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Changes in Nonnutritional Factors and Antioxidant Activity during Germination of Nonconventional Legumes

Yolanda Aguilera,[†] María Felicia Díaz,[‡] Tania Jiménez,[†] Vanesa Benítez,[†] Teresa Herrera,[†] Carmen Cuadrado,[§] Mercedes Martín-Pedrosa,[§] and María A Martín-Cabrejas*[,†]

[†]Instituto de Investigación de Ciencias de la Alimentación (CIAL), Facultad de Ciencias, Universidad Autónoma de Madrid, C/Nicolás Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain

[‡]Instituto de Ciencia Animal, Carretera Central, Km 47, 1/2, San José de las Lajas, La Habana, Cuba

[§]Departamento de Tecnología de Alimentos, SGIT-INIA, Ctra. de La Coruña km 7.5, 28040 Madrid, Spain

ABSTRACT: The present study describes the effects of germination on nonnutritional factors and antioxidant activity in the nonconventional legumes *Vigna unguiculata* (cowpea), *Canavalia ensiformis* (jack bean), *Lablab purpureus* (dolichos), and *Stizolobium niveum* (mucuna). Protease inhibitors and lectins were detected in raw legumes and were significantly decreased during the germination. Regarding total and individual inositol phosphates (IP5-IP3), important reductions of IP6 and high increases in the rest of inositol phosphates were also detected during this process. In addition, total phenols, catechins, and proanthocyanidins increased, accompanied by an overall rise of antioxidant activity (79.6 μmol of Trolox/g of DW in the case of mucuna). Germination has been shown to be a very effective process to reduce nonnutritional factors and increase bioactive phenolic compounds and antioxidant activities of these nonconventional legumes. For this reason, they could be used as ingredients to obtain high-value legume flours for food formulation.

KEYWORDS: *nonconventional legumes, germination, nonnutritional factors, antioxidant capacity*

INTRODUCTION

The role of seed legumes in the diets of animals and man in developed countries is well documented.[1] There is a lack of sufficient animal protein; hence it is necessary to search for alternative sources of protein from lesser-known legumes in lieu of expensive and scarce animal protein. The research efforts are being directed to this area to identify and evaluate underexploited legume food sources as alternative protein crops for the future. This development has stimulated research on the utilization of some underutilized legumes such as *Vigna unguiculata* (cowpea), *Canavalia ensiformis* (jack bean), *Stizolobium niveum* (mucuna), *Lablab purpureus* (dolichos), which are potential sources of plant protein for many developing countries.[2,3] The protein quality of these wild pulses seems to be similar to that of most edible legumes, and thus, they are advocated to be good sources of extending protein sources.[4] In addition, they provide a large amount of structural carbohydrates mainly due to their higher dietary fiber content when compared to other fiber rich plant foods such as cereals and tubers.[5,6] Many studies have been carried out to determine the benefits of legume dietary fiber such as prevention of obesity, cardiovascular disease, type 2 diabetes, and large intestine cancer. Thus, the role of legumes as therapeutic agents in the diet of healthy vulnerable populations (diabetes, metabolic disorders, etc.) is actually of great interest.[7]

Moreover, the uses of legumes in food formulation are assuming a greater importance and have attracted the attention of food processors, marketers, and consumers,[8] since their properties show a great impact on their utilization and are very important in the development of functional ingredients in some foods such as breads, cakes, and biscuits.[9,10]

However, their nutritional quality is limited by the presence of heat labile and heat stable nonnutritional factors (NNFs). The NNFs are structurally different compounds broadly divided into two categories: proteins (such as lectins and protease inhibitors) and others such as phytate, tannins, or proanthocyanidins, saponins, and alkaloids. The presence, distribution, and negative impact of the ingestion of NNFs in grain legumes have extensively been reported.[11] In general, raw legumes contain far higher levels of some NNFs than their processed forms, and hence processing is necessary before the incorporation of these grains into food or animal diets.[12]

In this regard, germination has been identified as an inexpensive and effective technology for improving the quality of legumes, by enhancing their digestibility increasing the content of soluble protein[13] and dietary fiber[5,6] and reducing the levels of nonnutritional factors.[14] Numerous investigations into the effects of germination on protein, starch, and dietary fiber have been carried out in common legumes. However, there is a paucity of literature about the changes of this process on underexploited legumes.[2,3] Moreover, germination conditions and their effects on legume composition can vary with the plant species, seed varieties, or cultivars.[15]

Thus, the present study was carried out with the aim to evaluate the influence of the germination process on nonnutritional factors and also antioxidant properties in four non-

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conventional legumes in order to make them more useful than the raw seeds for human and animal feed.

MATERIALS AND METHODS

Samples. Seeds of cowpea (*Vigna unguiculata* L.), jack bean (*Canavalia ensiformis* L.), dolichos (*Lablab purpureus* L.), and mucuna (*Stizolobium niveum* L.) were grown and supplied by the Instituto de Ciencia Animal (La Habana, Cuba).

Germination. The germination procedure for seeds was as follows: four portions of 150 g of seeds were washed with 0.7% sodium hypochlorite, soaked in 450 mL of distilled water at room temperature for 6 h, and shaken every 30 min. The water was then drained off, and the seeds were transferred to a separating funnel. The samples were germinated using 12 h of light daily.[4] The germination was carried out at 25 °C for 4 days, and seeds were sprayed daily with distilled water in order to maintain an adequate hydration level. The sprouts and seeds were ground and freeze-dried for analysis.

Enzyme Inhibitors. Seed flours were extracted (1:10 w/v) by stirring with 0.02 M sodium phosphate buffer pH 7.0 containing NaCl (8 g/L) overnight at +1 °C and centrifuged (5000*g* for 25 min). The resultant clear supernatants were stored at −20 °C. Estimations of protease-inhibitors content were carried out as described previously.[16] The α-amylase inhibitor content of the seed extracts was determined by the starch/iodine procedure of Piergiovanni.[17]

Lectins. Hemagglutinating activity in the pH 7.0 buffer extracts was estimated by a serial dilution procedure using rat blood cells.[18] The amount of material (g) that caused agglutination of 50% of the erythrocytes was defined as that containing 1 hemagglutinating unit (HU) and for comparison; values were expressed as HU/kg of seed meal. Trypsinized rat blood cells were used in order to detect the hemagglutination activity in legumes that showed lower activity.

Phytic Acid. The individual inositol phosphates (IP3-IP6) were extracted according Burbano et al.[19] with modifications and measured by HPLC. Analysis was with a Beckman System Gold HPLC equipped with a refractive index. The column was a macrosporous polymer PRP-1 (150 × 4.1 mm, 5 μm) heated at 45 °C and was equilibrated with the mobile phase for 1 h. The mobile phase was prepared by mixing methanol/water (51.5:48.5 v/v) with addition of 8 mL of tetrabutylammonium hydroxide (TBNOH), 1 mL of 0.5 M sulfuric acid, 0.5 mL of formic acid (ACS reagent, 91%), and 0.2 mL of a phytic acid solution (5 mg/mL). The pH was adjusted to 4.3. The mobile phase was filtered through a Millipore filter (0.45 μm) and degassed under a vacuum. The flow rate was 1.2 mL/min, and the injection volume was 20 μL. The standard used was sodium phytate (Sigma Chemicals, USA).

Polyphenolic Compounds. For extracting polyphenolic compounds, legume flours (5 g) were macerated with 3 × 50 mL of a solution of methanol-ClH ($1/_{1000}$)/water (80:20 v/v) using an orbital shaker (Stuart, Staffordshire, UK) at room temperature, separating the supernatants by centrifugation (3024*g*, 10 min, 5 °C). The three combined supernatants were taken to a fixed volume (150 mL) of the methanol solution, yielding a methanol extract in which the phenolic compound families and radical scavenging activity of the extract were determined. In the methanol solution, total polyphenols were quantificated by Folin-Ciocalteu reactant,[20] catechins with vanillin/HCl,[21] and proanthocyanidins by hydrolysis with butanol/HCl.[22]

Antioxidant Capacity. Ferric reducing antioxidant power (FRAP) assay was done according to Benzie and Strain[23] with some modifications. Legume flours (150 μL) were reacted with 2850 μL of the FRAP solution for 30 min in dark conditions. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in μmol of Trolox/g of dry weight (DW). Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

The DPPH assay (2,2-diphenyl-1-picrylhydrazyl) was done according to the method of Brand-Williams et al.[24] with some modifications. First, the absorbance of the disposable cuvette with 250 μL of the DPPH solution and 2.1 mL of 80% methanol was measured as blank. Then, the 80% methanol extracts (100 μL) were added to 250 μL of the DPPH solution and 2 mL of 80% methanol and allowed to stand at room temperature in the dark for 20 min. The decrease in absorbance of the resulting solution was monitored at 517 nm for 20 min. The Trolox standard solution (concentration 100−200 μM) in 80% methanol was prepared and assayed under the same conditions. DPPH-RSA-scavenging activity was expressed as μmol of Trolox/g of DW of sample.

Statistical Analysis. Germination was carried out in duplicate. Each sample was analyzed in triplicate. The data were analyzed by-one way analysis of variance (ANOVA) using Duncan test. Differences were considered to be significant at $P \leq 0.05$. The statistical analysis was performed by SPSS 17.0.

RESULTS AND DISCUSSION

Germination Process. The study of the effect of germination may provide useful information for optimizing of use of these legume seeds as food products, since germination has proved beneficial for the nutritional quality of common seeds. Table 1 indicates the changes in biomass and germination percentages with 12 h of light daily for 4 days. Fresh weight of seedlings increased from 113% to 340%. Cowpea showed the highest increase in fresh weight, while jack bean seeds exhibited the lowest increase. The obtained results are similar to those found for common legumes,[25] and few data were found in the literature for the nonconventional legumes.[2,5] The success of this process on these legumes was high, showing the good viability of cowpea and jack bean (98%) and dolichos (84%), while mucuna seeds only reached 63% of germination. Regarding radicle development, 96 h of the germination seemed to show high lengths in all studied legumes. Mucuna was the nonconventional legume with the smallest size of radicle (2.7 cm), while cowpea exhibited the greatest length (7.1 cm).

Table 1. Changes in Seed/Seedings Biomass and Percent of Germination at 25 °C for 4 Days

legume	% germination	development of radicle (cm)	% increase in fresh weight of seeds/seedings
cowpea	98	7.1 ± 0.3	340
jack bean	98	5.9 ± 0.2	113
dolichos	84	5.2 ± 0.5	204
mucuna	63	2.7 ± 0.2	200

Changes of Enzyme Inhibitors and Lectins during the Germination. α-Amylase and protease inhibitors are widely distributed in legumes, and their levels were influenced by the germination process (Table 2). No α-amylase inhibitor content was detected in raw legumes; only protease inhibitors that may have a major impact on nutritional value were identified. Trypsin inhibitors levels in raw seeds ranged from 1.4 mg/g of DW in mucuna to 6.9 mg/g of DW in dolichos. It was observed that colored cultivars showed lower trypsin inhibitor content, as Makkar et al.[26] established and also the different germination conditions modified the levels.[27] Regarding chymotrypsin inhibitor, the contents were lower than trypsin inhibitors, with cowpea, dolichos, and mucuna showing similar contents (1.5−1.6 mg/g of DW), higher than those exhibited by jack bean (0.7 mg/g of DW). Germination decreased significantly the trypsin inhibitor levels in all legumes except for mucuna, which did not show any change during process. Jack bean exhibited the highest reduction (78%), followed by dolichos and cowpea (54% and 38%, respectively). In the case of

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Table 2. Influence of Processing on α-Amylase and Protease Inhibitors (mg/g of DW) and Lectins Contents (mg/100 mg of DW of Lectin) in Raw and Germinated Non-Conventional Legumes[a]

samples	α-amylase inhibitor	trypsin inhibitor	chymotrypsin inhibitor	lectins[b]	lectins[c]
cowpea					
raw	n.d.	3.4 ± 0.13[b]	1.6 ± 0.08[a]	n.d.	n.d.
germinated	n.d.	2.1 ± 0.12[a]	1.5 ± 0.04[a]	n.d.	n.d.
jack bean					
raw	n.d.	3.2 ± 0.18[b]	0.7 ± 0.07[b]	5.1 ± 0.06[b]	
germinated	n.d.	0.7 ± 0.06[a]	0.4 ± 0.08[a]	2.5 ± 0.06[a]	
dolichos					
raw	n.d.	6.9 ± 0.14[b]	1.6 ± 0.10[b]	n.d.	10.2 ± 0.03[b]
germinated	n.d.	3.2 ± 0.09[a]	1.0 ± 0.10[a]	n.d.	2.5 ± 0.03[a]
mucuna					
raw	n.d.	1.4 ± 0.01[a]	1.5 ± 0.15[b]	n.d.	n.d.
germinated	n.d.	1.3 ± 0.10[a]	0.9 ± 0.08[a]	n.d.	n.d.

[a]Values are means of three analyses. n.d. not detected. Mean values within a column and legume followed by different superscript letter were significantly different at $p < 0.05$. Mean ± SD ($n = 3$). [b]Hemagglutinating unit (HU) or lectin equivalent (mg)/100 mg in legume seeds using rat blood cells. [c]Hemagglutinating unit (HU) or lectin equivalent (mg)/100 mg in legume seeds using trypsinized rat blood cells.

chymotrypsin inhibitor activity, the decreases by germination were also significant; dolichos, mucuna, and jack bean had reductions of 38%, 40%, and 43%, respectively, while cowpea did not show any noticeable decrease. Although these legumes contain significant levels of these protease inhibitors, the germination process seemed to decrease their contents. This fact might be related to different activities of endogenous proteases, due to an increase of pre-existent proteases that are activated.[14]

An initial evaluation of lectin content in all legume samples was carried out by using the hemagglutination assay. The results indicated that lectins were only detected in raw jack bean (Table 2), with levels similar to those in the literature;[16−18] in germinated jack bean a 50% decrease was observed. To detect the hemagglutination activity in the rest of legumes, trypsinized blood cells were used. Dolichos was the only legume that showed hemagglutination activity, exhibiting 76% of reduction in their germinated seeds. Hence, lectins in jack bean and dolichos were reduced by the germination treatment, improving the biological and nutritional value for their utilization in human foods and animal feeds.

Quantification of Inositol Phosphates during the Germination. Results of individual and total inositol phosphates of legume flours are shown in Table 3 and Figure 1, respectively. The total inositol phosphates contents determined by ion-pair HPLC exhibited differences among legumes ranging from 6.7 mg/g of DW (jack bean) to 8.8 mg/g of DW (dolichos). Similar contents were found in cowpea, dolichos, and mucuna (~8.4 mg/g of DW, respectively). The phytic acid composition of the studied legumes agreed with previously published data on nonconventional legumes by Sridhar and Seena[28] and Shohag et al.[29] The relative percentage values obtained of IP3-IP6 indicate that the legumes contained more than 70% of their inositol phosphates in the IP6 form, and in the case of cowpea and jack bean the value reached 83% and gave results similar to those found in the literature.[11,28,29] IP5 is the second predominant inositol phosphates in all studied legumes, ranging from 15% in jack bean to 23% in mucuna. The relative percentages of IP3 and IP4 are low and never higher than 3%. Only the highly phosphorilated inositol phosphates IP6 and IP5 have a negative effect on the bioavailability of minerals; the other hydrolytic products formed had a poor capacity to bind mineral.[30]

Table 3. Influence of Processing on Inositol Phosphates Content (mg/g of DW) of Raw and Germinated Nonconventional Legumes[a]

samples	IP3	IP4	IP5	IP6
cowpea				
raw	n.d.	0.2 ± 0.01[a]	1.3 ± 0.09[a]	6.8 ± 0.09[b]
germinated	0.1 ± 0.00	0.3 ± 0.02[b]	1.5 ± 0.06[b]	5.0 ± 0.17[a]
jack bean				
raw	0.1 ± 0.00	0.1 ± 0.01[a]	1.0 ± 0.07[a]	5.6 ± 0.12[b]
germinated	n.d.	0.6 ± 0.02[b]	1.6 ± 0.05[b]	3.5 ± 0.17[a]
dolichos				
raw	0.1 ± 0.00	0.3 ± 0.06[b]	1.9 ± 0.30[b]	6.5 ± 0.35[a]
germinated	n.d.	0.2 ± 0.03[a]	1.3 ± 0.03[a]	6.7 ± 0.25[a]
mucuna				
raw	0.1 ± 0.00	0.2 ± 0.03[a]	1.9 ± 0.11[b]	6.0 ± 0.18[b]
germinated	n.d.	0.4 ± 0.02[b]	1.6 ± 0.10[a]	5.0 ± 0.13 [a]

[a]Values are means of three analyses. Mean values within a column and legume followed by different superscript letter were significantly different at $p < 0.05$. Mean ± SD ($n = 3$).

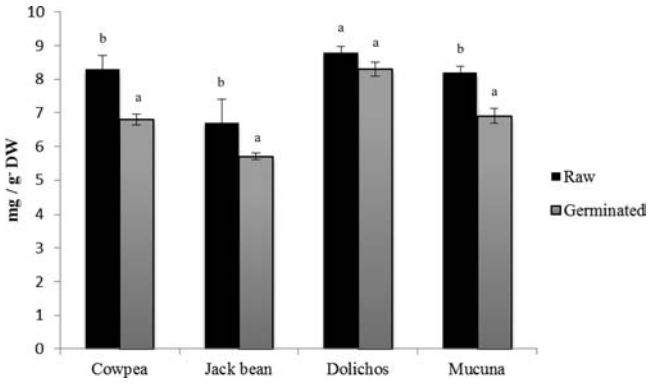

Figure 1. Influence of processing on total inositol phosphates content (mg/g of DW) of raw and germinated nonconventional legumes (mean ± SD, $n = 3$).

The germination process significantly affected the inositol phosphates contents in all seeds except for dolichos, which exhibited similar values after this treatment and the highest levels among germinated legumes. Germination caused a significant reduction ($p < 0.05$) of total inositol phosphates,

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Table 4. Influence of Processing on Total Phenols, Total Proanthocyanidins, and Total Catechins of Raw and Germinated Nonconventional Legumes[a]

samples	total phenols (mg gallic acid/g of DW)	total proanthocyanidins (mg/g of DW)	total catechins (mg/g of DW)	PC/CAT
cowpea				
raw	3.30 ± 0.11[a]	0.50 ± 0.02[a]	0.10 ± 0.01[a]	5.0
germinated	3.70 ± 0.03[b]	0.70 ± 0.02[b]	0.20 ± 0.01[b]	3.5
jack bean				
raw	2.30 ± 0.05[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	1.0
germinated	3.60 ± 0.02[b]	0.04 ± 0.01[b]	0.07 ± 0.01[b]	0.6
dolichos				
raw	0.72 ± 0.02[a]	0.20 ± 0.02[a]	0.04 ± 0.01[a]	5.0
germinated	1.70 ± 0.03[b]	0.20 ± 0.01[a]	0.10 ± 0.01[b]	2.0
mucuna				
raw	37.40 ± 0.42[a]	0.10 ± 0.02[a]	0.03 ± 0.01[a]	3.3
germinated	46.30 ± 0.12[b]	0.20 ± 0.03[b]	0.07 ± 0.01[b]	2.8

[a]Values are means of three analyses. Mean values within a column and legume followed by different superscript letter were significantly different at $p < 0.05$. Mean ± SD ($n = 3$).

being higher in cowpea (18%), followed by mucuna and jack bean (15%). The observed reduction in phytic acid content of legume seeds during germination may be partly due to the increase of endogenous phytase activity and the corresponding hydrolysis of the IP6. In fact, this enzyme makes soluble phytates and releases soluble proteins and minerals.[31] IP6 contents decreased significantly with a reduction of 17% to 37% of the original value; no change in IP6 was noted for dolichos, but there was an increase in the other inositol phosphates (IP4-IP5). Therefore, germinated nonconventional legumes seemed to present a better potential for mineral availability when compared to raw legumes; however, the extent of reduction of inositol phosphates is dependent on the type of legume.[32] A direct correlation between phytate reduction and phytase activity was found during germination of lentils; however, in chickpeas no correlation was established.[31]

Phenolics Contents during the Germination Process. Table 4 shows the influence of the germination process on total phenolics compounds (TPC), total proanthocyanidins (PC), and total catechins (CAT). Mucuna showed the highest amounts of TPC (37.40 mg/g of DW), followed by cowpea and jack bean, while dolichos exhibited the least total phenolic content (0.72 mg/g of DW). These results may be the cause of the different color of seed coats exhibited by these legumes. Similar behavior was observed in dark and highly pigmented bean varieties reported by Xu and Chang.[33] As was observed for other bioactive compounds, the influence of processing is relevant to total phenolic contents. Germination brought about further significant increases in TPC contents of all nonconventional legumes; increases varied from 12% (cowpea) to 136% (dolichos). These results are in agreement with those reported by earlier authors[13,34−36] who found relevant increases of total phenols in germinated legumes. In contrast, Khandelwal et al.[37] detected reductions in the concentration of total polyphenols in Indian pulses. During germination, endogenous enzymes are activated, promoting differences in the composition of legume varieties in terms of phenolic compounds.

Total proanthocyanidins have also been identified in raw legumes, representing from 28% to 19% of total phenols in dolichos, mucuna, and cowpea, respectively. After germination, PC increased significantly in mucuna (50%) and cowpea (40%); however, similar levels were found in the case of dolichos. During the germination process, proanthocyanidins could condense to highly polymerized forms through the activation of polyphenoloxidase.[32] The presence of catechins in raw legumes is low compared to common legumes as the levels represented <5% of total phenols in studied seeds (Table 4). As a consequence of germination, the CAT contents increased in all pulses. This could be attributed to the production of some secondary plant metabolites,[34,38] such as flavonoids. The ratio proanthocyanidin/catechin, a relative approximation of the polymerization degree of proanthocyanidins, showed decreases in all germinated samples (Table 4). It is important from a nutritional point of view that the degree of polymerization of proanthocyanidins should be low because the proanthocyanidin−protein interactions increases with the degree of polymerization.[39] The decrease in this process could be related to several factors such as changes in their real solubility and chemical reactivity that may modify the effectiveness of the analysis.[36] A decrease in the high polymerized proanthocyanidins was also reported for the germination and fermentation of lentils.[38]

Total Antioxidant Capacity during the Germination. The antioxidant capacity of ungerminated seeds, measured by FRAP assay (Figure 2a), ranged from 1.8 to 55.3 μmol of Trolox/g of DW, while the antioxidant capacity against DPPH-free radical (Figure 2b) ranged from 1.4 to 8.6 μmol of Trolox/g of DW (Table 4). In both assays, the highest antioxidant capacity corresponded to mucuna legume and the lowest to jack bean and dolichos. The initial antioxidant capacity in raw seeds is similar to common legumes reported by Vernaza et al.[13] and Xu and Chang.[33] An enhancement in antioxidant capacity by germination was exhibited in all pulses except in jack bean, and the increases were significant ($p < 0.05$) from 89% in cowpea to 22% in dolichos. The highest antioxidant activity was seen in germinated mucuna by both methods (79.6 μmol of Trolox/g of DW by FRAP and 10.70 μmol of Trolox/g of DW by DPPH), followed by cowpea and dolichos. A similar tendency after the germination process has been reported in the literature.[13,36] Mixtures of phenolics can exhibit an enhanced antioxidant activity since they can work together synergistically, and also the germination process might produce some secondary plant metabolites such as anthocyanins and flavonoids from seed coats and cotyledons due to the enzymatic reaction.[38] Germination has been reported to lead to the production of bioactive compounds with potent antioxidant properties.[15]

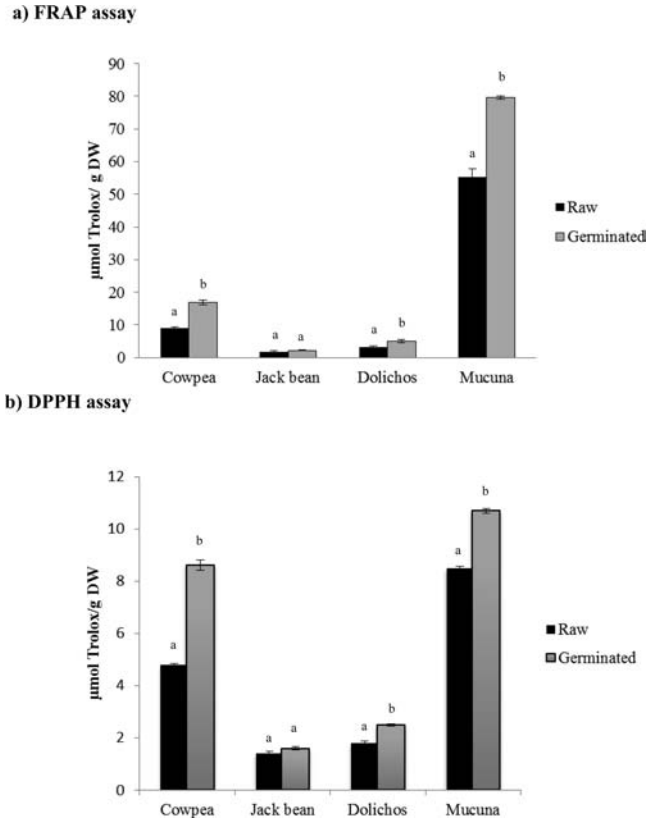

Figure 2. (a, b) Total antioxidant activity of raw and germinated nonconventional legumes (mean ± SD, n = 3).

Correlation analyses between total phenolic contents and antioxidant activities (FRAP and DPPH assays) were performed; these analyses exhibited significant linear correlations (r = 0.98, P < 0.05 and r = 0.97, P < 0.05, respectively). High correlation between these compounds and their antioxidant activity was also found in conventional legumes.[13,36] Moreover, significant linear correlation between DPPH and FRAP methods (r = 0.86, P < 0.01) was found with a combination of ungerminated and germinated legumes. Overall germinated legumes exhibited a more potent ability to inhibit reactions promoted by oxygen or peroxides than the raw seeds and showed a good correlation between antioxidant assays and total phenols content.

In conclusion, germination is a simple technological process that is easy to apply and has minimal cost. This process reduces enzyme inhibitors, lectins, and inositol phosphates, thereby improving the nutritional quality. In addition, a significant increase of phenolic compounds accompanied by an overall increase of antioxidant activity was observed in all germinated legumes. The germination process may be considered as an effective and promising method increasing the bioactivity of these nonconventional legumes. Nevertheless, further work to control the conditions of germination to obtain an optimal concentration of nonnutritional factors and antioxidant activity is needed to highlight the potential value of these nonconventional legumes as ingredients.

AUTHOR INFORMATION

Corresponding Author

*Tel: +34 91 0017913. Fax: +34 91 0017905. E-mail: maria.martin@uam.es.

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Notes

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REFERENCES

(1) Waterlow, J. C. Childhood malnutrition in developing nations: looking back and looking forward. *Ann. Rev. Nutr.* **1994**, *14*, 1−19.

(2) Janardhanan, K.; Vadivel, V.; Pugalenthi, M. Biodiversity in Indian underexploited/tribal pulses. In *Improvement Strategies for Leguminosae Biotechnology*; Jaiwal, P. K., Singh, R. P., Eds.; Kluwer Academic Publishers: Amsterdam, The Netherlands, 2003; pp 353−405.

(3) Alabi, D. A.; Alausa, A. A. Evaluation of the mineral nutrients and organic food content of the seeds of *Lablab purpureus, Leucaena Leucocaphala* and *Mucuna utilis* for domestic consumption and industrial utilization. *World J. Agric. Sci.* **2006**, *2*, 115−118.

(4) Díaz, M.; Martín-Cabrejas, M. A.; Gonzalez, A.; Torres, V.; Noda, A. Biotransformation of *Vigna unguiculata* during the germination process. *Cuban J. Agric. Sci.* **2007**, *41*, 161−165.

(5) Martín-Cabrejas, M. A.; Díaz, M. F.; Aguilera, Y.; Benítez, V.; Mollá, E.; Esteban, R. M. Influence of germination on the soluble carbohydrates and dietary fiber fractions in non-conventional legumes. *Food Chem.* **2008**, *107*, 1045−1052.

(6) Benítez, V.; Cantera, S.; Aguilera, Y.; Mollá, E.; Esteban, R. M.; Díaz, M. F.; Martín-Cabrejas, M. A. Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. *Food Res. Int.* **2013**, *50*, 64−69.

(7) Campos-Vega, R. M.; Loarca-Piña, G.; Oomah, B. D. Minor components of pulses and their potential impact on human health. *Food Res. Int.* **2010**, *43*, 461−482.

(8) Boye, J.; Zare, F.; Pletch, A. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Res. Int.* **2010**, *43*, 414−431.

(9) Anton, A. A.; Ross, K. A.; Lukow, O. M.; Fulcher, R. G.; Arntfield, S. D. Influence of added bean flour (*Phaseolus vulgaris* L.) on some physical and nutritional properties of wheat flour tortillas. *Food Chem.* **2008**, *109*, 33−41.

(10) Farooq, Z.; Boye, J. Novel food and industrial applications of pulse flours and fractions. In *Pulse Foods: Processing, Quality and Nutraceutical Applications*; Tiwari, B. K., Gowen, A., McKenna, B., Eds.; Elsevier: Amsterdam, The Netherlands, 2011; pp 283−323.

(11) Adebowale, Y. A.; Adeyemi, A.; Oshodi, A. A. Variability in the physicochemical, nutritional and antinutritional attributes of six Mucuna species. *Food Chem.* **2005**, *89*, 37−48.

(12) Hajós, G.; Osagie, A. U. Technical and biotechnological modifications of antinutritional factors in legumes and oilseeds. In *Proceedings of the Fourth International Workshop on Antinutritional Factors in Legume Seeds and Oilseeds*; Wageningen: EAAP, 2004; pp 293−305.

(13) Vernaza, M. G.; Dia, V. P.; González de Mejía, E.; Chang, Y. K. Antioxidant and anti-inflammatory properties of germinated and hydrolysed Brazilian soybeans flours. *Food Chem.* **2012**, *134*, 2217−2225.

(14) Vidal-Valverde, C.; Frías, J.; Sierra, I.; Blázquez, I.; Lambein, F.; Kuo, Y. H. New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *Eur. Food Res. Technol.* **2002**, *215*, 472−477.

(15) Paucar-Menacho, L. M.; Berhow, M. A.; Mandarino, J. M. G.; Chang, Y. K.; Mejia, E. G. Effect of time and temperature on bioactive compounds in germinated Brazilian soybean cultivar BRS 258. *Food Res. Int.* **2010**, *43*, 1856−1865.

(16) Martín-Cabrejas, M. A.; Esteban, R. M.; Waldron, K.; Maina, G.; Grant, G.; Bardocz, S.; Pusztai, A. Hard-to-cook phenomenon in beans: Changes in antinutrient factors and nitrogenous compounds during storage. *J. Sci. Food Agric.* **1995**, *69*, 429−435.

(17) Piergiovanni, A. R. Effect of some experimental parameters on activity of cowpea a-amylase inhibitors. *LWT-Food Sci. Technol.* **1992**, *25*, 321−324.
(18) Grant, G. Lectins. In *Toxic Substances in Crop Plants*; D'Mello, J. P. F.; Duffus, C. M.; Duffus, J. H., Eds.; The Royal Society of Chemistry: Cambridge, United Kingdom, 1991; pp 49−67.
(19) Burbano, C.; Muzquiz, M.; Osagie, A.; Ayet, G.; Cuadrado, C. Determination of phytate and lower inositol phosphates in Spanish legumes by HPLC methodology. *Food Chem.* **1995**, *52*, 321−325.
(20) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolibdic phosphotungstic acid reagent. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(21) Swain, T.; Hills, W. E. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* **1959**, *10*, 63−68.
(22) Ribéreau-Gayón, P.; Stonestreet, E. Dosage des tannins du vin rouges et détermination du leur structure. *Chem. Anal.* **1965**, *48*, 188−196.
(23) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.
(24) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **1995**, *22*, 25−30.
(25) Maass, B. L. Changes in seed morphology, dormancy and germination from wild to cultivated hyacinth bean germplasm (*Lablab purpureus*: Papilionoideae). *Gen. Res. Crop. Evol.* **2006**, *53*, 1127−1135.
(26) Makkar, H. P. S.; Becker, K.; Abel, H.; Pawelzik, E. Nutrient contents, protein degradability and antinutritional factors in colour- and white-flowering cultivars of *Vicia faba* beans. *J. Sci. Food Agric.* **1997**, *75*, 511−520.
(27) Dia, V. P.; Gomez, T.; Vernaza, G.; Berhow, M.; Chang, Y. K.; Gonzalez de, Mejia E. Bowman-Birk and Kunitz protease inhibitors among antinutrients and bioactives modified by germination and hydrolysis in Brazilian soybean cultivar BRS 133. *J. Agric. Food Chem.* **2012**, *60*, 7886−789.
(28) Sridha, K. R.; Seena, S. Nutritional and antinutritional significance of four unconventional legumes of the genus *Canavalia*-A comparative study. *Food Chem.* **2006**, *99*, 267−288.
(29) Shohag, M. J. I.; Wei, Y.; Yang, X. Changes of folate and other potential health-promoting phytochemicals in legume seeds as affected by germination. *J. Agric. Food Chem.* **2012**, *60*, 9137−9143.
(30) Lönnerdal, B. Phytic acid-trace element (Zn, Cu, Mn) interactions. *Int. J. Food Sci. Technol.* **2002**, *37*, 749−758.
(31) Greiner, R.; Pedrosa, M. M.; Muzquiz, M.; Ayet, G.; Cuadrado, C.; Burbano, C. Effect of germination on phytate content and phytase activity in legumes. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed*; Jansman, A. M., Hill, G., Hulsman, J., Van der Poel, A., Eds.; Wageningen Pers: Wageningen, The Netherlands, 1998; pp 82−83.
(32) Alonso, R.; Aguirre, A.; Marzo, F. Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chem.* **2000**, *68*, 159−165.
(33) Xu, B. J.; Chang, S. K. C. Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *J. Food Sci.* **2007**, *72*, 167−177.
(34) Dueñas, M.; Hernández, T.; Estrella, I.; Fernández, D. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). *Food Chem.* **2009**, *117*, 599−607.
(35) Germination dramatically increases isoflavonoid content and diversity in chickpea (*Cicer arietinum* L.) seeds Wu, Z.; Song, L.; Feng, S.; Liu, Y.; He, G.; Yioe, Y.; Liu, S. Q.; Huang, D. *J. Agric. Food Chem.* **2012**, *60*, 8606−8615.
(36) Guo, X.; Li, T.; Tang, K.; Liu, R. H. Effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts (*Vigna radiata*). *J. Agric. Food Chem.* **2012**, *60*, 11050−11055.
(37) Khandelwal, S.; Udipi, S. A.; Ghugre, P. Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. *Food Res. Int.* **2010**, *43*, 526−530.
(38) Bartolomé, B.; Estrella, I.; Hernández, T. Changes in phenolic compounds in lentils (*Lens culinaris*) during germination and fermentation. *Z. Lebensm.-Unters Forsch.* **1997**, *205*, 290−294.
(39) Ricardo-da-Silva, J. M.; Cheynier, V.; Souquet, J. M.; Moutounet, M. Interaction of grape seed procyanidins with various proteins in relation to wine fining. *J. Sci. Food Agric.* **1991**, *57*, 111−125.