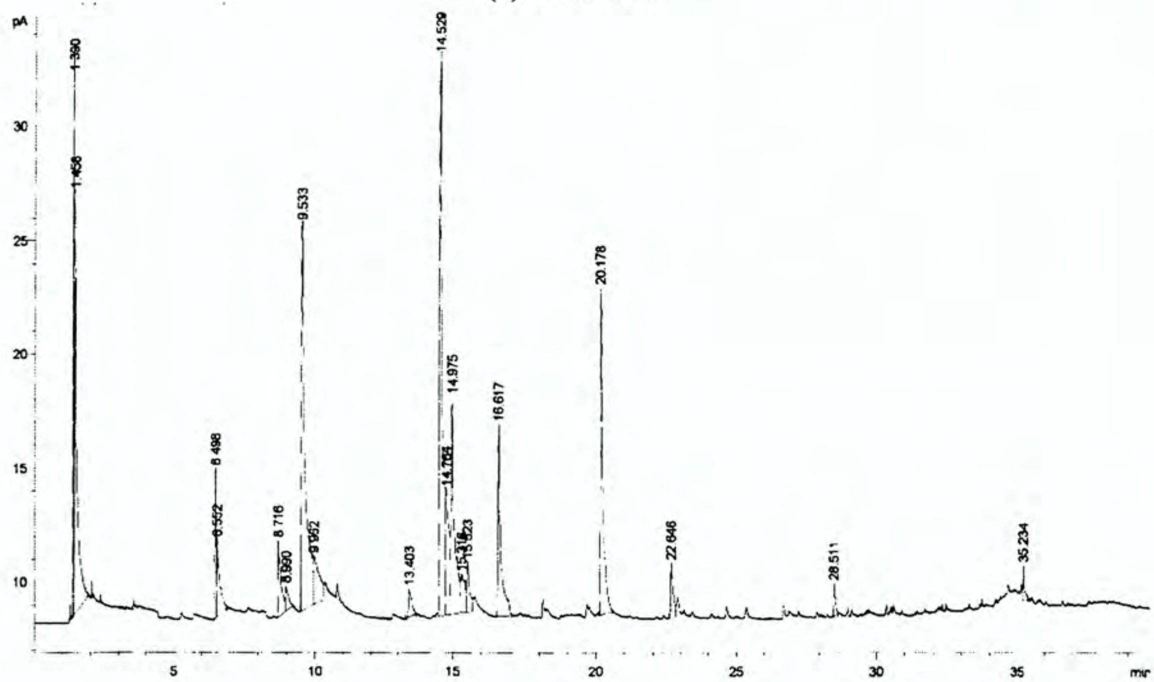


Figure 51. *Trans, trans*-2,4-decadienal detected in soymilk made from e-beam irradiated soybeans (Vinton 81, IA2032LS) at 0, 1, 5, 10 kGy.



(a) Vinton 81



(b) IA2032LS

Figure 52. Gas chromatogram of flavor analysis of two different soybean cultivars (a) Vinton 81, (b) IA2032LS.

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