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Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*

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Abstract

Germination is a method that can modify the presence of nutrients and antinutrients in legume seeds. In this study, the changes in trypsin inhibitors activity (TIA), phytic acid, tannins, ascorbic acid, thiamine, protein digestibility and minerals in germinated black beans (*Phaseolus vulgaris* L.), white beans (*Phaseolus vulgaris* L.) and pigeon beans (*Cajanus cajan* L. Millsp) were evaluated. The ungerminated grains were analysed as control. A significant decrease in the content of TIA of pigeon beans (19.2%), white beans (52.5%) and black beans (25%) was observed. The reduction of phytic acid was more than 40% for the three grains germinated; for the tannins, the reduction was of 14.3% for pigeon beans, 19% for black beans and 36.2% for white beans. Germination increased the protein digestibility in a 2–4% range, and also increased the ascorbic acid by 300% for white beans, by 33% for black beans and by 208% for pigeon beans. The thiamine content increased more than 26.7%. Germination affected the mineral content erratically, depending more on the grain and the type of mineral. These variations in the content of nutrients and antinutrients of the germinated grains are attributed to the joint effect of the germination and previous soaking the grains were subjected to.

Keyword: Phaseolus; Cajanus; Nutrients; Minerals; Protein digestibility

1. Introduction

Germination is a natural biological process of all superior plants by which the seed comes out of its latency stage, once the minimal environmental conditions needed for its growth and development, such as humidity, temperature, nutrients, etc., are given. For the seed to germinate, there are also external factors such as a humid substrate, availability of oxygen for aerobic respiration and an adequate temperature for the different metabolic processes and for the development of the plantlet (García & Primo, 1993). The process of germination has been developed in some countries as an alternative to defeat some of the disadvantages associated with untreated grains, such as undesirable tastes and smells, as well as the presence of trypsin inhibitors (Suberbie, Mendizabal, & Mendizábal, 1981).

During the germination there are certain changes that could occur as far as the quantity and type of nutrients within the seed. Those changes can vary depending on the type of vegetable, the variety of the seed and the conditions of germination (Bau, Villaume, Nicolas, & Méjean, 1997; Dhaliwal & Aggarwal, 1999). An increase in the bioavailability of minerals and vitamins has been observed. Germinated grains are good sources of ascorbic acid, riboflavin, choline, thiamine, tocopherols and pantothenic acid.

The objective of this study is to evaluate the effect of germination on some nutrients as well as on some antinutritional factors of white beans (*Phaseolus vulgaris* L.), black beans (*Phaseolus vulgaris* L.) and pigeon beans (*Cajanus cajan* L. Millsp.)

2. Materials and methods

2.1. Samples

Black beans and white beans (*Phaseolus vulgaris* L.) and pigeon beans (*Cajanus cajan* L. Millsp) were used. Beans were acquired at a local market.

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2.2. Germination

Grains were rinsed using tap water, disinfected by soaking in a solution of 1% sodium hypochlorite for 20 min and rinsed twice with distilled water. Sanitized grains were soaked in distilled water (1:3 w/v) for 5 h. Soaked grains were drained, dried with paper, placed on perforated aluminium trays and germinated in darkness at 25±2 °C for 5 days. In order to maintain an adequate hydration level, grains were sprayed daily with distilled water (Machaiah, Pednekar, & Thomas, 1999).

2.3. Flour preparation

Germinated grains were dried at 60 °C for 10 h using an oven (Felisa, Mexico) with forced ventilation. Dry grains were ground in a mill (HQ Analyser M.C., Argentina) and sieved, and a 0.5 mm fraction was collected. Flours were stored in glass containers at refrigeration temperatures for later analysis. For comparative purposes, flours of ungerminated grains were also prepared.

3. Analysis

The trypsin inhibitors activity (TIA) was determined according to Kakade, Rackis, Mcghee, and Puski (1974). The flour was suspended in 50 ml NaOH 0.01 N and homogenized for 2 min in a Polytron disintegrator (Kinematica, Switzerland) before the extraction phase. Results were expressed as trypsin inhibitory units (TIU) per milligrams of dried sample. Phytic acid was determined according to the method proposed by Mohamed, Perera, and Hafez (1986). Tannins were quantified by the method proposed by Price and Butler (1977).

In vitro protein digestibility was determined by the multi-enzymatic technique described by Hsu, Vavak, Satterlee, and Miller (1977). Three enzymes (trypsin, chymotrypsin and peptidase) were used. The extent of hydrolysis was determined by pH-drop after a 10 min incubation period (X) and the percentage of protein digestibility (Y) was calculated by the regression equation:

$$Y = 210.469 - 18.10X,$$

where X is the pH of protein suspension at 10 min digestion with the multi-enzymatic system.

Ascorbic acid was determined by a 2,6-dichloroindophenol titrimetric method (A.O.A.C., 1990). Thiamine was determined by a spectrofluorometric method (A.O.A.C., 1990). Minerals were determined by inductively coupled plasma-atomic emission spectroscopy (Spectroflame XL ICP, GBC, Australia). From the ash, an acidic solution was prepared and the contents of calcium, magnesium, zinc, iron and copper were quantified. All results are given as means of triplicate analysis, expressed on dry matter basis.

3.1. Statistical analysis

The Statgraphics Plus (version 1.4 for Windows 98) software was used for statistical analysis. Mean and standard deviations were calculated from the results of the analyses performed. The data were subjected to analysis of variance (ANOVA). Means comparison was performed using Duncan's Multiple Range Test. Significance was defined as $P<0.05$.

4. Results and discussion

A significant statistical decrease in the TIA of black and white beans (*P. vulgaris*) and pigeon beans (*C. Cajanus* L. Millsp) was observed as an effect of the germination (Table 1). For the white beans, the reduction was 52.5%. Likewise, the black beans displayed a reduction of 25.6%, whereas it was 41% for pigeon beans. Batra, Vasjshta, and Dhindsa (1986) determined that the germination of lentil grains reduced the TIA by about 50%. Deshpande, Sathe, Salunkhe, and Comforth (1982) reported a reduction for *Vigna umbellata* of approximately 30%. According to Urbano et al. (1995) in *Lens culinaris* the TIA was reduced by 22%, and for *V. umbellata* and *Phaseolus*

Table 1
Trypsin inhibitor activity, ascorbic acid, phytic acid and thiamine in ungerminated and germinated *P. vulgaris* and *C. cajan*

Sample	TIU/mg	Ascorbic acid (mg/100 g)	Phytic acid (mg/100 g)	Thiamine (mg/100 g)
P. vulgaris (white beans)				
Ungerminated	4.13±0.06[a]	3.30±0.01[a]	7.80±0.34[a]	0.82±0.03[a]
Germinated	1.90±0.02[b]	13.63±0.05[b]	4.30±0.05[b]	0.93±0.01[b]
P. vulgaris (black beans)				
Ungerminated	4.37±0.08[a]	9.14±0.07[a]	9.11±0.10[a]	0.72±0.02[a]
Germinated	3.22±0.01[b]	12.12±0.40[b]	4.32±0.10[b]	0.91±0.02[b]
C. cajan				
Ungerminated	4.75±0.10[a]	4.40±0.01[a]	7.34±0.20[a]	0.82±0.01[a]
Germinated	2.70±0.20[b]	13.60±0.01[b]	4.32±0.10[b]	0.93±0.01[b]

Results are means of triplicate±standard deviation, expressed on dry matter basis. Different letters in the same column means significant differences ($P<0.05$).
TIU: trypsin inhibitory units.

tetragonolobus, the reduction was 30% and 50%, respectively (Verma & Mehta, 1988). Such results are in agreement with this study. Akpapunam and Sefa-Dedeh (1997) found out that the germination of *Cicer ensiformis* was more effective than cooking on the reduction of the TIA. Germination is a mainly catabolic process as the reserved substances present in the cotyledon are used for the development and growth of the embryo. However, certain studies have shown some contradictory results. Khaleque, Elías, Braham, and Bressani (1985) reported that germination of *Cicer arietinum* did not significantly modify the TIA, while Oloyo (2004) determined an increase in the TIA in legumes, which was attributed to an increase in the content of phenolic compounds in the germinated seed. Burbano, Muzquiz, Ayet, Cuadrado, and Pedrosa (1999) indicate that there is a possibility that the trypsin inhibitors could be utilized as an energy source during the early stages of germination.

A significant statistical reduction was observed in the phytic acid content of white beans, black beans and pigeon beans due to the effect of germination (Table 1). The reduction percentages were 44.7% for the white beans, 52.9% for the black beans and 40.7% for pigeon beans. According to Sathe, Desphande, Reddy, Gell, and Salunkhe (1983) the reduction percentage was 57.8% for the *P. vulgaris* L. germinated for 5 days. For *V. faba* (var. Equina) and *P. vulgaris* (var. Athropurpurea), the germination reduced the phytic acid content by 61% and 30%, respectively (Alonso, Aguirre, & Marzo, 2000), whereas 42.6% and 55.4% were observed for *P. angularis* and *P. calcaratus*, respectively (Chau & Cheung, 1997). Phytic acid is a source of phosphorus and cations for the seeds that begin to sprout. Such acid is also a source of phosphates and inositol, which can both be generated by the hydrolysis taken place within the same acid, mediated by the phytases. Bau et al. (1997) point out that the decrease of phytic acid is a consequence of the increase in phytase activity during the germination process. This explains the importance of determining not only the phytic acid, but also the products of its hydrolysis. Several authors indicate that although the amount of phytic acid generally decreases, what happens with the products of the hydrolysis is going to depend on the legume and on the conditions of the germination (Honke, Kozlowska, Vidal-Valverde, Frías, & Górecki, 1998; Trugo, Donangelo, Trugo, & Bach, 2000; Vidal-Valverde et al., 2002).

A significant statistical reduction was observed in the tannins content of white beans, black beans and pigeon beans due to germination (Table 2). The levels of reduction were 36.2%, 19% and 14.3%, respectively. Prior to germination, the dried grains were soaked in water. Once the grains were drained, the soaking water displayed a dark colour, allegedly due to the tannins from the grains released to the water. Reddy, Pierson, Sathe, and Salunkhe (1985) reported that the overnight soaking of the grains followed by their germination for 48 h significantly reduced the tannins content by 50% in a wide variety of legumes studied. Besides, an enzymatic hydrolysis by polyphenolase causes loss of tannins in grains during germination (Reddy et al., 1985). The observed reduction level may be the joint effects of the soaking and germinating of the grains.

Table 2
Tannins and in vitro protein digestibility in ungerminated and germinated *P. vulgaris* and *C. cajan*

Sample	Tannins (g/100 g)	In vitro protein digestibility (%)
P. vulgaris (white beans)		
Ungerminated	1.7 ± 0.1^{a}	77.9 ± 0.2^{a}
Germinated	1.1 ± 0.1^{b}	79.2 ± 0.3^{b}
P. vulgaris (black beans)		
Ungerminated	2.1 ± 0.1^{a}	76.3 ± 0.2^{a}
Germinated	1.7 ± 0.1^{b}	78.9 ± 0.2^{b}
C. cajan		
Ungerminated	1.4 ± 0.1^{a}	76.8 ± 0.1^{a}
Germinated	1.2 ± 0.1^{b}	80.1 ± 0.2^{b}

Results are means of triplicate ± standard deviation, expressed on dry matter basis. Different letters in the same column means significant differences ($P < 0.05$).

A significant increase was observed in the in vitro protein digestibility of white beans, black beans and pigeon beans as effect of germination (Table 2). The increase was 2% for the white beans, 3% for the black beans and 4% for the pigeon beans. Alonso et al. (2000) reported that the in vitro protein digestibility of the *P. vulgaris* increased to 10.3% because of germination. Such an increment could be attributed to the partial or complete reduction of different antinutrients. The phytic acid and the condensed tannins interact with protein molecules, forming complexes and therefore reducing the protein susceptibility to enzymatic attacks (Alonso et al., 2000). In this study, significant reductions of the content of phytic acid and of tannins were observed, which could explain the increase of the protein digestibility.

The germination significantly increased the content of ascorbic acid of the white beans, black beans and pigeon beans (Table 1). For the white beans the increase was 300%, 208.4% for pigeon beans and 33.2% for black beans. The germination process is an effective method for the increment of ascorbic acid. For Kavas and Nehir (1992), the observed increase was 17.5 and 8.5 times in *L. esculenta* M. and *P. aureus*, respectively, whereas the ascorbic acid increment in soybeans Brag variety germinated for 4 days was 90% (Ahmad & Pathak, 2000). Therefore, the germinated legume seeds can be considered an excellent source of ascorbic acid. The differences of the effect of germination on the ascorbic acid could be attributed to the genetic variety, maturity, climatic conditions, lighting conditions, harvesting and storage methods (Macrae, Robinson, & Sadler, 1993). During germination, the respiration process is triggered by the ascorbic acid. This could be related to the observed increase as a consequence of germination.

Table 3
Minerals in ungerminated and germinated *P .vulgaris* and *C. cajan* (mg/100 g)

Sample	Ca	Mg	Zn	Fe	Cu
P. vulgaris (white beans)					
Ungerminated	343.8±5.9[a]	249.5±7.3[a]	3.2±0.2[a]	30.6±5.3[a]	1.1±0.4[a]
Germinated	372.8±2.5[b]	190.3±1.9[b]	7.3±0.1[b]	6.6±0.2[b]	1.1±0.5[a]
P. vulgaris (black beans)					
Ungerminated	179.1±0.5[a]	198.0±2.7[a]	3.1±0.1[a]	10.1±0.2[a]	0.9±0.2[a]
Germinated	187.4±1.1[b]	199.8±4.7[a]	9.3±0.3[b]	5.8±0.2[b]	1.1±0.3[a]
C. cajan					
Ungerminated	126.4±3.3[a]	131.2±6.2[a]	6.1±0.2[a]	14.9±3.3[a]	0.9±0.1[a]
Germinated	148.2±1.1[b]	138. 4±1.7[a]	8.4±0.7[b]	2.6±0.1[b]	0.9±0.1[a]

Results are means of triplicate±standard deviation, expressed on dry matter basis. Different letters in the same column means significant differences ($P<0.05$).

The germination significantly increased the thiamine content of the white, black and pigeon beans (Table 1). The increment was 12.8%, 26.5% and 7.4%, respectively. Thiamine content of germinated soybeans increased 22.1% (Ahmad & Pathak, 2000). Augustin and Klein (1989) observed that germination of a wide array of legumes significantly improved the thiamine content between 7.2% and 147.7%. However, Vidal-Valverde, Frias, Lambein, and Kuo (2001) reported a 6.8% reduction of thiamine content when *P. vulgaris* L. grains were germinated. No significant variation in the thiamine content was observed when seeds of *P. vulgaris* L., *L. culinaris* and *P. sativum* were germinated for 4 days, but it was observed after 6 days.

The content of mineral elements in the seeds of black beans, white beans and pigeon beans were significantly modified by germination (Table 3). The germination significantly increased the content of calcium of white beans by 8.4%, 5% for black beans and 17.2% for pigeon beans. Trugo et al. (2000) reported an increase in the calcium levels of 7.1% for *P. vulgaris* L. Contradicting results have been previously reported by Kumar, Venkataraman, Jaya, and Krishnamurthy (1978) who observed a significant decrease of calcium of 36% in germinated *P. aureus, V. sinensis* and *C. arietinum* grains. Germination significantly reduced the magnesium content by 23.7% in white-casing *P. vulgaris*, while black casing *P. vulgaris* L. and *C. cajan* did not show significant differences between ungerminated and germinated seeds. Kumar et al. (1978) indicated that the germination of legumes such as *P. aureus, V. sinensis* and *C. arietinum* caused a significant loss in the content of magnesium, which was attributed to the lixiviation during the germination process.

Moreover, the germination of legumes significantly increased their zinc content to 128.8% in white beans, 200% in black beans and 37.7% in pigeon beans. A decrease in iron content was also observed due to the effects of germination. The white-beans showed a decrease of 78.3%, while the black beans and pigeon beans showed meaningful decreases of 42.6% and 82.6%, respectively, compared to the iron content of ungerminated grains. Regarding the copper levels, there were no significant differences between ungerminated and germinated grains. This was a common fact in all the three types of legumes studied. In germinated grains, copper content diminished to 25.7% for the white beans, 34.5% for the black beans and 36.8% for *C. cajan*. Augustin and Klein (1989) reported that the content of phosphorus, potassium, zinc and copper increased significantly in various legumes. Donangelo, Trugo, Trugo, and Eggum (1995) observed that germination of *L. albus, P. vulgaris* and *G. max* did not significantly modify the concentration of minerals such as zinc, calcium, phosphorus and manganese but reduced iron and copper content. These results may be a consequence of previous soaking and lixiviation of the grains during germination. A fact to be considered is that the phytic acid tends to bind cations such as magnesium, calcium, iron and zinc. The phytic acid is hydrolysed as a consequence of germination, which gives way to suppose that these elements should now be more bioavailable for the human body (Augustin & Klein, 1989). This is mainly an additional benefit attributed to germinated seeds (Trugo et al., 2000).

The germination process significantly reduced the trypsin inhibitors, phytic acid and tannins. On the contrary, this process also increased the ascorbic acid and thiamine contents while improving the protein digestibility. The changes in mineral content varied according to the type of legume and mineral. The observed effect in the functionality changes of the germinated grains is affected by previous soaking.

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