

Radiation Physics and Chemistry 57 (2000) 289-293

Radiation Physics and Chemistry

www.elsevier.com/locate/radphyschem

Effect of irradiation on anti-nutrients (total phenolics, tannins and phytate) in Brazilian beans

Anna Lucia C.H. Villavicencio^{a,*}, Jorge Mancini-Filho^b, Henry Delincée^c, Ralf Greiner^c

^aIPEN/CNEN-SP, Depto of Engineering and Industrial Applications, Travessa R, No. 400 - Cidade Universitária, São Paulo, CEP 05508-910, Brazil

^bF.C.F. USP, Food and Experimental Nutrition Depto, São Paulo, Brazil
^cFederal Research Centre for Nutrition BFE, Haid-und-Neu-Str. 9, D-76131, Karlsruhe, Germany

Abstract

The Brazilian bean varieties *Phaseolus vulgaris* L. var. Carioca and *Vigna unguiculata* (L.) *Walp* var. Macaçar were irradiated with doses of 0.5, 1.0, 2.5, 5.0 and 10 kGy and subsequently stored at ambient temperature for 6 months. The anti-nutrients phenolic compounds, tannins and phytate were determined to be 0.48 mg g⁻¹ dry basis, 1.8 mg g⁻¹ dry basis and 13.5 μmol g⁻¹ dry basis in the raw non-irradiated Carioca beans and 0.30 mg g⁻¹ dry basis, 0.42 mg g⁻¹ dry basis and 7.5 μmol g⁻¹ dry basis in the raw non-irradiated Macaçar beans. After soaking and cooking a higher content of phenolic compounds and a lower phytate content was observed in both bean varieties. Tannin content was not affected by soaking and cooking of Carioca beans, but higher after soaking and cooking of Macaçar beans. Using radiation doses relevant for food did not effect the content of the anti-nutrients under investigation in both bean varieties. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Food irradiation; Beans; Total phenolics; Tannins; Phytate

1. Introduction

The extension of shelf life and improvement of technological qualities are aims of radiation processing of foods (Ahmed, 1993). Dry beans of *Phaseolus vulgaris* L. are important food sources, especially in developing countries, where they are a major source of dietary protein. Besides protein, legumes provide complex carbohydrate, dietary fiber and minerals in human

E-mail addresses: villavic@net.ipen.br (A.Lucia C.H. Villavicencio), jmancini@usp.br (J. Mancini-Filho), ralf.greiner@bfe.uni-karlsruhe.de (R. Greiner).

diet. In tropical countries, storage of common beans at high temperature and high humidity renders them susceptible to the hard-to-cook phenomenon and a greater insect infestation, causing losses around 31% in storage (Martín-Cabrejas et al., 1997; Farrar and Going, 1994). Irradiation treatment could reduce these losses, thereby maintaining the nutritional quality of beans (Delincée et al., 1998). However, several factors including phytate and certain phenolic compounds contribute to a lower nutritional value of legumes due to a reduced bioavailability of minerals and trace elements, especially zinc and iron (Ummadi et al., 1995; Gustafsson and Sandberg, 1995). Besides, this antinutritional property also some positive effects are discussed by consuming phytate and phenolic compounds

0969-806X/00/\$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved. PII: S0969-806X(99)00393-X

^{*} Corresponding author. Fax: +55-11-816-9186.

Table 1
Effect of irradiation on the concentration of phenolic compounds and Tannins in Carioca and Macaçar beans

	Phenolics (mg/g) ^a		Tannins (mg/g)		
Dose (kGy)	Raw	Cooked	Raw	Cooked	
Carioca					
0	$0.48 \pm 0.00^{\mathrm{a}}$	0.72 ± 0.00^{a}	1.8 ± 0.00^{a}	1.8 ± 0.00^{a}	
0.5	$0.49 \pm 0.00^{\mathrm{a}}$	0.73 ± 0.05^{a}	$1.8 \pm 0.00^{\rm a}$	1.8 ± 0.00^{a}	
1.0	0.48 ± 0.01^{a}	$0.67 \pm 0.01^{\mathrm{b}}$	1.7 ± 0.00^{a}	1.6 ± 0.00^{b}	
2.5	$0.48 \pm 0.00^{\mathrm{a}}$	0.63 ± 0.02^{bc}	$1.6 \pm 0.00^{\mathrm{b}}$	1.5 ± 0.00^{b}	
5.0	0.49 ± 0.01^{a}	$0.57 \pm 0.00^{\rm cd}$	$1.4 \pm 0.00^{\circ}$	$1.3 \pm 0.00^{\circ}$	
10.0	0.52 ± 0.01^{a}	0.51 ± 0.02^{e}	$1.4 \pm 0.00^{\circ}$	1.0 ± 0.00^{d}	
Macaçar					
0	$0.30 \pm 0.00^{\mathrm{a}}$	0.42 ± 0.01^{a}	0.42 ± 0.00^{a}	0.64 ± 0.00^{a}	
0.5	0.30 ± 0.01^{a}	$0.39 \pm 0.00^{a,b}$	$0.40 \pm 0.00^{\mathrm{a}}$	0.61 ± 0.00^{b}	
1.0	0.30 ± 0.01^{a}	$0.38 \pm 0.00^{\mathrm{b}}$	0.39 ± 0.00^{b}	$0.59 \pm 0.00^{\circ}$	
2.5	0.31 ± 0.01^{a}	$0.34 \pm 0.01^{\circ}$	$0.35 \pm 0.00^{\circ}$	$0.53 \pm 0.00^{\circ}$	
5.0	0.30 ± 0.00^{a}	0.32 ± 0.01^{cd}	$0.34 \pm 0.00^{\circ}$	0.49 ± 0.00^{d}	
10.0	0.25 ± 0.04^{b}	0.29 ± 0.01^{de}	0.29 ± 0.00^{d}	0.44 ± 0.00^{e}	

^a Means \pm SD (n = 3). In the same column, means with different superscript are statistically different (P < 0.05).

(Richard-Forget et al., 1995; Frühbeck et al., 1995). The main objective of this study was to investigate the effect of irradiation on the content of phenolic compounds, tannins and phytate in the two brazilian bean varieties Macaçar and Carioca.

2. Experimental

2.1. Material

Brazilian bean varieties *P. vulgaris* L. var. Carioca and *Vigna unguiculata* (L.) *Walp* var. Macaçar were bought in a local supermarket. All reagents were of analytical grade and the solutions were prepared with bidistilled water.

2.2. Irradiation of the beans

The samples were packed in plastic bags and irradiated in a Gammacell (AECL) with doses of 0.5, 1.0, 2.5, 5.0 and 10 kGy using a 60 Co source (IPEN, São Paulo) with a dose rate of 0.44 kGy h $^{-1}$.

2.3. Cooking of the beans

The beans were soaked for 10 h at 25°C in distilled water and then cooked at 100°C in the soaking water. The optimal cooking time was determined for each radiation dose and variety (Villavicencio et al., 1997).

2.4. Determination of phenolic compounds, tannins and myo-inositol phosphates

The beans including the cooking water were lyophilized. 2.5 g of each lyophilized samples were used to extract the phenolic compounds and the tannins. The determination of the concentration of phenolic compounds was performed according to Swain and Hills (1959) and the tannins were quantified as described by Price et al. (1978). The quantification of the different myo-inositol phosphates was performed by a combination of the AOAC-method and the ion-pair chromatography according to Sandberg and Ahderinne (1986).

2.5. Statistical analysis

The statistical analyses was performed using ANOVA (Yaname, 1977), *t*-test and the multiple comparison test described by Tukey–Kramer using the program GraphPad Instat, version 2.01.

3. Results and discussion

The content of phenolic compounds was determined to be 0.48 mg g⁻¹ dry basis in the raw non-irradiated Carioca beans and 0.30 mg g⁻¹ dry basis in the raw non-irradiated Macaçar beans (Table 1). Radiation doses up to 5 kGy do not have an effect on the concentration of phenolic compounds in the raw beans. Only a slight decrease in the concentration of phenolic compounds was observed in Macacar beans using 10

Table 2 Effect of irradiation on myo-inositol phosphate concentration in Carioca and Macaçar beans

Dose (kGy)	$IP_6 (\mu mol/g)^a$		$IP_5 (\mu mol/g)^a$		$IP_4 \; (\mu mol/g)^a$		$IP_3 \ (\mu mol/g)^a$	
	Carioca	Macaçar	Carioca	Macaçar	Carioca	Macaçar	Carioca	Macaçar
Raw								
0	13.5 ± 0.0^{a}	8.7 ± 0.0^{a}	1.9 ± 0.0^{a}	1.3 ± 0.0^{a}	0	0	0	0
0.5	13.5 ± 0.0^{a}	8.7 ± 0.0^{a}	1.9 ± 0.0^{a}	1.3 ± 0.0^{a}	0	0	0	0
1.0	13.2 ± 0.1^{a}	8.6 ± 0.0^{a}	1.9 ± 0.0^{a}	$1.0 \pm 0.0^{\rm b}$	0	0	0	0
2.5	13.3 ± 0.0^{a}	8.7 ± 0.0^{a}	1.9 ± 0.0^{a}	1.3 ± 0.0^{a}	0	0	0	0
5.0	13.2 ± 0.0^{a}	8.6 ± 0.0^{a}	1.9 ± 0.0^{a}	$1.5 \pm 0.0^{\circ}$	0	0	0	0
10.0	12.2 ± 0.0^{b}	8.6 ± 0.0^{a}	1.9 ± 0.0^{a}	1.3 ± 0.0^{a}	0	0	0	0
Cooked								
0	10.7 ± 0.0^{a}	7.5 ± 0.0^{a}	3.0 ± 0.0^{a}	1.9 ± 0.0^{a}	0.8 ± 0^{a}	0.4 ± 0^{a}		Traces
0.5	10.6 ± 0.0^{a}	7.4 ± 0.0^{a}	3.2 ± 0.0^{b}	1.9 ± 0.0^{a}	1.2 ± 0^{b}	0.4 ± 0^{a}		Traces
1.0	10.4 ± 0.0^{a}	7.5 ± 0.0^{a}	3.2 ± 0.0^{b}	2.1 ± 0.0^{b}	$1.3 \pm 0^{\rm b}$	0.4 ± 0^{a}		Traces
2.5	10.6 ± 0.0^{a}	7.5 ± 0.0^{a}	3.2 ± 0.1^{b}	2.1 ± 0.0^{b}	$1.3 \pm 0^{\rm b}$	0.4 ± 0^{a}		Traces
5.0	10.6 ± 0.0^{a}	7.4 ± 0.0^{a}	$3.3 \pm 0.0^{\circ}$	2.1 ± 0.0^{b}	1.2 ± 0^{b}	0.4 ± 0^{a}		Traces
10.0	10.3 ± 0.0^{a}	7.4 ± 0.0^{a}	3.4 ± 0.0^{c}	2.1 ± 0.0^{b}	1.6 ± 0^{c}	0.4 ± 0^{a}		Traces

^a Means \pm SD (n = 3). In the same column, means with different superscript are statistically different (P < 0.05).

kGy. This is in accordance to published studies by Oigiangbe and Onigbinde (1996) and Pinn et al. (1993), since these authors did not observe an effect of irradiation on the concentration of phenolic compounds. After cooking a decrease in the concentration of phenolic compounds was found by increasing the radiation dose (Table 1). Using the radiation dose allowed for insect disinfestation (up to 1 kGy) a 7% reduction in the concentration of phenolic compounds was observed. Compared to raw beans the soaked and cooked ones showed a higher content in phenolic compounds. This could be explained by a better extractability of those compounds due to alteration of cell wall components at higher temperature or by a decomposition of certain insoluble phenolic compounds.

The content of tannins was determined to be 1.8 mg g⁻¹ dry basis in raw non-irradiated Carioca beans and 0.42 mg g⁻¹ dry basis in raw non-irradiated Macaçar beans (Table 1). Ene-Obong (1995) reported a variation in the tannin content in different V. unguiculata varieties. No tannins were found in beans with a white skin, darker beans contain tannins up to a concentration of 1.42 mg g⁻¹ dry basis. Several authors (Martín-Cabrejas et al., 1997; Desphande et al., 1982; Barampama and Simard, 1994) reported tannin contents in different bean varieties of 0.03-1.56 mg g⁻¹ dry basis. The tannin content of the two bean varieties decreases with the increase in the radiation dose in raw as well as in cooked beans. This is in accordance to the results published by Pinn et al. (1993). Tannin content was not affected by soaking and cooking of Carioca beans, but higher after soaking and cooking of Macaçar beans.

Table 2 summarize the myo-inositol phosphate values for the raw and cooked beans. The most abundant myo-inositol phosphate in raw beans was phytate (IP₆), representing about 87% of the myo-inositol phosphates in both bean varieties. The concentration of IP₆ in the raw material ranges from 8.7 μ mol g⁻¹ dry basis in Macaçar beans to 13.5 μ mol g⁻¹ dry basis in Carioca beans. Of the lower myo-inositol phosphates only myo-inositol pentakisphosphate (IP₅) was found in significant amounts in raw beans. The concentration of IP₅ ranges from 1.3 µmol g⁻¹ dry basis in Macaçar beans to 1.9 μ mol g⁻¹ dry basis in Carioca beans. These results are in good agreement with recently published data (Morris and Hill, 1996). Myoinositol phosphate concentrations were not affected by the radiation process. After cooking a significant reduction in IP6 content with a concomitant increase in the concentrations of IP₅, myo-inositol tetrakisphosphate (IP₄) and myo-inositol trisphosphate (IP₃) was observed. Phytate reduction was determined to be 21% in Carioca beans and 14% in Macaçar beans. 8 to 40% phytate reduction was reported after soaking and cooking of different legumes (Bhatty, 1989; Fernandez et al., 1994; Ganesh et al., 1978; Ologhobo and Fetuga, 1984; Tabekhia and Luh, 1980). The rate of phytate reduction depends on the kind of legume and the preparation procedure used. Therefore, soaking and cooking the beans has only a limited effect on the content of myo-inositol phosphates with adverse effects on mineral absorption, because the degree of inhibitory action of the myo-inositol phosphates on mineral utilization is directly proportional to the degree of phosphorylation (Brune et al., 1992; Han et al., 1994;

Table 3
Effect of irradiation on phytase activity in Carioca and Macaçar beans

	Phytase (mU/g) ^a				
Dose (kGy)	Raw	Cooked			
Carioca					
0	38.50 ± 0.00^{a}	0			
0.5	39.00 ± 0.00^{a}	0			
1/0	38.50 ± 0.36^{a}	0			
2.5	37.00 ± 0.21^{a}	0			
5.0	30.40 ± 0.34^{b}	0			
10.0	$27.60 \pm 0.15^{\circ}$	0			
Macaçar					
0	15.40 ± 0.21^{a}	0			
0.5	15.00 ± 0.15^{a}	0			
1.0	13.50 ± 0.06^{a}	0			
2.5	$12.70 \pm 0.17^{\rm b}$	0			
5.0	11.20 ± 0.06 ^{bc}	0			
10.0	9.10 ± 0.17^{c}	0			

^a Means \pm SD (n=3). In the same column, means with different superscript are statistically different (P < 0.05).

Lönnerdal et al., 1989; Sandström and Sandberg, 1992). It was reported that only IP₆ and IP₅ show negative effects on zinc and iron absorption in humans, while IP₄ and IP₃ have no effect. Furthermore, the radiation process has no significant effect on the concentrations of the different myo-inositol phosphates after soaking and cooking, but the radiation process shows an impact on the activity of phytate-degrading enzymes (phytases). With an increase in radiation dose a decrease in phytase activity was observed in both bean varieties (Table 3). Since it was shown by Greiner and Konietzny (1998) that phytase is responsible for phytate degradation during soaking and cooking of beans the radiation process should have an effect on phytate degradation after soaking and cooking. An explanation for the missing effect is that apart from the activity of the enzymes, the free access of the enzyme to phytate must be taken into account. This access is connected with the partial breakdown of cell walls and the liberation of phytate from complexes with other plant tissue. The missing effect could be explained by a partly prevention of access of those enzymes to phytate. Hence, plant tissue degrading activities seems to be a very important factor (Zyla et al., 1992).

4. Conclusions

The content of the anti-nutrients phenolic compounds, tannins and phytate in the brazilian bean varieties *P. vulgaris* L. var. Carioca and *V. unguiculata* (L.) *Walp* var. Macaçar were of the same magnitude as

reported in the literature. Irradiated with doses of 0.5, 1.0, 2.5, 5.0 and 10 kGy has no or only a very limited effect on the content of the anti-nutrients phenolic compounds, tannins and phytate. Therefore, irradiation does not result in a better nutritional quality of the beans under investigation in respect to an increased bioavailability of minerals and trace elements

Acknowledgements

We are thankful to the International Atomic Energy Agency (IAEA) for the financial support and also to Ms S. Delincée, Ms A. Wachs, S. Felleisen and E. Haller, for their technical help during this study, our gratitude.

References

Ahmed, M., 1993. Up-to-date status of food irradiation. Radiat. Phys. Chem. 42, 245–251.

Barampama, Z., Simard, R.E., 1994. Oligosaccharides, antinutritional factors, and protein digestibility of dry beans as affected by processing. J. Food Sci. 59, 833–838.

Bhatty, R.S., 1989. Cooking quality and losses of phytic acid, calcium, magnesium and potassium of lentils soaked in different solutions. Can. Inst. Food. Sci. Technol. J. 22, 450–455.

Brune, M., Rossander-Hulten, L., Hallberg, L., Gleerup, A., Sandberg, A.S., 1992. Iron absorption from bread in humans: inhibition effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J. Nutr. 122, 442–449.

Delincée, H., Mancini-Filho, J., Villavicencio, A.L.C.H., 1998. Protein quality of irradiated Brazilian beans. Radiat. Phys. Chem. 52, 43–47.

Desphande, S.S., Sathe, S.K., Salunkhe, D.K., Cornforth, D.P., 1982. Effects of dehulling on phytic acid, polyphenols, and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.). J. Food Sci. 47, 1846–1850.

Ene-Obong, H.N., 1995. Content of antinutrients and in vitro protein digestibility of the African yambean, pigeon and cowpea. Plant Foods Hum. Nutr. 48, 225–233.

Farrar, I.V.H., Going, T., 1994. Commercial application of food irradiation in Brazil, An FAO/IAEA mission report IAEA, pp. 1–41, Vienna.

Fernandez, M.M., Lopez-Jurado, M., Moreu, M.C., Porres, J., Garcia-Fuentes, M.A., Frias, J., Aranda, P., Urbano, G., 1994. Effect of processing on phyate phosphorus of faba beans (*Vicia faba* L., var. major). Influence on bioavailability of phosphorus in rats. In: Proc. Of the Int. EURO Food Tox IV Conference on Bioactive Substances in Food from Plant Origin, vol. 2, pp. 32–34.

Frühbeck, G., Alonso, R., Marzo, F., Santidrián, S.A., 1995. Modified method for the indirect quantitative analysis of phytate in foodstuffs. Anal. Biochem. 225, 206–212.

Ganesh, K., Venkataraman, L.V., Jaya, T.V., Krishnamurthy,

- K.S., 1978. Cooking characteristics of some germinated legumes: changes in phytins, Ca²⁺, Mg²⁺ and pectin. J. Food Sci. 43, 85–88.
- Greiner, R., Konietzny, U., 1998. Endogenous phytatedegrading enzymes are responsible for phytate reduction while preparing beans (*Phaseoulus vulgaris*). J. Food Process. Preserv. 22, 321–331.
- Gustafsson, E.-L., Sandberg, A.-S., 1995. Phytate reduction in brown beans (*Phaseolus vulgaris* L.). J. Food Sci. 60, 149– 152.
- Han, O., Faila, M.L., Hill, A.D., Morris, E.R., Smith Jr, J.C., 1994. Inositol phosphates inhibit uptake and transport of iron and zinc by human intestinal cell line. J. Nutr. 124, 580–587.
- Lönnerdal, B., Sandberg, A.-S., Sandström, B., Kunz, C.J., 1989. Inhibitory effect of phytic acid and other inositol phosphates on zinc and calcium absorption. J. Nutr. 199, 211–214.
- Martín-Cabrejas, M.A., Esteban, R.M., Perez, P., Maina, G., Waldron, K.W., 1997. Changes in physicochemical properties of dry beans (*Phaseolus vulgaris* L.) during long-term storage. J. Agric. Food Chem. 45, 3223–3227.
- Morris, E.R., Hill, A.D., 1996. Inositol phosphate content of selected dry beans, peas, and lentils, raw and cooked. J. Food. Comp. Anal. 9, 2–12.
- Oigiangbe, N.O., Onigbinde, A.O., 1996. The association between some physico-chemical characteristics and suscepbility of cowpea (*Vigna unguiculata* (L.) *Walp*) to *Callosobruchus maculatus* (F.). J. Stored Prod. Res. 32, 7–11
- Ologhobo, A.D., Fetuga, B.L., 1984. Distribution of phosphorus and phytate in some Nigerian varieties of legumes and some effects of processing. J. Food Sci. 49, 199–201.
- Pinn, A.B.O., Colli, C., Mancini-Filho, J., 1993. Beans Phaseolus vulgaris irradiation. I. Iron bioavailability. In:

- Bioavailability'93. Nutritional, Chemical and Food Processing Implications of Nutrient Availability Karlsruhe, 1993. Karlsruhe, BFE, vol. 2, pp. 195–199.
- Price, M.L., Van Scoyoc, S., Butler, L.G., 1978. Critical evaluation of the vanillin reaction as an assay for tannin in sorghu grain. J. Agric. Food Chem. 26, 1212–1218.
- Richard-Forget, F., Gauillard, F., Hugues, M., Jean-Marc, T., Boivin, P., Nicolas, J., 1995. Inhibition of horse bean and germination Barley lipoxygenases by some phenolic compounds. J. Food Sci. 60, 1325–1329.
- Sandberg, A.S., Ahderinne, R., 1986. HPLC Method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. J. Food Sci. 51, 547–550.
- Sandström, B., Sandberg, A.-S., 1992. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. J. Trace Elem. Electrolytes Health Dis. 6, 99–103.
- Swain, T., Hills, W.E., 1959. Determinação de fenólicos totais. J. Sci. Food Agric. 10, 63–68.
- Tabekhia, M.M., Luh, B., 1980. Effect of germination, cooking, and canning on phosphorus and phytate retention in dry beans. J. Food Sci. 45, 406–408.
- Ummadi, P., Chenoweth, W.L., Uebersax, M.A., 1995. The influence of extrusion processing on iron dialyability, phytates and tannins in legumes. J. Food Process. Preserv. 19, 119–131.
- Villavicencio, A.L.C.H., Mancini-Filho, J., Delincée, H., Bognár, A., 1997. Analytical determination of thiamine (vitamin B1) in irradiated and stored Brazilian beans. In: Proceedings of IV ENAN, Brazil, vol. 1, pp. 918–921.
- Yaname, T., 1977. Estatística. Harla, Ciudad de México.
- Zyla, Z., Ledoux, D.R., Garcia, A., Veum, T.L., 1992. An in vitro procedure for studying dephosphorylation of phytate in maize-soybean feeds for turkey poults. Br. J. Nutr. 74, 3–17.