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Nutritional and antinutritional factors during the storage process of common bean

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Abstract

The common bean (*Phaseolus vulgaris* L.) is one of the most important elements of the diet of the Brazilian population, being an excellent source of protein, having good content of carbohydrates and being rich in iron. In addition to providing essential nutrients, it is used as an alternative to meat or other protein products for the population with a low-income. The aim of this study was to assess the influence of storage on the nutrient and antinutritional factors that the beans undergo during their process of cooking. Beans of the Carioca variety IAPAR 81 were assessed at 0, 45, 90, 135 and 180 days of storage and three processes of cooking: baked beans without soaking water (FCSAM), baked beans with soaking water (FCCAM) and baked beans without soaking (FCSM) and raw bean (control). Each sample was analyzed for moisture, total solids, protein, phytic acid, tannin and minerals (calcium, iron and manganese), which were compared to results from raw beans within five days of storage. The results were subjected to analysis of variance (ANOVA) and differences between means were analyzed by Tukey's test at 5% probability ($p < 0.05$). There was significant increase in the cooking time of the stored grain, which indicated hardening of the grain stored at room temperature. Moisture content showed a lower result within 45 days of storage. There was less concentration of protein in beans cooked without soaking water. When analyzing the protein as a function of storage time, it was observed that the beans with 45 days of storage had the highest protein content. By analyzing the content of phytates, it was found that the months of storage decreased the phytate content of the grain, and the best treatment to reduce phytate would be baked beans without soaking water. Since the tannin content showed an increase with storage, and the period of 180 days had the highest concentration of tannins in the grain, its best treatment was the FCSAM. The content of tannin increased in the broth with FCSAM. The storage time was a major factor and influenced the content of protein, phytates, tannins and calcium, reducing or increasing their values as a function of time.

Key words: Phytates, tannins, protein, *Phaseolus vulgaris* L.

Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important components of Brazilian population diet [1, 2], as well as of other countries of South America [3], once it is an excellent source of protein, besides possessing suitable carbohydrate content and is rich in iron [4].

Among the nutritional characteristics of common bean, it contains positive factors, such as high contents of protein and lysine [5]. However, it also contains limiting factors such as difficult cooking; phenolic compounds; phytic acid; flatulence factors and low protein digestibility. Proteins present reduced digestibility when consumed "in natura"; however, their digestibility increase after thermal treatment. Even though, the digestibility is still limited, in the light of the change of their primary structure and the permanence of thermostable protease inhibitors that interact with polyphenolic or digestive enzymes and/or with the common bean proteins, forming complexes and decreasing the degree of hydrolysis [6].

The tannins are phenolic compounds found in plants, foods and beverages [7]. Polyphenols of Leguminosae and cereals are predominantly tannins of flavonoid origin [8]. The term was originally used to describe the substance contained in plant extracts and used in tanning leather, mainly by their capacity of combining with proteins of animal skins, inhibiting the putrefaction process[9].

After harvest, the respiration and other metabolic processes of the grains continue actives and, in the majority of cases, causing significant losses on quality, which are irreversible and cumulative; and whose intensity depends on different factors [10]. Such processes can be diminished and/or delayed by reducing moisture; but even with low moisture content, the grains lose their quality due to weight loss, and energy consumption through respiratory process; what may lead to reduction of its consumption as grain[11-15].

In face of the foregoing, the need of further information concerning the changes of the nutritional and antinutritional factors during the product storage should be emphasized, aiming at the best utilization of their nutrients.

In this context, the main objective of this study was to verify the influence of storage period as well as different culinary techniques for the preparation of common bean, on the content of nutrients and antinutritional factors in the grains of this leguminous plant.

Material and Methods

Material: The seed samples of the "carioca" type of cultivar IAPAR 81 of common bean (*Phaseolus vulgaris* L.), cultivated during the 2009/2010 rain season, were obtained in a commercial production area, in municipality of Santa Tereza do Oeste, State of Paraná, Southern Region of Brazil. The grains were harvested mechanically with the aid of a combine-harvester and dried until reaching 13% moisture content, before the storage.

Grains storage: The seed samples were packaged into kraft paper bags for a total period of 180 days, without control of temperature and relative humidity (laboratory normal environment). Assessments were performed in five different storage periods (SP) of day 0 (before storage), 45, 90, 135, and 180 days.

Analyses of whole grains: Before storage (day 0) and after each storage period, subsamples of approximately 16 g were removed for determining the cooking time of grains, with the aid of an adapted Mattson Cooker, according to the method proposed by Proctor and Watts [16]. Determination of moisture content was performed by the gravimetric method, in a convection oven [17].

Procedures for obtaining meal and broth of beans: Before (day 0) and after each storage period, the whole bean grains were selected and subjected to four different processing procedures for obtaining the meals and broth: bean cooked in the maceration water (BCIMW); bean cooked without the maceration water (CBWMW); and non-macerated and cooked bean (NMCB). Non-cooked grains, or raw beans (RB), were used as control.

The treatments with maceration were performed with immersion of grains in deionized water, during 16 h, in a ratio 1:4 (beans:water), under environmental temperature. The samples were cooked into lidded Becker cups for a mean time of obtained in each storage period, maintaining during all the cooking time the same ratio of beans: water used in the maceration process (1:4). The separation of the cooked beans from the broth was performed with the aid of a sieve. Immediately after cooking, the percentage of water absorption by the grains was determined according to Carbonell *et al.* [18].

The cooked grains were dried in a forced air circulation oven, at 60ºC, until constant weight and then ground and sieved through a 50 mesh sieve. The grains of control treatment (RB), were also ground after each storage period and the meal obtained was, in the same manner, sieved through a 50 mesh sieve. The broth obtained after the cooking process was packed in plastic pipes and frozen until the moment of analyses.

Chemical analyses: The assessment of crude protein was performed using the method of semi-micro-Kjeldahl, with conversion factors of 6.15x N% [19]. The phytate analysis was carried out by colorimetric method, according to methodology proposed by Latta and Eskin [20], and tannin determination was performed by the Folin-Denis spectrophotometric method [21]. The samples so obtained were stored into refrigerator, at 5ºC, during analyses execution. For determination of minerals (Fe, Mn, and Ca), the determination of ashes by calcination was performed. For this, the samples were dissolved in 25 ml of 0.1 N HCl [22]. The reading of sample results was performed with the aid of an atomic absorption spectrophotometer and results were expressed in mg/100 g of of beans. All analyses were carried out in triplicates and expressed in dry basis.

Chemical analyses of the cooking broth: The content of total solids in the broth was determined gravimetrically in a forced air circulation oven. The crude protein content in the broth was assessed using the same procedure previously described for the grain meal.

Statistical analyses: The experiment was carried out in a completely randomized experimental design, in a split plot systems of arrangement for treatments and four replications. To each processing method (BCIMW, CBWMW, NMCB, and RB) were arranged in the plots; and the storage periods (0, 45. 90, 135, and 180 days) were arranged in the subplots.

Data were submitted to ANOVA and means of treatment were compared by the F test of Snedecor (F(n1-n2-1; 2) and Tukey test, at 5% probability, using the Sisvar software [23]. For the data without normality by the Anderson-Darling test, the descriptive statistics analysis of means (n = 8) was performed.

When the interaction was statistically significant between the factors: type of processing x storage period, at 5% probability level; data indicate that indeed occurred influence of storage period on the cooking time. However, if the interaction was not statistically significant and the processing types behave similarly to storage period, the comparisons were performed among general means of treatments with culinary processing types and the means of storage period.

Results and Discussion

Whole grains: Data on cooking time and on moisture content of whole grains during all storage period are shown in Table 1. Analyzing these data, it is possible to verify that from 90 days of storage on, there was increase in the cooking time; which in 135 days reached 80% of increase, and reached 300% in 180 days of storage, in relation to time of cooking of the control period (day 0). Such increase is normally expected; but its intensity, however, may vary with genetic and environmental factors [14, 24].

The moisture content of grains also presented variations along the storage period (Table 1); although such variation is related to variation on temperature and relative humidity [25], which in this experiment was maintained below 14%, thus favoring the quality of product. In this case, the metabolism of seeds is reduced due to respiratory process, and there is inhibition of microorganisms and insects development [26].

Table 1. Cooking time (min) and moisture content (%) of "carioca" type bean grains, cultivar IAPAR 81, after 180 days storage without control of environmental conditions (temperature and RH).

	Storage time (days)				
	0	45	90	135	180
Cooking time	30	30	46.5	54	128
Moisture content (%)	11.5	10.74	11.77	10.88	13.27

The values of water absorption by whole bean grains, which were subjected to different processing procedures after the cooking (Table 2), presented statistically significant interaction between the treatments of cooking time x storage period. The highest values for percent of water absorption were obtained

Table 2. Percentage of water absorption by grains of common bean, “carioca” type, cv. IAPAR 81, after cooking; and then dried and subjected to different types of culinary processing and different storage periods.

ST (days)	NMCB	BCIMW	CBWMW	Média
0	104.01eB	106.64dB	111.56eA	107.40
45	108.56dB	117.87cA	117.46dA	114.63
90	131.30aB	138.32aA	141.82aA	137.17
135	115.20cC	128.48bA	122.36cB	122.03
180	123.10bB	136.60aA	135.53bA	131.77
Means	116.48	125.58	125.74	

*Means followed by the same small letters in the columns and capital letters in the lines do not statistically differ between each other by the Tukey test, at 5% probability. ** (ST) = storage time. (NMCB) = non-macerated cooked bean; (BCIMW) = beans cooked in the maceration water; (CBWMW) = beans cooked without the maceration water.

starting from the 90 days storage period, in all types of culinary processing; the same behavior occurred for the values of cooking time.

Grains with high values for water absorption after cooking are recommended for cafeterias and industrial kitchens, once provides higher yield; although the increase on the cooking time could be representing an undesirable factor. Despite the values found in this study be similar to values found in other studies [27, 28], a direct correlation between cooking time and water absorption was not found [18] in grains assessed by cooking without previous maceration. In this study, however, the highest water absorption was found in the treatments using previous maceration of grains what may indicate the effect of domestic normal procedure in water absorption.

Assessment of grains subjected to domestic processing: The content of proteins, during the storage of raw beans, as well as the beans subjected to different domestic processing modes (Table 3), presented statistically significant differences, with interactions likewise statistically significant between culinary processings modes and storage period. It was observed that storage of grains, from 45 days, caused reduction on protein content of raw beans. In addition, the processing of beans cooked without the maceration water presented lower protein contents in relation to the remaining treatments, when compared to non-processed beans.

Ramírez-Cárdenas *et al.* [29] found increase of crude protein of until 7% in beans cooked without the maceration water, when compared to raw beans; what is the contrary to results herein obtained. In this study it was verified reduction of circa 2% for the treatment CBWMW; it was also verified protein contents close to 2%, or even lower than that, in the broth (Table 2); what indicates that losses of this nutrient occurred in the cooking broth. Despite the differences be not statistically significant, it was observed higher absolute value for loss of protein in the product cooked with the maceration water, what testifies that such loss occurs during maceration and cooking processes. That loss can be confirmed by the total content of solids in the broth (Table 2), where higher losses can be observed for treatment BCIMW, as compared to other remaining treatments.

In the assessment of the storage period variable (Table 4), it was observed that freshly-harvested beans presented higher phytate contents than the grains stored, thus indicating reduction of such antinutritional factor with the storage. Silva *et al.* [30] found

Table 3. Percentage of proteins in grains and total solids in grains and broth of common bean, “carioca” cv. IAPAR 81, subjected to different types of culinary processing and different storage periods.

ST (days)	NMCB	BCIMW	CBWMW	Média	
		Grains protein			
0	25.84bA	26.24bA	27.38bA	27.16bA	26.65
45	29.16bA	24.15bC	25.72bAB	26.38aB	23.36
90	27.88aA	26.2bAB	26.49aAB	25.48aB	26.64
135	26.46bAB	28.06aA	27.70aA	25.03bB	26.80
180	25.90bA	24.09bAB	23.31bB	23.74aB	24.20
Média	27.05	25.85	26.12	25.56	
		Broth protein			
TA (dias)	FC	FCSM	FCCAM	FCSAM	Média
0	-	0.34	0.45	0.36	0.38 c
45	-	0.59	0.91	0.67	0.73 b
90	-	0.99	1.07	1.06	1.04 a
135	-	1.00	1.13	0.86	0.96 ab
180	-	0.97	0.81	0.79	0.86 ab
Média		0.78a	0.87a	0.75a	
		Broth Total Solids			
TA (dias)	FC	FCSM	FCCAM	FCSAM	Média
0	-	4.07aA	4.69aA	4.02aA	4.26
45	-	2.97bB	4.58aA	2.91bB	3.49
90	-	3.92aA	3.27bA	1.77cB	2.99
135	-	3.95aB	4.93aA	3.87aB	4.25
180	-	4.83aA	4.58aA	4.36aA	4.59
Média		3.95	4.41	3.39	

* Means followed by the same small letters in the columns and capital letters in the lines do not statistically differ between each other by the Tukey test, at 5% probability. ** (ST) = storage time. (NMCB) = non-macerated cooked bean; (BCIMW) = beans cooked in the maceration water; (CBWMW) = beans cooked without the maceration water**

Table 4. Percentage of phytates and tannins in grains and total solids in grains and broth of common bean, “carioca” cv. IAPAR 81, subjected to different types of culinary processing and different storage periods.

		Phytates			
ST (days)	ST	NMCB	BCIMW	CBWMW	Means
0	0.44aB	0.53aA	0.56aA	0.57aA	0.52
45	0.18bC	0.23bB	0.36bA	0.15bD	0.23
90	0.23bA	0.22bA	0.17cA	0.09cB	0.18
135	0.02cA	0.05cA	0.07dA	0.07cdA	0.06
180	0.04cA	0.02cA	0.01dA	0.02dA	0.02
Means	0.18	0.21	0.24	0.18	
		Tannins			
0	3.59dB	4.52dA	1.99cAB	1.56dB	2.91
45	3.28cA	3.30cA	3.00cA	1.65cA	2.81
90	9.04bA	8.83bA	8.48bA	6.92bA	8.32
135	20.74aA	15.43aA	27.74aA	16.48aA	19.98
180	34.46aA	34.82aA	26.71aB	25.10aB	30.27
Means	14.22	13.38	13.18	10.65	

* Means followed by the same small letters in the columns and capital letters in the lines do not statistically differ between each other by the Tukey test, at 5% probability. ** (ST) = storage time. (NMCB) = non-macerated cooked bean; (BCIMW) = beans cooked in the maceration water; (CBWMW) = beans cooked without the maceration water; and (RB) = raw beans (control).

contents of phytates of 0.564%, a value similar to what was found in this study. However, the storage of grains was not assessed and results indicated that this was the main factor related to phytate reductions in the grains. Granito *et al.* [31] stored common bean during 150 days, and found reductions in phytate content of circa 20% lower than what was found herein, but confirming that the storage causes reduction in this antinutritional factor.

Effect of thermal treatment on the content of phytates was also not found (Table 4), once there was no reduction of this component with the domestic use of the different processing procedures. Ertas [32] observed reduction of phytates with the cooking of grains; although with application of high pressure and maceration with antioxidant agents, which may explain the result opposite to what was herein obtained, once the cooking in this study was performed under normal atmospheric pressure.

The content of tannins in the bean grains stored and afterwards subjected to different types of processing (Table 4), presented inverse effect of phytate content in the grains, once increases in this antinutritional factor were observed in the beans after storage. The maceration process of the grains is associated with the reduction of tannins, since the beans, cooked with or without the maceration water, presented lower values of tannins in the bean meal. According to Oliveira *et al.* [33], the domestic processing of common beans, including the maceration prior to cooking, caused reduction of 88% in tannin content, as compared to the non-processed product. Beans cooked without the maceration water, presented lower content of phytates and tannins; although the maceration of the grains, prior to the cooking, has not interfered on its nutritional value; such fact was also observed in studies carried out by Moura and Canniatti-Brazaca [34] and Granito *et al.* [31].

There was not statistically significant variation in the ash content of the bean grains (Table 5); however, certain reduction on ash content was observed for the beans cooked without the maceration water; thus indicating that such process influences the final content of minerals.

Table 5. Percentage of ash in grains of common bean, "carioca" cv. IAPAR 81, subjected to different types of culinary processing and different storage periods.

ST (days)	RB	NMCB	BCIMW	CBWMW	Means
0	4.63	3.68	3.58	3.20	3.77 a
45	5.41	3.96	3.20	3.54	4.03 a
90	3.95	4.21	4.20	3.70	4.02 a
135	3.24	4.25	4.00	3.75	3.81 a
180	4.48	3.49	3.25	3.74	3.74 a
Means	4.34a	3.92a	3.65b	3.59b	

* Means followed by the same small letters in the columns and capital letters in the lines do not statistically differ between each other by the Tukey test, at 5% probability. ** (ST) = storage time. (NMCB) = non-macerated cooked bean; (BCIMW) = beans cooked in the maceration water; (CBWMW) = beans cooked without the maceration water; and (RB) = raw beans (control).

In a study [35] on total amount of ash, the values obtained varied from 3.36% to 4.17%.; values that are very close to the results found in this research work. The ash content (4.63%) herein found for raw beans was very similar to the value reported by Mechi *et al.* [36] (4.9%) to the same variable. In a study carried out by Mesquita *et al.* [37], the content of ash for 21 breeding lines of common beans varied between 3.0% and 4.9%. According to the same author, such differences may be inherent to the lines, or caused by cultivation conditions, such as climate and soil fertility.

Table 6 shows the contents of Fe, Mn, and Ca in the bean grains. To Fe and Mn, higher contents were found in the grains cooked without the maceration water. In verifying the values of Ca in the grains, it was found that there was statistically significant correlation among the values of these factors; thus showing that the storage period influenced the content of Ca, in relation to cooking method.

Studies by Mesquita *et al.* [37], who evaluated 21 breeding lines of common beans, demonstrated that manganese contents were between 14.93 and 28.90 mg.kg^{-1} of beans. The iron content was between 71.37 and 126.90 mg.kg^{-1} of beans; and calcium content between 0.03 and 0.28 g/100 g of beans. These values were similar

Table 6. Iron (Fe), manganese (Mn) and calcium (Ca) (mg kg^{-1}) of common bean, "carioca" cv. IAPAR 81, subjected to different types of culinary processing and different storage periods.

ST (days)	Fe			
	NMCB	BCIMW	CBWMW	Means
0	70.05	86.38	61.31	71.58ab
45	100.95	67.77	74.72	77.29ab
90	80.85	85.47	67.06	77.69ab
135	62.80	81.57	265.77	117.77a
180	53.99	32.28	29.55	45.58b
Means	73.70 a	70.70 a	99.68b	
	Mn			
0	16.79	22.13	16.14	16.25b
45	11.69	12.51	10.80	9.65c
90	25.51	31.17	30.02	26.25 a
135	23.98	22.83	29.46	22.23 a
180	23.16	23.41	26.09	22.45a
Means	20.23a	22.41a	22.50b	
	Ca			
0	376.59cA	321.47bA	188.80cB	299.37
45	234.38dB	347.65bA	338.17bA	282.06
90	611.87abA	679.38aA	675.21aA	584.67
135	691.37aA	649.81aA	583.14aA	572.77
180	529.68bA	589.11aA	615.12aA	487.56
Means	488.78	517.48	480.09	

* Means followed by the same small letters in the columns and capital letters in the lines do not statistically differ between each other by the Tukey test, at 5% probability. ** (ST) = storage time. (NMCB) = non-macerated cooked bean; (BCIMW) = beans cooked in the maceration water; (CBWMW) = beans cooked without the maceration water; and (RB) = raw beans (control).

to the values found within this study.

Barrueto-Gonzalez [38] concluded that leguminous sources are subject to presence of substances, which promote or not the absorption of minerals, and whose concentrations vary according to the type of Leguminosae. Nevertheless, it is possible to attenuate these negative effects with the adequate culinary procedures, on preparing these grains; once the majority of these compounds are water-soluble.

Conclusions

In reference to domestic preparation and in relation to its nutritional value, the quality of the common beans increases with the disposal of the maceration water before the cooking process, once this procedure reduces the limitations inherent to grains of this leguminous, such as the antinutritional factors, thus providing increase in the digestibility of the proteins contained within them.

The storage of common bean grains for long periods also alters their nutritional quality. The storage time was a major factor and influenced the content of protein, phytates, tannins and calcium, reducing or increasing their values as a function of time.

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