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EFFECT OF IRRADIATION AND DIELECTRIC HEATING ON SOYBEAN ULTRASTRUCTURE, TRYPSIN INHIBITOR, AND LIPOXYGENASE ACTIVITIES

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Abstract

Scanning electron microscopy (SEM) was used to observe the protein bodies of soybeans after irradiation (0-10 kGy dose range), dielectric heating and storage. Microbiological changes, lipoxygenase and trypsin inhibitor activities and biological value were also studied after different treatments and storage. There were no differences in the ultrastructure of control and irradiated (3, 6 and 10 kGy) soybeans immediately after irradiation. The effect of storage depended on whether whole or milled soybeans had been stored. There was no direct effect of dielectric heating on soybeans ultrastructure, however, during storage it changed the same way as the milled and irradiated soybean. Irradiation reduced the number of microbes, but had no effect on the biological values or the activities of trypsin inhibitor and lipoxygenase (0-10 kGy). Dielectric heating had no effect on microbes, but inactivated the trypsin inhibitor and lipoxygenase and increased the biological values of the soybeans.

Introduction

Most soybeans are processed for oil and high protein meal which are important constituents of both human and animal diets. Raw soybeans contain many biologically active factors. Trypsin inhibitor and lipoxygenase are major factors responsible for poor protein digestibility and a beany off-flavour, respectively (Tanteeraratarm et al., 1989; Asbi et al., 1989). Gamma irradiation has long been known as a method of food preservation (reduction of microbial contamination, extension of shelf life). Gamma radiation also effects protein solubility, trypsin and chimotrypsin inhibitors, lipoxygenase activities, and fatty acid composition in soybean seeds (Hafez et al., 1985; Hafez et al., 1989). However, thermal denaturation of lipoxygenase and trypsin inhibitor is more effective than irradiation (Wang and Toledo, 1987; Alsoe and Adler-Nissen, 1988; Yoshida and Kajimoto, 1989).

Dielectric heating (high frequency) treatments at 42 and 2450 MHz, of intact soybeans containing only their innate moisture can produce products of potentially high nutritional value. The biological properties of soybeans treated by dielectric heating are dependent on the minimum energy absorbed (Demeczky, 1982). Trypsin inhibitor, urease and lipoxygenase activities are all reduced to low levels by treatments. Protein solubility and dispersibility are reasonable indicators of trypsin inhibitor activity for dry soybeans exposed to dielectric heating. Some treated samples with low trypsin inhibitor and lipoxygenase activities still retained relatively high peroxidase activities that may be advantageous for bleaching effects (Pour-El et al., 1981). Borchers and coworkers (1972) used dielectric heating for rapid inactivation of trypsin inhibitor and urease and at the same time the water solubility of the soybean protein decreased.

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KEY WORDS: soybean, irradiation, dielectric heating, storage, scanning electron microscopy (SEM), lipoxygenase, trypsin inhibitor, nutritive value, microbes

The present study was conducted on soybean to determine the effects of gamma radiation and dielectric heating on microbiological counts, ultrastructure, trypsin inhibitor and lipoxygenase activities, and biological value.

Materials and Methods

Raw material

Soybean variety McCall, grown in Hungary was used.

Irradiation conditions and dielectric heating

The irradiation was carried out in the Institute of Isotopes of the Hungarian Academy of Sciences with a K-120 type panoramic Co-60 gamma-source /activity 3,7 PBq, dose rate 1 kGy.h⁻¹). The dielectric (high frequency) treatment was carried out in the CPRI with Brown Boveri equipment of 10 kW useful capacity, 27.12 MHz frequency, having a horizontal treatment area. The anode voltage was 10 kV, the final temperature 110°C, and the absorbed electric energy 0.18 kWh.kg⁻¹ soybean (Wetzell and Zehnder, 1980).

Storage

The flour was stored for 10 months at room temperature, before investigation. The whole beans, treated and untreated, were in glass jars of 5 kg, covered with linen cloth and stored at ambient (20-25°C) temperature for 8 months. The samples were milled before the investigations.

Investigations

a/ Investigation immediately after irradiation (Irr) (0, 3, 6 and 10 kGy). Samples were milled after irradiation, before scanning electron microscopy (SEM) (fresh Irr soybean).

b/ Irradiated (0, 3, 6 and 10 kGy) soybeans were stored for 8 months, then investigated. Samples were milled before SEM (stored Irr soybean).

c/ Soybeans were irradiated (0, 2, 4, 6, 8.5 and 10 kGy) milled and the flour was stored for 10 months then investigated (Irr and stored soyflour).

d/ Soybeans were irradiated (0, 2, 4, 6, 8.5 and 10 kGy), milled, defatted then stored for 10 months before investigation (Irr, stored and defatted soyflour).

e/ Soybeans were dielectrically heated (DH), milled and investigated (fresh DH soybean).

f/ Soybeans were DH treated, then stored for 8 months, milled and investigated (stored DH soybean).

g/ Soybeans were irradiated (3 kGy), DH treated (combined treatment), stored for 8 months, and then milled and investigated (Irr+DH+stored soybean).

h/ Soybeans were irradiated (3 kGy), stored for 8 months then DH treated. Samples were milled before investigation (Irr + stored + DH).

Microbiological investigation

20 g whole soybean was added to 80 cm³ peptone water (9 g NaCl, 1 g peptone, 1 cm³ Tween 80, 1000 cm³ distilled water), shaken for 10 min in an ELPAL Shaker type 357, then serial dilutions were made and the mesophilic aerobic plate count, aerobic spore count and mold count were determined (Kiss, 1984).

SEM

Three samples were taken from milled soybeans. The samples were then coated with gold in a Zeiss HBA vacuum evaporator and examined in a JEOL JSM 25-SII type scanning electron microscope at 12.5 kV accelerating voltage. Pictures were taken automatically.

Determination of lipoxygenase activity

Lipoxygenase activity was assayed by the method of Hafez et al. (1985), using linoleic acid as substrate. Soybean samples were defatted prior to assay. Analysis of variance was applied in the evaluation.

Determination of nutritional value

Net protein utilization (NPU), net protein ratio (NPR), true digestibility (TD) and biological value (BV) were determined (Hegedüs et al., 1981). The basic test feed contained soya oil (15 %), potato starch (10 %), glucose (15 %) vitamin premix (2.0 %), mineral premix (0.5 %), NaCl (0.5 %), corn starch (varying between 17.0 and 21.8 % to adjust the weight of feed), and the test samples (35.2-40.0 %) as protein sources. Whole egg powder was used as protein source for the control group. Another control group feeding on protein-free diet was also applied. Twenty one day-old male, albino rats of Sprague-Dawley CFY strain were used, ten in each group. The feeding period was 10 days.

Determination of trypsin inhibitor activity

The method of Kakade and coworkers (1974) was applied. The milled powders were defatted with petroleum ether prior to assay.

Results and Discussion

Microbiology

A relatively low dose of irradiation (3 kGy) reduced the number of vegetative bacteria and spores, but had no direct effect on molds (Fig. 1). The dielectric heating also had no direct effect on microbes, however during

Irradiation and dielectric heating on soybean

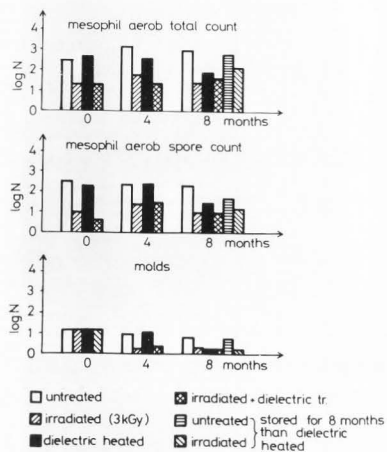


Fig 1. The effect of irradiation and dielectric heating on microbes of soybeans.

storage there was a slow decrease in all counts, irradiation being the main factor. When the untreated and irradiated soybeans stored for 8 months were treated with a high frequency, the low effect of dielectric heating was also obvious.

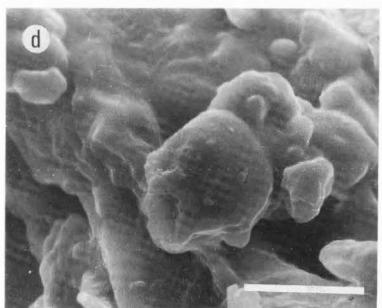
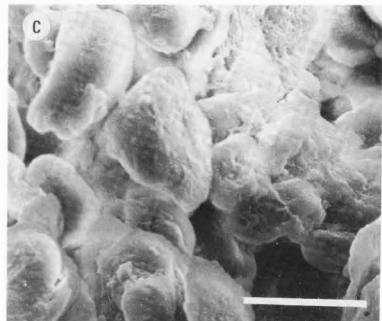
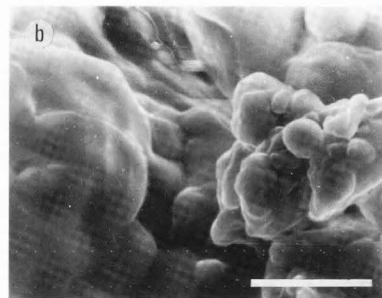
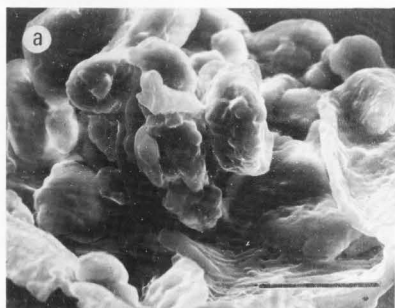


Fig 2. Soybeans immediately after irradiation (Samples were milled after irradiation (a: control; b: 3 kGy; c: 6 kGy; d: 10 kGy). Bar 10 μ m

The results concerning the effect of irradiation are in good agreement with those of Beczner and coworkers (1983), who found that irradiation up to 5 kGy reduced the cell count; however, the favourable effect becomes effective only under low temperature and low moisture and/or equilibrium relative humidity conditions. The fungi are generally more resistant to radiation than bacteria, their growth can be retarded by this relatively low dose of irradiation for a short storage period only; retardation time is strongly dependent on storage conditions.

Seed coats play a significant role in the relationship between structure and quality of legumes. The seed coat is an effective barrier to fungi under normal storage conditions. Seed coat defects such as cracks occur naturally and also result from harvesting or subsequent handling of the seed (Swanson et al., 1985).

Some soybeans have damaged areas in their seed coats, in which the palisade cell and hourglass cell layers are pulled apart (Wolf and Baker, 1980). Similar cracks can be formed in intact seed coats by soaking the beans in water and then air drying them. Because of the openness of the hourglass cell layer, damage of this type permits rapid penetration of moisture into the interior of the seed coat and is also a likely site for entry of insects and microorganisms (Wolf et al. 1981).

SEM

In Fig. 2 soybean flour can be seen immediately after irradiation. There were no differences among the 0, 3, 6 and 10 kGy treatments (Fig. 2a, 2b, 2c and 2d). The cotyledon cells of soybean cannot be distinguished clearly. Surface details appear to be obscured by a layer of lipid-like material. Round protrusions are seen which are thought to be protein bodies. Allen and Arnott (1981) observed a similar structure in untreated sunflower cotyledon surfaces. The control (Fig. 3a) and irradiated (Fig. 3b and 3c) soybeans, milled and defatted showed that the oil from lipid bodies and left only the cytoplasmic network.

Our results are in good agreement with those of Wolf and Baker (1980), who investigated freeze fractured soybeans. They established, that protein bodies were covered with a sponge-like cytoplasmic network with embedded lipid bodies. Hexane dissolved the oil from lipid bodies and left only the cytoplasmic network, with depressions corresponding to the former location of the lipid bodies.

In a further experiment soybean seeds were irradiated, milled (stored as flour) and investigated 10 months later (Fig. 4). Control (Fig. 4a) and 10 kGy (Fig. 4e) samples did not change during storage. Small pits are seen on the surface of protein bodies of 2, 6 and 8.5 kGy samples (Fig 4b, 4c, 4d).

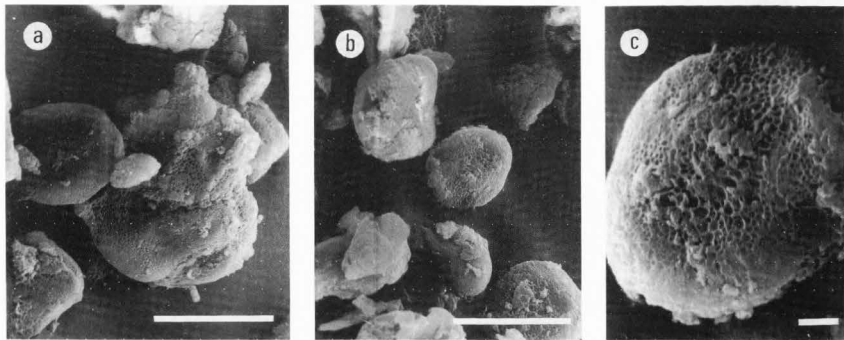


Fig 3. Defatted soybean flour, milled immediately after irradiation of whole beans (a: control; b,c: 3 kGy). Bar: 10 μ m (a, b), 1 μ m (c)

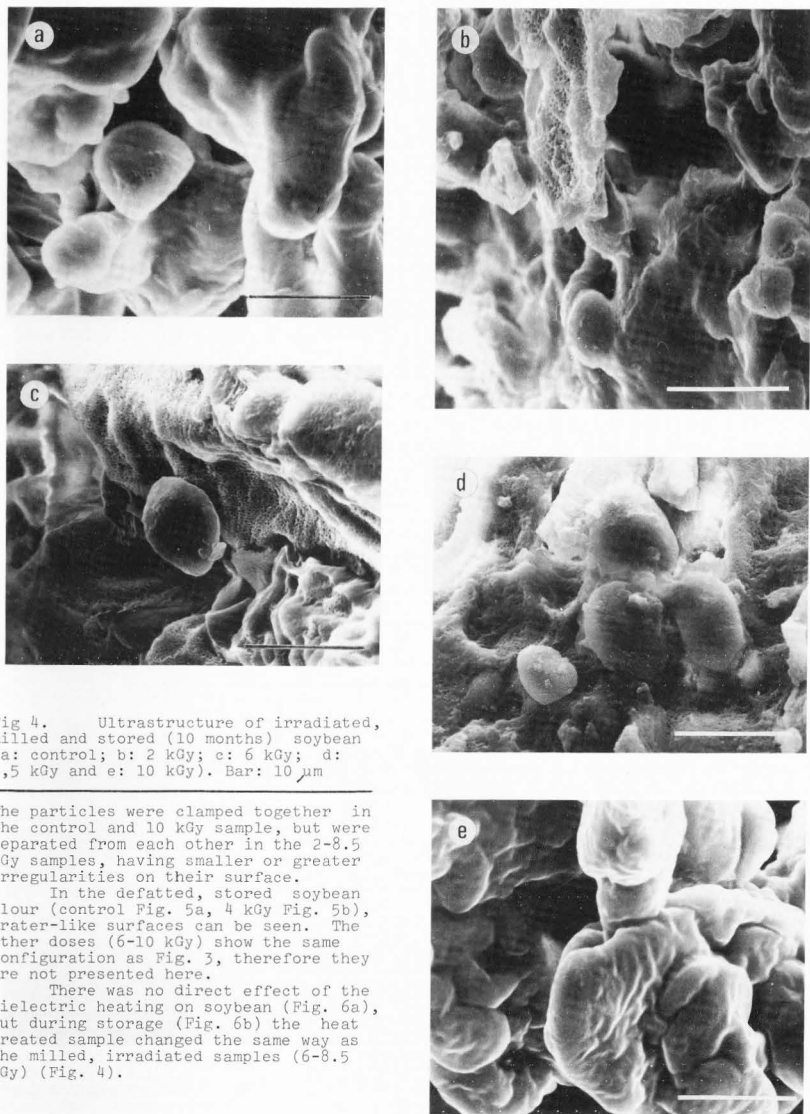


Fig 4. Ultrastructure of irradiated, milled and stored (10 months) soybean (a: control; b: 2 kGy; c: 6 kGy; d: 8,5 kGy and e: 10 kGy). Bar: 10 μ m

The particles were clamped together in the control and 10 kGy sample, but were separated from each other in the 2-8.5 kGy samples, having smaller or greater irregularities on their surface.

In the defatted, stored soybean flour (control Fig. 5a, 4 kGy Fig. 5b), crater-like surfaces can be seen. The other doses (6-10 kGy) show the same configuration as Fig. 3, therefore they are not presented here.

There was no direct effect of the dielectric heating on soybean (Fig. 6a), but during storage (Fig. 6b) the heat treated sample changed the same way as the milled, irradiated samples (6-8.5 kGy) (Fig. 4).

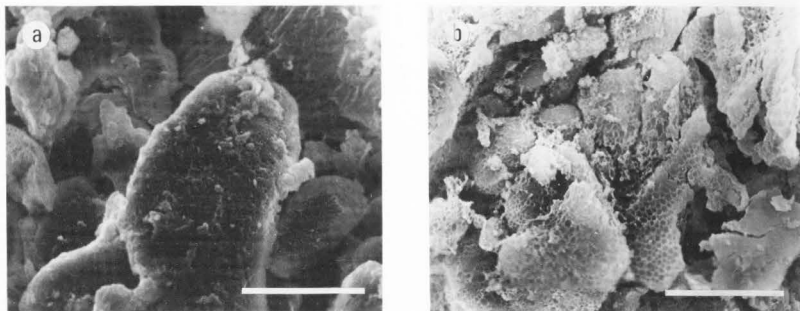


Fig 5. Ultrastructure of irradiated, milled, defatted and stored (10 months) soybean (a: control; b: 4 kGy). Bar: 10 μ m

There were no differences among the control and irradiated samples (Fig. 7a-c) after 8 months of storage.

The effect of combined treatment on the ultrastructure of soybeans depended on the chronological order of treatments, only, when the soybeans were stored (Fig. 8). The dielectric heat treatment caused structural changes in the sample (Fig. 8a) during storage. The nutritional value of this sample (NPU, BV and NPR) was higher after treatment and after storage, too, as it will be dis-

cussed later. It seems that the structural changes influenced the nutritional value of soybean.

Lipoxygenase activity

Our results are in good agreement with those of others (Pour-El et al., 1981; Hafez et al., 1985; 1989). Irradiation (0-10 kGy) in this study had no effect on lipoxygenase activity, whereas the dielectric heating inactivated it (Table 1) (Yoshida and Kajimoto, 1989; Wang and Toledo, 1987).

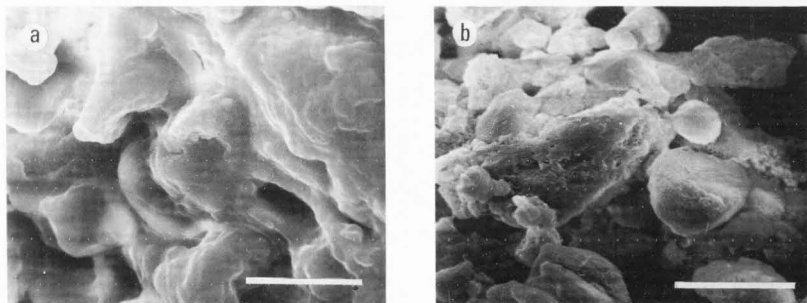


Fig 6. Ultrastructure of dielectric heated soybean immediately after treatment, and after storage (a: fresh; b: stored). Bar: 10 μ m

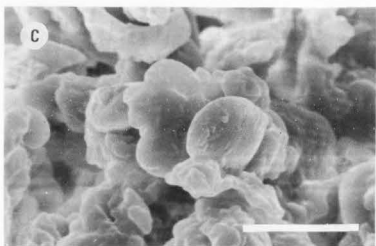
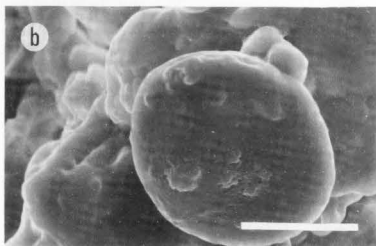
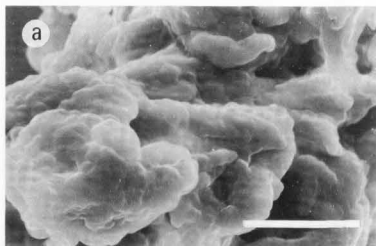


Fig 7. The effect of storage time (8 months) on the ultrastructure of control and irradiated samples (3 and 6 kGy). (a: control, b: 3 kGy and c: 6 kGy). Bar 10 μ m

Fig 8. Ultrastructure of combined treated (dielectric heating and irradiation) and stored soybean. (a: irradiated and dielectrically heated then stored for 8 months; b: irradiated (3 kGy) then stored for 8 months and then dielectrically heated). Bar 10 μ m

Table 1

Lipoxygenase activity of soybeans as a function of treatments (stored for 10 months).

Treatments	Lipoxygenase activity (units. mg ⁻¹ defatted solid)	
	\bar{x}	s
0 kGy	45.3	± 6.5
3 kGy	30.3	± 6.8
6 kGy	35.0	± 6.2 x
10 kGy	43.0	± 7.2
DH	4.0	± 1.2 xx
I + DH	10.7	± 1.5 xx

LSD_{95%} 9.8 ; LSD_{99%} 13.7

DH: Dielectrically heated, I + DH: Irradiated and dielectrically heated. Significant at 5% (x) and at 1% (xx) level; \bar{x} : average; s: standard deviation.

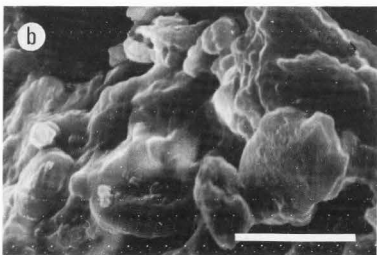
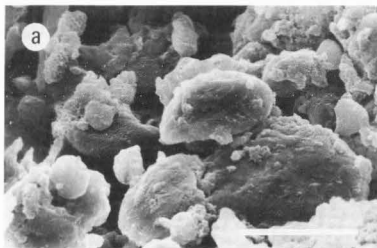


Table 2

Nutritional properties of irradiated and dielectrically heated soybeans

Treatment	NPU		BV		TD		NPR	NSI		
	AB (%)		AB (%)		AB (%)					
(a) Fresh (0 month)										
	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s
A	36.4 [±] 1.4		49.1 [±] 1.3		74.3 [±] 0.7		16.5 [±] 0.1		64.5 [±] 1.1	
B	35.3 [±] 2.7		46.4 [±] 5.6		76.3 [±] 3.4		14.7 [±] 0.1		62.6 [±] 7.7	
C	<u>52.1[±]1.2</u>		65.3 [±] 0.4		79.8 [±] 1.4		19.9 [±] 0.1		22.5 [±] 1.5	
D	47.4 [±] 3.0		61.5 [±] 3.0		77.1 [±] 1.1		20.7 [±] 2.2		24.0 [±] 1.0	
S	96.4 [±] 2.2		103.9 [±] 1.7		92.7 [±] 0.7		35.0 [±] 0.1			
(b) Stored (8 months)										
A	40.8 [±] 12.2		49.6 [±] 16.4		82.8 [±] 2.8 x		16.1 [±] 4.6		54.4 [±] 2.2	
B	43.2 [±] 6.1		53.6 [±] 10.3		80.7 [±] 4.8		17.4 [±] 2.2		50.6 [±] 2.2	
C	<u>67.3[±] 0.9</u> x		<u>80.6[±] 0.3</u>		81.2 [±] 2.5		<u>28.1[±]0.7</u> xx		17.9 [±] 0.6	
D	<u>69.2[±] 5.1</u> xx		<u>80.8[±]15.4</u>		80.2 [±] 1.1		<u>27.0[±]0.8</u> xx		15.5 [±] 0.6	
Analysis of variance (treatments and storage) NPU, BV, TD, NPR separately										
\bar{x} : average; s: standard deviation ;										
LSD _{95%}	15.0		20.9		5.4		4.6			
LSD _{99%}	21.8		30.4		7.8		6.7			

AB = Mean of subgroup A and B

NPU= Net protein utilization

BV = Biological value

TD = True digestibility

NPR= Net protein ratio

A= Untreated

B= Irradiated (3 kGy)

C= Dielectric heated

D= Irradiated than dielectric heated (18)

S= Standard (egg protein)

The underlinings mean significant differences between control and treated samples

x, xx The asterisks mean significant differences between fresh and stored samples

Nutritional value

Results of the feeding tests (Table 2) showed that the raw and irradiated soybeans hardly increased the body weight of the test animals. However, the soybeans treated with dielectric heating alone or in combination with irradiation showed a significant effect. On the basis of NPU, BV, TD and NPR value it can be stated that the biological value of the raw and irradiated soybeans was much lower than that of the standard egg protein.

Concerning the protein value, the treatments involving dielectric heating were more favourable; they improved digestibility and the biological value (Table 2a and b).

The different treatments influence the technological properties and biological values of soybeans (non-reversible aggregation during heating to 110 and 120° C (Lewis, 1986)). The age and storage of soybeans influence both (Table 2b). Loss of membrane integrity may be related to increased peroxidation within the cytoplasm (Swanson et al., 1985). Aggregation and gelation developed with increased fragmentation of soybean protein (Fuke et al., 1985).

Table 3

Trypsin inhibitor activity of soybeans as a function of irradiation (measured within 1 month after treatment)

Dose (kGy)	Trypsin inhibitor activity (mg.g ⁻¹) dry material	
	\bar{x}	s
0	94.0 ± 5.1	
3	93.8 ± 3.2	
6	87.8 ± 8.3	
10	96.4 ± 11.6	

LSD_{99%} 16.7; \bar{x} : average; s: standard deviation

Trypsin inhibitor activity

Our results showed that the trypsin inhibitor activity does not change as a function of radiation treatment up to 10 kGy (Table 3), but decreased greatly on dielectric heating (Table 4).

Increased irradiation dose to 100 kGy caused a 25 % decrease in trypsin inhibitor activity. Similar reduction was observed when the moisture content was increased and the radiation dose was lower (19 kGy) (Hafez et al., 1985).

Table 4

Trypsin inhibitor activity of soybeans as a function of treatment and storage

Treatments	Trypsin inhibitor activity (mg.g ⁻¹ dry material)	
	\bar{x}	s
fresh	A	75.1 ± 1.6
	B	76.1 ± 2.3
	C	27.9 ± 6.8 xx
	D	19.8 ± 2.4 xx
	E	19.8 ± 2.4
stored (8 months)	\bar{x}	
	s	
	A	81.7 ± 14.7
	B	78.8 ± 18.2
	C	17.8 ± 1.2 xx
D	24.0 ± 2.8 xx	
E	23.8 ± 0.9 xx	

LSD_{95%} 13.6; LSD_{99%} 18.5

A: Control; B: Irradiated (3 kGy); C: Dielectrically heated; D: Irradiated and dielectrically heated, E: Irradiated (3 kGy) stored for 8 months then dielectrically heated (compared with D) Significant at the 1% (xx) level

Conclusions

Irradiation (0-10 kGy) does not influence the ultrastructure of soybean seeds neither immediately, nor after storage (8 months). If the irradiated sample was milled then stored, the ultrastructure changed (in the dose range of 6-8.5 kGy) the same way as in samples dielectrically heat treated and stored.

Irradiation reduced and kept the number of microbes low on soybeans. Irradiation has no effect on lipoxygenase and trypsin inhibitor activities, so we propose to use additional treatments i.e. dielectric heating, which inactivates both of them. A dose of 3 kGy does not influence the biological value of soybean, but dielectric heating increases it. We think that heat treatment (microwave or dielectric heating) is more favourable to improve nutritional values of soybean than irradiation, but irradiation has high economic importance in the elimination of insects (at doses less than 1 kGy) or inhibition of fungi under extreme storage conditions. Practically irradiation and heat treatment should be combined to gain a favourable results.

Acknowledgements

We thank Ms I. Csányi for the help with SEM, the Biological Department of CPRI for the animal feeding test and Ms Zs. Márkus for the dielectric heat treatment.

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Discussion with Reviewers

W.J. Wolf: How is NPR defined?

Authors: The NPR index shows the difference in body weight between rat groups feeding on test-feed and protein-free feed, referred to the amount of nitrogen consumed by the test group (Campbell, J.A. (1963). Method for determination of PER and NPR. In: Evaluation of Protein Quality. Nat. Res. Council Publ. 1100 Washington, 31. p.).

W.J. Wolf: Dielectric heating inactivated lipoxygenase and trypsin inhibitors as well as decreased the NSI values and yet did not reduce the microbial contamination. If the ambient moisture was sufficient to proteins, why weren't the microbes killed?

H.E. Snyder: The lack of dielectric heating on microbial population might be due to the dryness of the soybeans. But dielectric heating does have an effect on lipoxygenase and trypsin inhibitor. How do you account for the heating apparently destroying proteins but not having an influence on the microorganisms present?

Authors: The heat and radiation resistance of microbes is different from that of plant or animal cells. In addition, certain food components have protective effect against heat and radiation.

H.E. Snyder: Why should irradiation or storage cause changes in soybean flour structure that mimic lipid extraction?

Authors: No explanation can be given at the moment. Probably due to enzymic changes the structure of lipids has changed and an artifact forming during the preparation for SEM has been noticed.