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Technical note

Effects of gamma radiation on total phenolics, trypsin and tannin inhibitors in soybean grains

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Abstract

The objective was determining possible radiation-induced alterations (with doses of 2, 4 and 8 kGy) in raw or cooked grains from five soybean cultivars through the analysis of some antinutrient. Total phenolic ranged from 2.46 to 10.83 mg/g, the trypsin inhibited from 18.19 to 71.64 UTI/g and tannins from 0.01 to 0.39 mg/g. All the antinutrient studied underwent reduction with increases in the doses and cooking process was effective too.

Keywords: Gamma radiation; Antinutritional factors; Soybean; Total phenolic compounds; Trypsin and tannin inhibitors

1. Introduction

Proteins originating from leguminous seeds correspond to 20% of vegetal proteins consumed by man (Vasconcelos et al., 2001). However, some legumes, particularly soybeans, contain significant amounts of bioactive compounds that may change the utilization of nutrients by the organism, when consumed. The main protein responsible for the low nutritional value of raw soybean grains are the trypsin and lectin inhibitors, but there are other natural compounds that may contribute for the harmful effects observed.

In order to minimize losses occurring during harvest and storage of these grains, the radiation process emerges as an attractive and healthy alternative when compared to chemical conventional treatments. The application of ionizing radiation with the objective of preserving and disinfesting grains emerges as promising practice used to extend the shelf life and to reduce losses of grains during storage. The estimated costs and benefits obtained with the commercial radiation seem to be competitive when compared to other fumigation methods and physical and thermal treatments.

The sum of observations exposed in previous chapters leads to the proposal of determining the possible alterations promoted by different cultivars through the use of gamma radiation in the alteration of antinutrients as well as evaluating the after-cooking behavior.

2. Methods and materials

2.1. Material

The cultivars used in the present work are products of the EMBRAPA Soybean—Londrina/PR and are part of the 2003/2004 agricultural harvest as follows: BRS 212, BRS 213, BRS 214, BRS 231 and E48.

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2.2. Irradiation

The raw grains were treated with gamma rays at doses of 0, 2, 4 and 8 kGy. Multipurpose gamma irradiator with a Cobalt^{60} source was used (compact-type commercial radiator) at the Nuclear and Energetic Researches Institute (IPEN) in the city of São Paulo, Brazil. The dose effectively received by the material ranged from 2.09 to 3.02 in samples radiated with 2 kGy, from 3.50 to 5.08 for those radiated with 4 kGy and from 7.04 to 9.12 for those radiated with 8 kGy, measured through the Amber dose meter Batch: P., spectrophotometer Genesis – 20, reading 603 nm and at a temperature of 35 °C.

2.3. Cooking of grains

The samples were soaked for 10 h in distilled water in the proportion of 1:3; the water was changed by distilled water in the proportion of 1:2. The grains were cooked in autoclave at 121 °C for 10 min (Molina et al., 1975). After cooking, the samples were placed in aluminum trays and dried in stove with forced circulation of air at 50–55 °C until constant weight (approximately 24 h).

2.4. Determination of the phenolic compounds, trypsin inhibitors and tannins

The determination of the total phenolic concentration was performed according to methodology described by Swain and Hillis (1959), the trypsin inhibitory activity was determined according to Kakade et al. (1969), using benzoyl-DL-arginine-ρ-nitroanilide (BAPA) as substrate and tannins were determined according to the methodology described by Price et al. (1980).

2.5. Statistical analysis

The results obtained were submitted to analysis of variance through the F test and if significant, the Tukey test was performed ($p \leqslant 0.05$) with the use of the SAS program (Statistical Analysis System Institute, 1996).

3. Results and discussion

The results are presented in Tables 1–3.

Table 1 presents the percentage of phenolic compounds in the different cultivars for raw and cooked grains. The cooking process decreased the content of phenolic compounds in all cultivars. The dose of 8 kGy promoted an increase in the content of total phenolic compounds in all raw samples and in cooked samples from some cultivars. Villavicencio et al. (2000) presented higher contents of phenolic compounds when compared with raw samples. The authors attribute this result to the higher extractability of these compounds in cooked samples due to alterations in cellular compounds at high temperatures or due to the decomposition of some insoluble phenolic compounds. This can explain the increase of total phenolic compounds T 8 kGy too. The higher dose increases decomposition of phenolic compounds which can reorganize in others ways.

One observes through Table 2 that all cultivars presented the same behavior in relation to radiation for inhibited trypsin units both for raw and cooked samples, with significant differences ($p \leqslant 0.05$) between all doses used. Controls presented the highest values, followed by doses of 2 and 4 kGy and dose of 8 kGy presented the lowest value. It is possible that increases in the radiation dose used promoted decreases in the trypsin contents of the samples.

The cooking treatment promoted significant decrease in the trypsin inhibitor contents of all cultivars. Savage et al. (1995) observed that the inactivation of 80% of the trypsin inhibitory activity was obtained in decorticated soybeans submitted to bleaching at 100 °C for 12 min. According to Rackins et al. (1975), only 50–60% of reduction on the trypsin inhibitory activity is required to avoid pancreatic hypertrophy in rats, and the inactivation of 70–80% resulted in a maximum value of protein efficiency rate (PER) of diet containing soybean flour.

Carvalho et al. (2002), found variation from 122 to 206 UTI/mg of raw soybean grain samples, values far above those found in the present work for nonradiated cultivars analyzed.

However, Miura et al. (2001), evaluated cultivar BRS36 and strain BRM 95-5262 genetically modified in order to present low trypsin inhibitory activity and absence of Kunitz inhibitor and found values of 43.06 UTI/mg of raw sample in cultivar BRS36 and 28.15 UTI/mg of raw sample from strain genetically modified. Cultivars 214 and 231 presented similar values (Table 2).

Abu-Tarboush (1998), found reduction of 34.9% on the trypsin inhibitory activity in soybean flour radiated with 10 kGy. The author attributed this reduction to the breakage of the trypsin Inhibitory structure with the radiation treatment.

Farag (1998), found increase in the inactivation level with increase in the doses used (41.8%, 56.3%, 62.7% and 72.5% of loss in the trypsin inhibitory activity) for doses of 5, 15, 30 and 60 kGy, respectively. In the present work, radiation with dose of 2 kGy promoted reduction of 11.19% in average on the trypsin inhibitory activity, dose of 4 kGy reduced 28.59%, and 8 kGy reduced 37.60%.

It is possible to observe in Table 3 that the cooking process reduced the tannin concentration in all cultivars. This result is not in agreement with results found by Villavicencio et al. (2000), where the tannin concentration remained unchanged with soaking and cooking in variety Carioca and increased in variety Macacar. However, Mechi et al. (2005), found reduction in the tannin contents in black beans radiated with the cooking process, results in agreement with results found in the present work.

Amaya et al. (1991) suggest that the reduction in the tannin contents due to the cooking process is clear and is related to changes in solubility and chemical reactivity.

Table 1
Effect of radiation on the phenolic compounds concentration (mg/g) in the different cultivars analyzed

Cultivar	Treatment	Dose 0 kGy	Dose 2 kGy	Dose 4 kGy	Dose 8 kGy	Average cultivars
212	Raw	7.15 ± 0.02^{1b2}	5.87 ± 0.01^{c}	5.33 ± 0.01^{d}	7.60 ± 0.01^{a}	6.48 ± 0.96^{A3}
	Cooked	6.25 ± 0.03^{a}	5.27 ± 0.02^{b}	4.91 ± 0.05^{d}	5.01 ± 0.00^{c}	5.37 ± 0.56^{B}
213	Raw	8.07 ± 0.01^{b}	7.61 ± 0.01^{c}	6.98 ± 0.01^{d}	10.83 ± 0.01^{a}	8.37 ± 1.54^{A}
	Cooked	7.19 ± 0.00^{b}	5.52 ± 0.02^{c}	5.09 ± 0.02^{d}	8.56 ± 0.02^{a}	6.58 ± 1.44^{B}
214	Raw	6.60 ± 0.00^{b}	5.78 ± 0.01^{c}	5.00 ± 0.03^{d}	8.07 ± 0.00^{a}	6.36 ± 1.19^{A}
	Cooked	5.70 ± 0.00^{a}	4.99 ± 0.01^{b}	3.29 ± 0.01^{d}	4.88 ± 0.01^{c}	4.71 ± 0.92^{B}
231	Raw	7.54 ± 0.01^{a}	5.90 ± 0.01^{b}	5.11 ± 0.03^{c}	7.58 ± 0.01^{a}	6.53 ± 1.11^{A}
	Cooked	5.85 ± 0.02^{a}	3.83 ± 0.01^{c}	3.24 ± 0.01^{d}	4.26 ± 0.00^{b}	4.29 ± 1.01^{B}
E48	Raw	7.33 ± 0.00^{b}	6.24 ± 0.01^{c}	5.34 ± 0.03^{d}	7.84 ± 0.01^{a}	6.68 ± 1.01^{A}
	Cooked	4.95 ± 0.01^{a}	3.73 ± 0.03^{b}	2.46 ± 0.03^{d}	2.76 ± 0.01^{c}	3.47 ± 1.02^{B}

[1]Average ± standard deviation.
[2]Averages with different small letter (s) in horizontal are significantly different at level of ($p \leqslant 0.05$).
[3]Averages with different capital letter (s) in vertical within each cultivar (raw or cooked) are significantly different at level of ($p \leqslant 0.05$).

Table 2
Effect of radiation on the inhibited trypsin units (UTI/mg of sample) in the different cultivars analyzed

Cultivars	Treatment	Control	Dose of 2 kGy	Dose of 4 kGy	Dose of 8 kGy	Average cultivars
212	Raw	71.64 ± 0.4^{1a2}	67.69 ± 0.6^{b}	51.74 ± 0.4^{c}	43.95 ± 0.4^{d}	58.76 ± 11.8^{A3}
	Cooked	43.42 ± 0.8^{a}	32.84 ± 0.1^{b}	30.98 ± 0.1^{c}	29.10 ± 0.1^{d}	34.09 ± 5.8^{B}
213	Raw	56.17 ± 0.1^{a}	51.22 ± 0.2^{b}	41.61 ± 0.1^{c}	37.59 ± 0.1^{d}	46.65 ± 7.7^{A}
	Cooked	32.74 ± 0.1^{a}	26.68 ± 0.1^{b}	21.27 ± 0.2^{c}	18.19 ± 0.1^{d}	24.72 ± 5.7^{B}
214	Raw	48.51 ± 1.2^{a}	45.04 ± 0.1^{b}	32.98 ± 0.1^{c}	30.13 ± 0.1^{d}	39.09 ± 8.0^{A}
	Cooked	36.38 ± 0.1^{a}	31.66 ± 0.2^{b}	24.80 ± 0.1^{c}	22.96 ± 0.1^{d}	28.95 ± 5.6^{B}
231	Raw	42.60 ± 0.4^{a}	39.25 ± 0.1^{b}	36.11 ± 0.2^{c}	29.68 ± 0.1^{d}	36.91 ± 4.9^{A}
	Cooked	29.62 ± 0.4^{a}	26.91 ± 0.3^{b}	22.54 ± 0.1^{c}	19.81 ± 0.1^{d}	24.72 ± 3.9^{B}
E48	Raw	70.66 ± 0.2^{a}	62.58 ± 0.3^{b}	47.38 ± 0.3^{c}	43.32 ± 0.1^{d}	56.01 ± 11.6^{A}
	Cooked	41.41 ± 0.1^{a}	36.81 ± 0.1^{b}	28.35 ± 0.2^{c}	20.34 ± 0.1^{d}	31.73 ± 8.4^{B}

[1]Average ± standard deviation.
[2]Averages with different small letter (s) in horizontal are significantly different at level of ($p \leqslant 0.05$).
[3]Averages with different capital letter (s) in vertical within each cultivar (raw or cooked) are significantly different at level of ($p \leqslant 0.05$).

Table 3
Effect of radiation on the tannin concentration (mg/g) in the different cultivars analyzed

Cultivar	Treatment	Control	Dose of 2 kGy	Dose of 4 kGy	Dose of 8 kGy	Average cultivars
212	Raw	0.33 ± 0.02^{1a2}	0.21 ± 0.01^{b}	0.08 ± 0.01^{c}	0.07 ± 0.01^{c}	0.17 ± 0.11^{A3}
	Cooked	0.16 ± 0.03^{a}	0.10 ± 0.01^{b}	0.07 ± 0.01^{bc}	0.04 ± 0.01^{c}	0.09 ± 0.05^{B}
213	Raw	0.37 ± 0.06^{a}	0.28 ± 0.01^{b}	0.18 ± 0.02^{c}	0.07 ± 0.02^{d}	0.22 ± 0.12^{A}
	Cooked	0.27 ± 0.01^{a}	0.23 ± 0.02^{b}	0.14 ± 0.02^{c}	0.02 ± 0.01^{d}	0.17 ± 0.10^{B}
214	Raw	0.39 ± 0.01^{a}	0.25 ± 0.02^{b}	0.11 ± 0.01^{c}	0.11 ± 0.01^{c}	0.21 ± 0.12^{A}
	Cooked	0.23 ± 0.01^{a}	0.15 ± 0.02^{b}	0.08 ± 0.01^{c}	0.05 ± 0.01^{d}	$0.12 \pm 0.\ 07^{B}$
231	Raw	0.31 ± 0.02^{a}	0.22 ± 0.01^{b}	0.14 ± 0.01^{c}	0.08 ± 0.01^{d}	0.18 ± 0.09^{A}
	Cooked	0.19 ± 0.01^{a}	0.16 ± 0.02^{a}	0.10 ± 0.00^{b}	0.03 ± 0.02^{c}	0.11 ± 0.06^{B}
E48	Raw	0.28 ± 0.00^{a}	0.15 ± 0.01^{b}	0.13 ± 0.01^{b}	0.04 ± 0.02^{c}	0.15 ± 0.09^{A}
	Cooked	0.19 ± 0.01^{a}	0.11 ± 0.00^{b}	0.09 ± 0.01^{c}	0.01 ± 0.00^{d}	0.10 ± 0.07^{B}

[1]Average ± standard deviation.
[2]Averages with different small letter (s) in horizontal are significantly different at level of ($p \leqslant 0.05$).
[3]Averages with different capital letter (s) in vertical within each cultivar (raw or cooked) are significantly different at level of ($p \leqslant 0.05$).

Thus, this reduction is due to combination properties including proteins and other organic substances or to alterations in their chemical structure.

The same results were obtained by Brigide and Canniatti-Brazaca (2006). Mechi et al. (2005) and Villavicencio et al. (2000) found that gamma radiation also promoted reduction in the tannin contents as the radiation dose increased until a limited dose.

This reduction in the tannin contents is very favorable, once this antinutritional factor presents the capacity of decreasing the protein digestibility. When this antinutritional factor is found at the proportion of 5:1 tannin/protein, all protein is precipitated due to the tannin action (Pino and Lajolo, 2003).

According to results obtained, one may conclude that in relation to the antinutritional factors, all underwent reduction with increases in the radiation doses both for raw and cooked samples; the cooking process also promoted reduction in the antinutritional factors analyzed. Thus, the use of radiation treatment resulting in an improvement in the nutritional quality of the soybean grains was investigated.

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