Effects of Pretreatment with Gamma Rays or Microwaves on Storage Stability of Dry Beans

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The objective of this work was to study the effects of gamma radiation or microwave treatment on physical, chemical, and sensorial properties of dry beans during storage. Microwave treatment for 2 min or gamma irradiation at 2 kGy was applied. Samples were stored at 4–5 °C in a refrigerator and at 30 °C, 75% relative humidity (RH), for 6 months. The hydration capacity of samples stored at 4–5 °C for 6 months was about 60% that of samples stored at 30 °C, 75% RH. Gamma radiation increased the hydration rate and decreased cooking time and hardness of the seeds, whereas microwave treatment resulted in increased hardness and cooking time. Sensorial attributes were preserved in all samples kept under refrigeration (4–5 °C) but deteriorated considerably at 30 °C, 75% RH. Overall, the sensory properties of the irradiated samples did not differ ($p \leq 0.05$) from those of the controls, while the microwave-treated samples presented inferior sensorial properties. Storage for 6 months at 30 °C, 75% RH, reduced phytate to 50% of the original values in the control and irradiated samples and to 60% in the microwave-treated sample. For the same storage condition and time, methionine was reduced to 72, 75, and 63% of original value, in the control, irradiated, and microwave-treated samples, respectively.

INTRODUCTION

Deterioration of legume seed quality as a function of time and condition of storage has been described in the literature by many investigators (Morris and Wood, 1956; Quast and Silva, 1977; Kon, 1979; Kon and Sanshuck, 1981; Antunes and Sgarbieri, 1979). The factors responsible for the deterioration have been identified as high water content in the beans or high relative humidity and temperature in the storage environment. Primarily observed deteriorations have been hardness of the cotyledons and loss of cookability, deterioration of texture and flavor, and loss of nutritive value (Antunes and Sgarbieri, 1979; Mejia, 1982; Molina et al., 1975).

More recently, some hypotheses have been proposed to explain the hardening and related phenomena in dry beans (Stanley and Aguilera, 1985; Jones and Boulter, 1983; Rivera et al., 1989). Some attempts have also been made to prevent hardening (Rivera et al., 1989; Molina et al., 1976) or to reverse hardening once it had occurred (Neme et al., 1975a,b; Reedy et al., 1979; Carvalho et al., 1991).

In this paper the effects of pretreatment with microwave or gamma radiation on storage stability of dry beans are described. The main objective was to determine if these treatments would prevent the beans from hardening during storage under two different sets of conditions.

MATERIALS AND METHODS

Bean Cultivar. The beans utilized in this study were from the cultivar Carioca 80 SH grown at one of the Agricultural Experimental Stations of the Agronomic Institute of Campinas, State of São Paulo, Brazil. After harvest, the beans were kept under refrigeration (10 °C) prior to use.

Microwave Treatments. Bean samples in Petri dishes (15-cm diameter × 2-cm height) were submitted to microwaves in a Panasonic oven of 2450 MHz for 30, 60, 90, 120, 150, 180, 210, and 240 s. The temperature inside the oven after each heating time was 40, 60, 85, 105, 120, 135, 160, and 190 °C, respectively. The time of 120 s (105 °C) was selected as the best pretreatment for storage on the basis of complete inactivation of trypsin inhibitor and peroxidase and on cooking time. Heating for more than 120 s increased the cooking times for the beans exponentially.

Gamma Radiation. Six 500-g samples were packed in plastic bags and submitted to gamma radiation at final dosages of 1.0, 2.0, 3.0, 4.0, 5.0, and 10.0 kGy. A cobalt-60 source of 18-cm diameter at a radiation rate of 3.5 kGy/h was used. The selected treatment for the storage study was 2.0 kGy total dosage on the basis of sensory evaluation of the various treatments. Dosage above 2 kGy caused deterioration of flavor detected by the panelists in the triangular test (Moraes, 1988).

Conditions and Time of Storage. Samples treated with microwave (2 min) or with gamma rays (2 kGy) plus control samples (not treated) were stored under refrigeration (4–5 °C) or at 30 °C, 75% RH. Analyses were performed at 0, 2, 4, and 6 months of storage.

Hydration. Velocity and capacity of hydration were determined according to the method of Morris and Wood (1956). Bean seeds of pretreated samples and control were initially wetted and weighed to find the initial weight of the sample. Then the samples were soaked in distilled water (1:5 w/v) for 10 h. Absorbed water was indirectly determined by measuring the remaining volume of water at different times. Evaporation losses were determined by maintaining identical beakers with the same volume of distilled water under the same conditions. Remaining volumes in these beakers were measured at the various time intervals. Hydration capacity was expressed as milliliter of water absorbed per 100 g of seeds, referred to on a dry basis.

Cooking Time. Cooking time was determined in a puncturometer of the type originally designed by Burr et al. (1968). The seeds previously soaked in distilled water for 16 h (1:4 w/v) were put under the needles of the equipment and cooked submerged in boiling water under atmospheric pressure. Cooking time was estimated by the time (minutes) when 50% plus one of the needles had penetrated the seeds. Estimation was done in triplicate samples.

Sensory Evaluation. For the sensory evaluation tests a nonstructured scale of 9 cm (Moraes, 1988) was used with contrasting words anchored at the extremities. For evaluation of flavor (taste and aroma) the words characteristic and noncharacteristic were adopted. Noncharacteristic was anchored to the left side corresponding to lower numerical values, and characteristic was anchored to the right side corresponding to higher values. The same principle was used with the words weak (left) and strong (right) for firmness of cooked beans. The seeds previously soaked in filtered water for 16 h were cooked for 13

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0021-8561/93/1441-1710$04.00/0

min in a pressure cooker and then were served in 50-mL beakers which were kept in a thermal tray at 45–50 °C during the tasting period. Samples were offered to the tasters in a balanced incomplete block design, as proposed by Cochran and Cox (1957).

Water Content of the Samples. Water content was determined according to the AOAC (1975) method in triplicate samples at 105 °C to constant weight, and the results were expressed as percent, on a dry basis.

Phytate Determination. Phytate was determined according to the procedure described by Latta and Eskin (1980) which is based on the property of phytate to chelate minerals, such as iron from $FeCl_3 \cdot 6H_2O$.

Determination of Methionine. Methionine was determined in the samples after *in vitro* enzyme digestion by pepsin and pancreatin according to the procedure of Akeson and Stahmann (1964). Methionine quantification in the hydrolysate was done by the colorimetric method of McCarthy and Sullivan (1941) as modified by Lunder (1973).

Trypsin Inhibitor Activity. Trypsin inhibitor activity was determined according to the method of Kakade et al. (1969) using a 2% solution of casein (Hammersten) as substrate.

Peroxidase Activity. This was determined according to the procedures described by Plhak et al. (1987). Bean flour (10 g) was extracted in 100 mL of 0.1 M citrate buffer, pH 6.2, containing 2.5% polyvinylpolypyrrolidone (PVPP) and 0.4 M $CaCl_2$, under continuous agitation in an ice bath for 30 min. The extracts were centrifuged (16000*g*, 30 min, 0–4 °C). The enzyme unit was arbitrarily defined as the increase of 0.01 absorbance unit under the assay condition. Results were expressed in peroxidase units per minute per milliliter of extract.

Statistical Analysis. Statistical analysis obeyed an experimental factorial design in balanced incomplete blocks (Pimentel, 1982). Results of the sensory evaluation were submited to analysis of variance and to Tukey's test utilizing a computer program.

RESULTS AND DISCUSSION

Preliminary experiments were performed to establish appropriate treatment with gamma radiation or microwaves prior to storage. The rationale was that pretreatment with gamma radiation or microwaves could prevent or retard hardening and other deteriorations which occur during storage. By generation of heat, microwaves should inactivate enzymes possibly involved in the deteriorative reactions; gamma radiation could interfere with the hardening phenomenon by splitting certain chemical bonds and disrupting structural tissues that might be involved in the hardening process. The microwave treatment (2 min) was adopted because it inactivated peroxidase completely and about 80% of the activity of the Bowman–Birk-type trypsin–chymotripsin inhibitor, used as indicators due to the well-known heat resistance of these proteins. Furthermore, this treatment did not affect the cooking time of the beans, whereas microwave treatment for more than 2 min increased cooking time drastically (data not presented). Gamma radiation treatment (2 kGy) was adopted on the basis of sensory evaluation. It was the maximum dosage that could not be detected by the tasters in triangular tests, although the initial cooking time of the beans was reduced to 36% of the nontreated (control) sample.

The two conditions of storage, refrigeration and 30 °C, 75% RH, were chosen on the basis of reported data showing that refrigeration prevents beans from hardening (Moscoso et al., 1984) and that high environmental temperatures and humidities accelerate hardening (Antunes and Sgarbieri, 1979; Burr et al., 1968; Quast and Silva, 1977). Furthermore, 30 °C and 75% RH are conditions commonly found in tropical countries where dry beans are an important staple food.

The water contents of the samples at the beginning of and during the storage period of 6 months are shown in Table I. At zero time the water content of the sample treated with microwaves (2 min) was about 10% lower than that of the irradiated and control samples because microwave heating caused rapid water evaporation from the seeds. In the first 2 months under refrigeration there was a decrease in seed moisture from 9.75 to 8.21% for the control and from 9.67 to 7.82% for the irradiated samples, with subsequent stabilization up to 6 months of storage, except for the irradiated sample, which showed a statistically significant increase ($p \leq 0.05$) at 6 months. On the other hand, at 30 °C, 75% RH, a continuous increase in the water content of the seeds was observed. At 6 months of storage the percent increases of water (w/w) were 25.5, 32.7, and 43.2% for the control, irradiated, and microwave-treated samples, respectively.

The hydration velocity and hydration capacity of the beans at the beginning (prestorage) and during the 6 months of storage under the two sets of different conditions are given in Figure 1. Prior to storage, the control and the microwave-treated samples showed a slower initial rate of hydration than the irradiated sample. However, after 9 h of soaking in distilled water, the volumes of water absorbed by the three samples were essentially the same

Table I. Changes in Water Content[a] [Percent (w/w)] of Dry Beans Stored for 6 Months under Refrigeration (4–5 °C) or at 30 °C, 75% Relative Humidity (RH)

time of storage, months	conditions of storage	treatments: control	irradiated (2 kGy)	microwave (2 min)
0		9.75 ± 0.21[a]	9.67 ± 0.24[a]	7.78 ± 0.24[b]
2	refrigeration	8.21 ± 0.41[aB]	7.82 ± 0.14[bB]	7.46 ± 0.09[cB]
	30 °C, 75% RH	10.12 ± 0.29[bA]	10.29 ± 0.11[bA]	11.08 ± 0.24[aA]
4	refrigeration	8.93 ± 0.16[aB]	7.38 ± 0.17[bB]	7.62 ± 0.10[bB]
	30 °C, 75% RH	12.85 ± 0.35[aA]	12.25 ± 0.26[bA]	11.40 ± 0.26[cA]
6	refrigeration	8.09 ± 0.30[aB]	8.00 ± 0.23[aB]	7.76 ± 0.26[bB]
	30 °C, 75% RH	13.09 ± 0.14[cA]	14.37 ± 0.23[aA]	13.71 ± 0.09[bA]

[a] Results are means ± SD of four determinations. [b] a–c (rows) indicate statistically significant differences, a > b > c ($p \leq 0.05$), among treatments for each condition and time of storage. A,B (columns) indicate statistically significant differences, A > B ($p \leq 0.05$), between storage conditions at each storage time.

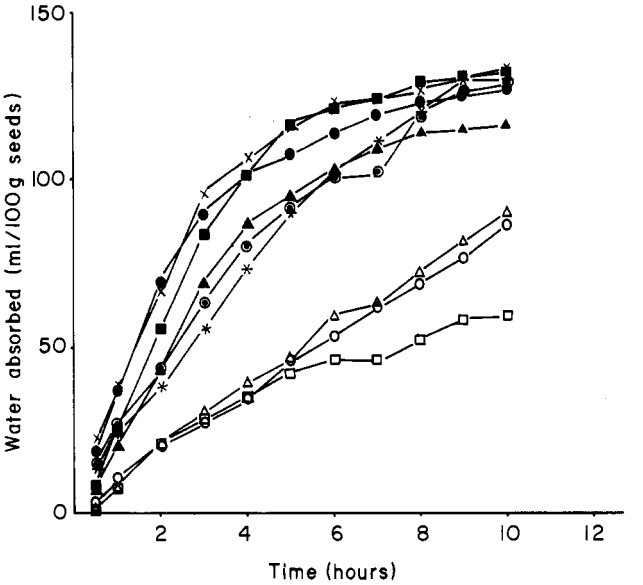

Figure 1. Hydration curves of dry beans submitted to various treatments: pretreated with gamma rays, 2 kGy (×), with microwaves, 2 min (⊙); control, not treated (*); pretreated and stored at 30 °C, 75% RH, for 6 months with gamma rays (●), microwaves (■); control stored, not treated (▲); pretreated and stored under refrigeration (4–5 °C) with gamma rays (○), microwaves (□); control stored, not treated (△).

Table II. Changes in Cooking Time (Minutes) of Dry Beans Stored for 6 Months under Refrigeration (4–5 °C) or at 30 °C, 75% Relative Humidity (RH)

time of storage, months	conditions of storage	treatments[a] control	irradiated (2 kGy)	microwave (2 min)
0		47^{b}	17^{c}	63^{a}
2	refrigeration	46^{aB}	16^{bB}	49^{aB}
	30 °C, 75% RH	65^{bA}	56^{bA}	122^{aA}
4	refrigeration	42^{aB}	18^{bB}	45^{aB}
	30 °C, 75% RH	233^{aA}	128^{bA}	224^{aA}
6	refrigeration	41^{aB}	17^{bB}	46^{aB}
	30 °C, 75% RH	292^{bA}	156^{cA}	412^{aA}

[a] a–c (rows) indicate statistically significant differences, a > b > c ($p \leq 0.05$), among treatments for each condition and time of storage. A,B (columns) indicate statistically significant differences, A > B ($p \leq 0.05$), between storage conditions at each storage time.

(Figure 1). After 6 months of storage at 30 °C, 75% RH, the hydration rate and hydration capacity of the samples pretreated with gamma rays and microwaves were comparable to those of the sample treated with gamma rays prior to storage. The samples stored at 30 °C, 75% RH, exhibited a lower rate of water absorption and a lower hydration capacity as compared to the irradiated and microwave-treated samples. The samples stored under refrigeration showed, consistently, lower hydration rate and hydration capacity compared with the ones stored at 30 °C, 75% RH. Up to 5 h of soaking in distilled water, the three samples behaved almost identically. From 5 to 10 h, the control and the irradiated samples continued to absorb water at about the same rates; however, the microwave-treated samples exhibited a considerable decrease in water absorption (Figure 1).

Table II shows the cooking times, in minutes, for the control and for the irradiated and microwave-treated samples before and during the storage period, evaluated at 2, 4, and 6 months of storage. When the effect of treatment is compared (rows), in each storage condition, it becomes clear that irradiation (2 kGy) contributed significantly ($p \leq 0.05$) to soften the bean seeds, therefore decreasing the cooking time. At zero storage time the cooking time of the irradiated sample was 36 and 27% of that of the control and microwave-treated samples, respectively. At 6 months of storage at 30 °C, 75% RH, the cooking time relation for the irradiated to the control and microwave-treated samples was 53.4 and 37.8%, respectively. Therefore, while irradiation contributed to decrease the cooking time, microwave treatment contributed to increasing it. The softening effect of gamma radiation on beans and other cereal grains has been reported by Neme et al. (1975a,b) and Carvalho et al. (1991). Nevertheless, we have not found any reported data on the application of microwaves or gamma rays on beans prior to storage with the purpose of preventing the hardening phenomenon. Microwave pretreatment (2 min) as well as gamma radiation (2 kGy) failed to prevent dry beans from hardening; however, because of the initial softening effect of gamma radiation, the cooking time of the irradiated sample at 6 months of storage was only 53% of that for the control sample.

Acceleration of hardening and other undesirable changes in dry beans stored at high temperatures and humidities has been reported by several investigators (Antunes and Sgarbieri, 1979; Burr et al., 1968; Mejia, 1982; Quast and Silva, 1977; Rozo et al., 1990).

Sensory attributes for the samples were evaluated before and after 2, 4, and 6 months of storage at refrigeration (4–5 °C) or 30 °C, 75% RH. The results for aroma, taste, and firmness are presented in Tables III, IV, and V, respectively.

Table III. Influence of Pretreatment and Storage Time on the Aroma[a] of Dry Beans Stored under Refrigeration (4–5 °C) or at 30 °C, 75% Relative Humidity (RH)

time of storage, months	conditions of storage	treatments[b] control	irradiated (2 kGy)	microwave (2 min)
0		7.08^{a}	6.91^{a}	3.45^{b}
2	refrigeration	6.23^{aA}	6.76^{aA}	6.66^{aA}
	30 °C, 75% RH	5.91^{aA}	6.49^{aA}	5.45^{aB}
4	refrigeration	7.30^{aA}	6.79^{abA}	5.90^{bA}
	30 °C, 75% RH	6.20^{aB}	6.14^{aA}	3.96^{bB}
6	refrigeration	7.18^{aA}	7.11^{aA}	6.75^{aA}
	30 °C, 75% RH	6.05^{aB}	5.44^{aB}	3.36^{bB}

[a] Lower values = less characteristic aroma; higher values = more characteristic aroma. [b] a, b (rows) indicate statistically significant differences, a > b ($p \leq 0.05$), among treatments for each condition and time of storage. A,B (columns) indicate statistically significant differences, A > B ($p \leq 0.05$), between storage conditions at each storage time.

Table IV. Influence of Pretreatment and Storage Time on the Taste[a] of Dry Beans Stored under Refrigeration (4–5 °C) or at 30 °C, 75% Relative Humidity (RH)

time of storage, months	conditions of storage	treatments[b] control	irradiated (2 kGy)	microwaves (2 min)
0		7.21^{a}	6.58^{a}	2.58^{b}
2	refrigeration	6.35^{aA}	6.51^{aA}	5.91^{aA}
	30 °C, 75% RH	5.51^{aA}	5.59^{aA}	4.50^{bB}
4	refrigeration	7.24^{aA}	6.44^{abA}	5.61^{bA}
	30 °C, 75% RH	6.11^{aB}	5.85^{aA}	2.78^{bB}
6	refrigeration	7.69^{aA}	7.33^{abB}	6.59^{bA}
	30 °C, 75% RH	5.10^{aB}	4.08^{bB}	1.79^{cB}

[a] Lower values = less characteristic taste; higher values = more characteristic taste. [b] a–c (rows) indicate statistically significant differences, a > b > c ($p \leq 0.050$, among treatments for each condition and time of storage. A,B (columns) indicate statistically significant differences, A > B ($p \leq 0.05$), between storage conditions at each storage time.

Table V. Influence of Pretreatment and Time of Storage on the Firmness of Dry Beans Stored Under Refrigeration (4–5 °C) or at 30 °C, 75% Relative Humidity (RH)

time of storage, months	conditions of storage	treatments[a] control	irradiated (2 kGy)	microwave (2 min)
0		1.85^{b}	1.71^{b}	6.53^{a}
2	refrigeration	2.00^{bB}	1.51^{bB}	3.64^{aB}
	30 °C, 75% RH	4.31^{aA}	4.00^{aA}	4.51^{aA}
4	refrigeration	1.38^{bB}	1.28^{bB}	4.14^{aB}
	30 °C, 75% RH	4.03^{bA}	5.06^{bA}	6.49^{aA}
6	refrigeration	2.28^{bB}	1.75^{bB}	3.79^{aB}
	30 °C, 75% RH	5.29^{bA}	6.25^{abA}	7.35^{aA}

[a] a,b (rows) indicate statistically significant differences, a > b ($p \leq 0.05$), among treatments for each conditions and time of storage. A,B (columns) indicate statistically significant differences, A > B ($p \leq 0.05$), between storage conditions at each storage time.

In Table III (rows) the effect of pretreatment on aroma is analyzed prior to storage (zero time) and at 2, 4, and 6 months, for each set of storage conditions. At zero time there was no statistical difference in aroma between control and irradiated samples, while both samples were superior ($p \leq 0.05$) to the microwave-treated sample. There was no statistical difference ($p \leq 0.05$) between control and irradiated samples stored in each set of conditions for 2, 4, and 6 months. The microwave-treated sample did not show statistical difference with the control and irradiated sample, in each storage condition, at 2 months of storage; it was statistically different at 4 months and at 6 months

(only for 30 °C, 75% RH). From a comparison of the effect of storage conditions on aroma (columns, Table III), it is observed that results for the microwave-treated samples stored at 30 °C, 75% RH, were consistently inferior ($p \leq 0.05$) for the samples stored under refrigeration. Storage at 30 °C, 75% RH, was unfavorable to the aroma at 6 months of storage only. The control samples stored at 30 °C, 75% RH, differed from the samples kept under refrigeration at 4 and 6 months of storage. As a whole, the influence of treatment and storage conditions on the aroma of bean was more pronounced in the microwave-treated samples than in the control and irradiated samples.

Table IV shows the effects of treatments with gamma rays (2 kGy) and microwaves (2 min) as well as conditions and time of storage on dry bean taste. The effect of treatments (rows) indicates that the results for the control and irradiated samples are not statistically different and that the microwave-treated samples were inferior ($p \leq 0.05$) to the control and the irradiated samples. For each storage condition the irradiated samples did not differ from the control samples up to 4 months of storage. Difference with the control was noticed only for the sample stored for 6 months at 30 °C, 75% RH. Except for the sample stored for 2 months under refrigeration, all of the other results obtained for the samples treated with microwaves were statistically inferior ($p \leq 0.05$) to the control and irradiated samples. When comparisons were made for conditions of storage (columns), the microwave-treated samples stored at 30 °C, 75% RH, were statistically inferior ($p \leq 0.05$) to the samples stored under refrigeration; there was no influence of storage conditions in the irradiated samples. In the control samples storage conditions showed no effect at 2 months of storage, but at 4 and 6 months, samples stored at 30 °C, 75% RH, were statistically inferior to those stored under refrigeration.

The attribute of firmness is described in Table V. Influence of treatments (rows) indicates that the firmness of irradiated samples did not differ statistically from the control samples, in each storage condition. On the other hand, the microwave-treated samples were consistently harder (lower quality) at statistical level of 5% probability. Regarding influence of storage conditions on firmness (columns), significantly higher values ($p \leq 0.05$) were found for all samples submitted to the various treatments and stored at 30 °C, 75% RH. These results confirm the well-known fact that high storage temperature and moisture greatly accelerate hardening in dry beans.

Few studies have evaluated the effects of gamma radiation on the sensory properties of dry bean and other legume seeds (Neme et al., 1975a,b; Rao and Vakil, 1985; Carvalho et al., 1991). Neme et al. (1975a,b) reported that legume seeds submitted to gamma radiation (10 kGy) showed reduction of cooking time in the range 8–39%, compared to nonirradiated samples. Still greater reductions were observed with dosages of 20 and 30 kGy, when undesirable changes were detected in the sensory properties, rendering the products unacceptable for human consumption.

Rao and Vakil (1985) reported that gamma radiation up to 5 kGy altered the sensory attributes of legume seeds as judged by trained panelists. They also observed reduction in cooking time and in starch viscosity on gelatinization which they attributed to starch depolymerization due to the action of gamma rays. Carvalho et al. (1991) verified that 5 kGy was sufficient to cause alteration of flavor in dry beans which was clearly detectable by the tasters.

Table VI. Changes in Phytate Contents [Percent (w/w), Dry Basis][a] of Dry Beans Stored for 6 Months under Refrigeration (4–5 °C) or at 30 °C, 75% Relative Humidity (RH)

sample/conditions of storage[b]	time of storage			
	0 months	2 months	4 months	6 months
C, refrigeration	0.97 ± 0.02	0.77 ± 0.01	0.81 ± 0.02	0.77 ± 0.02
C, 30 °C, 75% RH		0.88 ± 0.01	0.85 ± 0.01	0.49 ± 0.02
I, refrigeration	1.05 ± 0.02	0.74 ± 0.02	0.70 ± 0.01	0.85 ± 0.01
I, 30 °, 75% RH		0.96 ± 0.03	0.63 ± 0.01	0.53 ± 0.02
M, refrigeration	1.08 ± 0.01	0.85 ± 0.01	0.74 ± 0.02	0.75 ± 0.01
M, 30 °C, 75% RH		0.93 ± 0.01	0.73 ± 0.01	0.66 ± 0.01

[a] Results are mean ± SD of three determinations. [b] C, samples not pretreated; I, samples pretreated with gamma rays (2 kGy); M, samples pretreated with microwaves (2 min).

It seems to be clear that the dosage at which irradiated seeds become organoleptically different from nonirradiated is dependent on the kind and composition of the seeds. More studies should be conducted on various kinds of seeds to establish the relationship between gamma ray dosage and the sensory properties of cooked seeds. It is suggested that for dry beans the safe dosage, as far as keeping sensory qualities, lies in the range 2–5 kGy, although some regulatory international agencies (FAO/IAEA, 1982) established 10 kGy as the maximum dosage for human foods.

Table VI shows the changes in phytate content of the irradiated, microwave-treated, and control samples stored at refrigeration or 30 °C, 75% RH, for 6 months. The changes from 0 to 6 months under refrigeration storage were 20.6, 19.0, and 30.5% for the control, irradiated, and microwave-treated samples, respectively. For the samples stored at 30 °C, 75% RH, the changes were 50.9, 49.5, and 38.9%, respectively, for the control, irradiated, and microwave-treated samples. It is noticeable that the samples stored at high temperature and humidity showed higher phytate degradation than the samples stored under refrigeration. Highest degradation (~50%) was found in the control and irradiated samples stored at 30 °C, 75% RH, while for the microwave-treated samples degradation was only 38.9% of the phytate originally present. This may be due to partial inactivation of the phytase enzyme during the microwave heating.

Phytate and the action of the enzyme phytase have been implicated as part of the most acceptable hypothesis to explain the mechanism of legume seed hardening (Aguilera and Stanley, 1985; Jones and Boulter, 1983; Kon and Sanshuck, 1981; Moscoso et al., 1984). The essence of the hypothesis is that calcium liberated from phytate by the action of phytase would complex pectic acid at the middle lamella of the cell, cross-binding cotyledon cells and acting as a blockage to the separation of the cells, responsible for softening the seeds in the cooking process.

Two other components in this system are pectin and pectin methyl esterase, producing sufficient pectic acid to bind calcium.

Moscoso et al. (1984) found a high negative correlation between phytate content or solubility of pectic material and extent of hardening of dry beans. According to Jones and Boulter (1983), the splitting of phytate into phosphate and inositol by phytase prevents the binding of calcium and magnesium to phytate, therefore making these divalent ions available for binding to pectic acid and thus contributing to hardening.

Our data on phytate degradation do not seem to correlate well with hardening, on the basis of cooking time, although a general decrease in phytate is observed with a corresponding increase in cooking time.

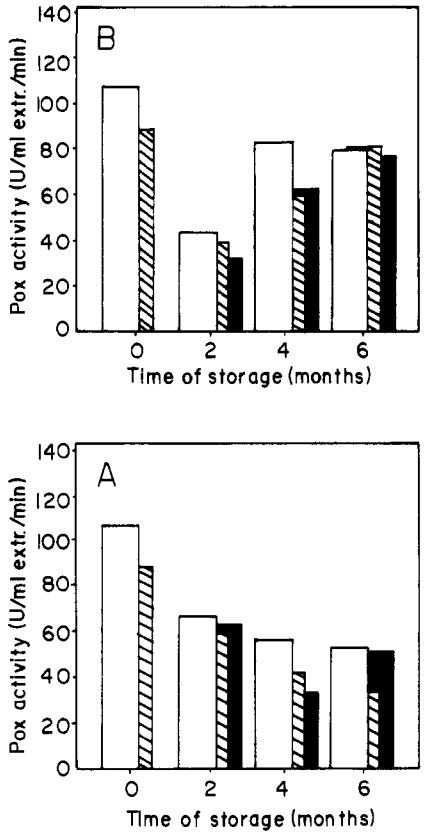

Figure 2. Peroxidase activity of beans stored for various times under two sets of conditions: (A) 30 °C, 75% RH (■) pretreated with microwaves (2 min), (▨) pretreated with gamma rays (2 kGy), (□) not treated (control); (B) refrigeration (■) pretreated with microwaves (2 min), (▨) pretreated with gamma rays (2 kGy), (□) not treated (control).

Other cell components such as polyphenols and peroxidase have also been suggested as possible counterparts in the hardening process.

Mejia (1982) found an increase in polyphenol oxidase activity and a decrease in polyphenols in dry beans stored at high temperature and humidity. Disappearance of polyphenols contrasted with the increase in hardening of the beans, which the authors interpreted as polymerization of polyphenols by polyphenol oxidase. Rivera et al. (1989) reported on a decrease of peroxidase activity with a concurrent lignification of the middle lamella of the cotyledon cells. The authors assume that lignification of the middle lamella by peroxidase in the presence of phenolic compounds could be an important component of the bean hardening process.

In this study a general decrease in peroxidase activity was demonstrated for the high-temperature/high-humidity conditions (Figure 2A). This general tendency was not observed for the refrigeration condition (Figure 2B).

Table VII shows the degradation of methionine of dry beans for the irradiated, microwave-treated, and control samples stored for 6 months under refrigeration or at 30 °C, 75% RH. Losses of methionine under refrigeration condition were 4.6, 17.0, and 27.4% for the control, irradiated, and microwave-treated samples, respectively. For the 6 months storage at 30 °C, 75% RH, the methionine losses were 27.6, 25.0, and 36.8% for the control, irradiated, and microwave-treated samples, respectively. Antunes and Sgarbieri (1979) reported rapid losses of methionine and methionine bioavailability in dry beans stored for 6 months under 37 °C, 76% RH, which resulted in drastic reduction of protein nutritive value.

Table VII. Changes in Methionine Content (Grams per 16 g of N, Dry Basis)[a] of Dry Beans Stored for 6 Months under Refrigeration (4–5 °C) or at 30 °C, 75% Relative Humidity (RH)

		6 months of storage	
treatment[b]	zero time	refrigeration	30 °C, 75 RH
C	0.77 ± 0.04	0.83 ± 0.04	0.63 ± 0.05
I	1.00 ± 0.05	0.83 ± 0.04	0.75 ± 0.02
M	0.95 ± 0.06	0.69 ± 0.04	0.60 ± 0.02

[a] Results are mean ± SD of three determinations. [b] C, samples not pretreated; I, samples pretreated with gamma rays (2 kGy); M, samples pretreated with microwaves (2 min).

As a whole, the results reported in this paper lead to the following conclusions. Dry beans stored under high temperature and high relative humidity undergo deteriorations characterized by hardening of the cotyledons, increase in cooking time, undesirable changes in texture and flavor, decrease in peroxidase activity, and degradation of phytate and methionine. Pretreatment of the beans with gamma rays (2 kGy) or with microwaves (2 min) did not prevent the beans from hardening. However, due to the pronounced initial softening effect of gamma radiation and the initial hardening effect of microwave treatment, cooking time at 6 months of storage under 30 °C, 75% RH, was shorter for the irradiated sample and longer for the microwave-treated beans, compared with the control. With regard to texture and flavor, the irradiated samples did not differ from the control samples, whereas the microwave-treated samples were of inferior quality. The results indicated an advantage of gamma radiation, but not microwave, pretreatment to prolong the life of dry beans stored at 30 °C, 75% RH.

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Received for review August 6, 1992. Revised manuscript received May 4, 1993. Accepted June 7, 1993.[⊕]

[⊕] Abstract published in *Advance ACS Abstracts*, August 15, 1993.