Efficacy of Soft-electron (Low-energy Electron Beam) for Soybean Decontamination in Comparison with Gamma-rays

O.K. KIKUCHI, S. TODORIKI, M. SAITO, AND T. HAYASHI

ABSTRACT: Soft-electron is a term referring to electrons with energies of 300 kV or lower. Enrei and Vinton soybeans were irradiated with gamma-rays and soft-electrons at 60 keV to compare the effectiveness of the treatments for decontamination purpose. The doses of 20 kGy of gamma-rays and 26 kGy of soft-electrons were effective to decontaminate the soybeans. The gamma radiation changed some properties of the grains: inhibited the germination capacity, increased the lipid oxidation and decreased the lipoxygenase activity, radical scavenging activity and carotenoid content. Soft-electron treatment caused less or none change in soybean quality, being considered a more appropriate procedure for decontamination.

Keywords: soft-electron, soybean, electron-beam, gamma-rays, decontamination

Introduction

SOYBEAN, ONE OF THE MOST IMPORTANT grains in the world, is used in a variety of food products like soymilk, tofu, miso, tempeh, soy sauce, nato, and so on. It is used as an ingredient in biscuits, chocolates, and animal feed too. Soybean protein is considered as a substitute of the animal protein when processed as soymilk and vegetable meat. Beyond the nutritional qualities, many researches indicate that soybean acts as preventive agent against some diseases like cancer, osteoporosis, diabetes, and high cholesterol (Anderson and others 1999, Messina 1999, Hulka and Moorman 2001, Kaneki and others 2001). Moreover, it is considered a unique nutritionally relevant source of isoflavones (Messina 1999) that have radical scavenger and antioxidant properties and can act as inhibitor of lipid oxidation (Cotelle and others 1996).

As in any other food, the presence of microbes in grains causes a decrease in the nutritional value and the formation of undesirable irritant metabolites, toxins, or allergenic spores that can be hazardous to the health. Besides, grains are normally infested by insects, and one of the procedures of disinfestation currently adopted consists of fumigation with methyl bromide, which will be phased out soon due to its depleting action of the Earth's ozone layer. The gamma-radiation treatment efficiency to eliminate insects has been already demonstrated and confirmed by many researches (Hallman 2001). Once the doses required for pasteurization are higher than for disinfestation, the radiation treatment of grains and other foods with pasteurization doses is also effective for disinfestation, and consequently, the fumigation procedure is not necessary.

The effectiveness of soft-electron treatment for decontamination purpose has been verified and confirmed in rice, wheat, and spices by Hayashi and others (1997; 1998a, b) and Hayashi and Todoriki (2000). Todoriki and others (2002) evaluated the sterility and some qualities of soymilk prepared from soft-electron treated soybeans with good results, indicating that the soymilk shelf-life can be extended with 60 keV energy of soft-electrons. It is known that conventional procedures used in the soymilk production like moist/dry heating blanching and wet/dry dehulling processes can affect the characteristics of the final product (Iwuoha and Umunnakwe 1997). Byun and others (1995) reported that the quality of soymilk and tofu prepared from gamma-irradiated seeds was improved with 2.5 kGy and 5 kGy, while 20 kGy was unfavorable to the soymilk color and tofu color, taste, and texture.

Recently, the Nissin-High Voltage Co., Ltd. (NHV, Kyoto, Japan) constructed a Soft Electron Processor with a process capacity of 500 kg/hr of grains, demonstrating the effectiveness to sterilize rice and wheat. Our previous report (Todoriki and others 2002) focused on the shelf-life extension of soymilk and the Tofu-gel production. Moreover, soybean is an important source of fat and fat-soluble compounds like isoflavones. In this paper, we analyze if the treatment using pasteurization dose of soft-electron and gamma-ray preserves also some soybean properties important to soy as a foodstuff. To evaluate it, we consider germination capacity for sprout production; lipid oxidation and pigment content for soymilk, soy oil, and tofu production; lipoxygenase activity responsible by soymilk off-flavor; and radical scavenging activity that prevent oxidation process. The aim of this research is to verify if radiation effects of soft-electrons are lower than that caused by gamma-rays, since penetration of electron beam in the product is small than gamma-ray.

Materials and Methods

Soybean samples

Soybean (Glycine max), Japanese variety Enrei and American variety Vinton harvested in 1999 were kindly gifted by Ryokokushoji Co., Ltd. (Hiroshima, Japan). After the treatments with radiation the samples were stored at 15 °C, for 6 mo.

Treatment with electron-beam

A Van der Graaff electron accelerator (Nissin High Voltage Engineering Co. Ltd.) with electrons at acceleration voltage of 170 kV was used to treat 25 g of soybeans placed in a tray of grain rotator (Hayashi and others 1998a) under a distance of 15 cm of the accelerator window. This rotator was operated at shaking speed of 1 reciprocation per second and a vibrating speed of 10 reciprocations per second. The beam current was 4 mA. The energy reaching the samples was estimated to be 60 keV, based on the stopping power of air and titanium (ICRU 1984).

The dose rate on the surface of the tray was 1.78 kGy/min when Radiachromic film Dosimeter (RCF) (FWT-60, Far West Technology, Inc., Calif., U.S.A.) was fixed on the tray of grain rotator and irradiated with electrons for 60 min (Hayashi and others 1998a). The average absorbed dose by the surface of rotating soybeans was estimated as half the dose absorbed by RCF during same irradiation period.

Treatment with gamma-rays

A gamma-cell 220 (Co-60) (Nordion International Inc., Kanata, ON, Canada) was used to irradiate 50 g of soybeans with a dose rate of 1.4 kGy/h (by Fricke dosimeter).

Determination of the number of microorganisms

Ten grams of soybean were homogenized with stomacher in a filter-bag containing 100 ml of 0.1% peptone and 0.85% NaCl. The homogenate (1 ml) was plated on nutrient agar (Nutrient Agar Nissui; Nissui Seyaku Co., Ltd., Tokyo, Japan), incubated at 30 °C and the colonies formed were counted after 48 h. The presence of coliform bacteria was also detected according to Todoriki and others (2002), by BGLB test and formation of red colonies in dezoxycholate agar.

Germination test

The roll-paper method according to Yano and others (2000) with some modifications was used to measure the sprout root length. Ten seeds were put on duplicated sheet of wet kitchen paper and rolled in. The roll was hung on a vinyl net put in a beaker with a clothespin. The beaker was covered with a vinyl bag and stored in the dark, at 25 °C. The number of germinated seeds and the root length were observed 4 d after.

Soybean powder preparation

Ten grams of soybean grains were grounded in a commercial mill, Shibata SCM-40A and sieved through 40 mesh. About 4 grams of powder were obtained.

Lipid oxidation measurement

Thiobarbituric acid (TBA) test to measure the lipid oxidation was done according to Hayashi and others (1998a). One hundred milligram of soybean powder (sample, sa) was mixed with 0.5 ml of 1.15% KCl, 3.0 ml of 1% phosphoric acid and 1.0 ml of 0.67% 2-thiobarbituric acid. The solution was heated for 45 min in boiling water, cooled, mixed with 4.0 ml of n-butanol and centrifuged at 9000 rpm (9000 *g*) for 10min. The supernatant fraction was used to measure the absorbance at 535 nm and 520 nm. As standard (st) was used 0.5 ml of 10 nmol/ml 1,1,3,3-tetraethoxypropane. TBA value (nmol/g of soybean) was calculated as follows: $(A_{535sa} - A_{520sa})/ (A_{535st} - A_{520st}) \times 5 \times 1000/$ sample weight (mg).

Table 1—Microbial load (CFU/g of grain) of Enrei and Vinton soybean exposed to gamma-rays and soft-electrons (EB, 60 keV).

	Enrei		Vinton	
Treatment	0 mo	6 mo	0 mo	6 mo
Control	4.6×10^3	9.7×10^2	1.7×10^3	2.7×10^2
g-rays, 5kGy	5.0×10^2	—*	1.8×10^2	—
g-rays, 10kGy	2.2×10^1	—	2.4×10^1	—
g-rays, 15kGy	5.0	—	5.0	—
g-rays, 20kGy	3.3	< 10**	< 10	< 10
EB,60keV,9kGy	4.5×10^1	—	5.0×10^1	—
EB,60keV,17kGy	1.7×10^1	—	7.0	—
EB,60keV,26kGy	11	< 10	< 10	< 10

Values are the mean of 6 repetitions (n = 6).
*—: not determined
**< 10: means that no colony was detected.

Determination of lipoxygenase (LOX) activity

LOX activity was measured according to Hafez and others (1985) with some modifications. Ten grams of soybean were soaked in water for 1 h and homogenized in 100 ml water with an ACS Homogenizer (Nihon Seiki Kaisha Ltd.) with 2000 rpm for 5 min. The homogenized sample was centrifuged in 17000 rpm (35000 *g*) for 20 min and 1 ml of the supernatant was added to 50 ml of 0.1M phosphate buffer at pH 7.0 and used as working sample solution. The reaction was done at 25 °C by mixing 0.05 ml of the working sample solution with 2.95 ml of the reaction mixture containing 0.29 μg/ml (1 μMole/ml) of linoleic acid that was used as working substrate. The absorbance was recorded at 234 nm for 24 s. One unit of lipoxygenase corresponded to the amount that caused an increase of 0.001 absorbance unit per minute at 234 nm.

Determination of radical scavenging activity

The free-radical scavenging activities was measured using as a stable free radical 0.2 mM DPPH (1,1-diphenyl-2-picrylhydrazyl, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Four hundred milligrams of soybean powder was submitted to extraction with 10 ml of ethanol. The solution was shaken at a speed of 170 min^{-1} for 20 min in dark condition. The sample was centrifuged at 5000 rpm (3000 *g*) for 10min, at 20 °C, and 4 ml of the supernatant was used to the reaction. At zero time, 4 ml of the DPPH solution was added and the mixture was incubated for 1 h, in the dark, at 25 °C. The absorbance was recorded at 517 nm.

Determination of lutein content

The method of Lichtenthaler (1987) was used to estimate the carotenoid content. The extraction was done with 10 ml of acetone and 1 gram of soybean powder, shaking the solution at a speed of 170 min^{-1} for 20 min and centrifuging at 5000 rpm (3000 *g*) for 10 min. The supernatant absorbance was recorded in a spectrum range of 400 to 500 nm. Lutein (Sigma) was used as standard and the content of lutein in the samples was calculated based on the absorbance at 476 nm.

Statistical analysis

The Graph Pad InStat v2.01 from Graph Pad Software Inc. (San Diego, Calif., U.S.A.) was used to the statistical analysis. One-way analysis of variance (ANOVA) was used to compare the treatments and when significant the Tukey-Kramer test was used.

Results and Discussion

Sterility

The doses of 5, 10, and 15 kGy of gamma-rays and 9 and 17 kGy of soft-electrons did not eliminate efficiently the microorganisms (Table 1). Enrei and Vinton soybeans were effectively sterilized when treated with 20 kGy of gamma-rays and with 26 kGy of soft-electrons with 60 keV energy (Table 1). These 2 doses were established as irradiation doses of the grains to measure the other parameters, except the germination capacity. The coliform bacteria was not detected in the control and treated samples (data not shown).

Germination

Table 2 shows that the germination of soft-electron irradiated seeds was not affected, but 20 kGy of gamma-rays inhibited totally the process. The root development (Table 2) of 4 d soybean sprouts was delayed by 5 kGy of gamma-rays but it was not affected

by the soft-electrons. It means that soft-electrons are also effective to decontaminate seeds used for the production of soybean sprouts, moyashi.

Lipid oxidation

TBA value (Table 3) increased significantly when the soybeans were gamma-irradiated and a little when treated with soft-electrons. After storage for 6 mo, the lipid oxidation of the control and treated Enrei soybean increased a little and the opposite effect occurred with Vinton soybean. Hayashi and others (1998a) also observed that rice treated with gamma and soft-electron beam had an increase of lipid oxidation, but the EB treated rice seeds had lipid oxidation only in the external layer that could be eliminated by milling procedure. The soft-electrons induced less lipid oxidation than gamma irradiation because the electron-beam did not reach the inner of the grain.

Lipoxygenase activity

Lipoxygenase activity (Table 4) was inhibited by gamma-rays, but when treated by soft-electrons, there was no alteration in Enrei soybean and a small decrease in Vinton variety. Our data of gamma-irradiation are in accordance with the results of Hafez and others (1985). Sung and Chiu (1995) also reported that the lipoxygenase activity was inhibited by seed aging, but the lipid oxidation was increased, depending of the storage conditions.

Radical scavenging activity

Radical scavenging activity (Table 5) was preserved in soft-electron treated soybeans, but the gamma-rays decreased the activity mainly in Vinton variety.

The radical scavenging activity using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) evaluates the decrease in absorption of the stable DPPH radical at 517 nm. The bleaching of DPPH absorption occurs when the odd electron of the radical is paired, being representative of the capacity of flavones to scavenge free radicals, independently from any enzymatic activity (Cotelle and others 1996). It can indicate that some isoflavones and other radical scavengers could be affected by gamma-radiation and that, consequently, the capacity of inhibiting the lipid oxidation would be affected. Soft-electron treatment is more advantageous because those important compounds remain intact.

Lutein content

Lutein content (Table 6) of gamma-irradiated soybeans decreased soon after the treatment, but after 6 mo of storage there was not significant change. The control and soft-electron treated samples presented some decrease in the lutein content after the storage but still retained higher levels than gamma-irradiated ones.

Carotenoids are important pigments with antioxidant properties, interacting with free radicals and inhibiting lipid peroxidation (Astorg 1997), and lutein is the major carotenoid component of mature nongreen soybean seed (Monma and others 1994). We observed that the soft-electron treat-

Table 2—Germination percentage (%) and sprout root length (cm) of Enrei and Vinton soybeans 4 d after treatment with gamma-rays (γ-rays) and soft-electrons (EB, 60 keV).

Treatment	Enrei germination (%)	Vinton germination (%)	Enrei root length (cm)	Vinton root length (cm)
Control	97.5 ± 5.0[a]	82.5 ± 5.0[a]	16.18 ± 1.12[a]	16.50 ± 1.12[a]
g-rays, 5 kGy	85.0 ± 17.3[a]	—*	5.28 ± 0.48[b]	—
g-rays, 20 kGy	0	0	0	0
EB, 60 keV, 26 kGy	97.5 ± 5.0[a]	87.5 ± 12.6[a]	16.80 ± 1.42[a]	16.70 ± 1.31[a]

Values are the mean ± standard deviation of 4 repetitions (n = 4). Means with the same letter in columns indicate no statistically significant differences at P ≥ 0.05.
*– : not determined

Table 3—TBA value (nmoles/g of soybean powder) of Enrei and Vinton soybean exposed to gamma-rays (γ-rays) and soft-electrons (EB, 60 keV).

	Enrei (nmoles/g)		Vinton (nmoles/g)	
Treatment	0 mo	6 mo	0 mo	6 mo
Control	103.65 ± 7.65[a]	109.88 ± 4.54[a]	154.70 ± 9.81[a]	150.97 ± 4.40[a]
g-rays, 20kGy	144.00 ± 1.23[b]	147.67 ± 0.59[b]	229.47 ± 9.68[b]	204.46 ± 4.73[b]
EB,60 keV,26 kGy	113.51 ± 1.99[a]	123.55 ± 6.99[c]	162.83 ± 7.02[a]	153.49 ± 0.82[a]

Values are the mean ± standard deviation of 3 repetitions (n = 3). Means with the same letter in columns indicate no statistically significant differences at P ≥ 0.05.

Table 4—Lipoxygenase activity (units/mg grain) of Enrei and Vinton soybean treated with gamma-rays (γ-rays) and soft-electrons (EB, 60 keV).

	Enrei (units/mg)		Vinton (units/mg)	
Treatment	0 mo	6 mo	0 mo	6 mo
Control	1825.58 ± 86.54[a]	1310.34 ± 134.00[a]	2040.93 ± 35.20[a]	1635.89 ± 24.50[a]
γ-rays, 20 kGy	1353.32 ± 68.48[b]	643.00 ± 70.44[b]	1522.44 ± 135.98[b]	556.33 ± 12.44[b]
EB,60 keV, 26 kGy	1875.20 ± 67.53[a]	1454.11 ± 30.74[a]	2004.67 ± 40.79[a]	1380.78 ± 26.10[c]

Values are the mean ± standard deviation of 3 repetitions (n = 3). Means with the same letter in columns indicate no statistically significant differences at P ≥ 0.05.

Table 5—Enrei and Vinton soybean DPPH radical scavenging activity (%) after treatment with gamma-rays (γ-rays) and soft-electrons (EB, 60keV).

	Enrei (%)		Vinton (%)	
Treatment	0 mo	6 mo	0 mo	6 mo
Control	45.13 ± 0.46[a]	38.88 ± 0.28[a]	40.32 ± 0.78[a]	36.20 ± 0.53[a]
γ-rays, 20kGy	42.80 ± 0.50[b]	37.03 ± 0.70[b]	32.71 ± 0.96[b]	30.69 ± 0.17[b]
EB,60keV,26kGy	44.71 ± 0.33[a]	39.81 ± 0.33[a]	38.26 ± 0.12[c]	35.98 ± 0.46[a]

Values are the mean ± standard deviation of 3 repetitions (n = 3). Means with the same letter in columns indicate no statistically significant differences at P ≥ 0.05.

Table 6—Lutein content (mg/g powder) of Enrei and Vinton soybean treated with gamma-rays (γ-rays) and soft-electrons (EB, 60keV).

	Enrei (μg/g)		Vinton (μg/g)	
Treatment	0 mo	6 mo	0 mo	6 mo
Control	6.87 ± 0.25[a]	6.58 ± 0.42[a]	12.17 ± 0.12[a]	10.68 ± 0.09[a]
γ-rays, 20kGy	2.99 ± 0.03[b]	3.07 ± 0.13[b]	5.20 ± 0.08[b]	5.89 ± 0.24[b]
EB,60keV,26kGy	7.09 ± 0.15[a]	5.54 ± 0.13[c]	13.44 ± 0.32[c]	9.91 ± 0.22[c]

Values are the mean ± standard deviation of 3 repetitions (n = 3). Means with the same letter in columns indicate no statistically significant differences at P ≥ 0.05.

ment did not change the color of the powder and homogenated soy.

Conclusion

THE TREATMENT WITH SOFT-ELECTRONS is more recommended for soybean decontamination than gamma-irradiation procedure, because it causes minimum or no quality deterioration of the product, since the soft-electrons do not reach the internal structures. No toxic agent is produced or released to the environment and no chemical residue is left in the treated product. As soft-electron treatment does not inhibit the germination process, it can be used to decontaminate seeds for sprout production, like moyashi (soybean sprout). Another advantage is that electrons with such low energy do not require a thick safety shield due to their low penetration capacity, thus enabling less expensive inline food processing plants.

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MS 20020493 Submitted 8/28/02, Revised 10/3/02, Accepted 11/4/02, Received 12/4/02

The present work was carried out at National Food Research Institute and Japan International Research Center for Agricultural Sciences. The authors are grateful to Dr. Sayuki Nikkuni of JIRCAS and Msc. Jose Renato Bordignon of the Brazilian Agricultural Research Cooperation (EMBRAPA-Soybean) for helping in the microbiology experiments. The first author was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), an entity of Brazilian Government dedicated to the science and technology development.

Author Kikuchi is with the Institute of Nuclear and Energy Research (IPEN-CNEN/SP), Travessa R 400, São Paulo-SP, Brazil, ZIP 05508-900. Author Todoriki is with the National Food Research Institute (NFRI), 2-1-12 Kannondai, Tsukuba, Ibaraki, Japan, ZIP 305-8642. Authors Saito and Hayashi are with the Japan International Research Center for Agricultural Sciences (JIRCAS), 1-2 Ohwashi, Tsukuba, Ibaraki, Japan, ZIP 305-8686. Direct inquiries to author Todoriki (E-mail: setsuko@nfri.affrc.go.jp)