Biotransformation of *Lablab purpureus* during the germination process

María F. Díaz[1], María Á. Martín-Cabrejas[2], Acela González[1], Verena Torres[1] and Aída Noda[1]

[1]*Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba*

[2]*Departamento de Química Agrícola, Universidad Autónoma de Madrid, Ciudad Universitaria de Cantoblanco 28049, Madrid*

Correo electrónico: mdiaz@ica.co.cu

Some changes in *Lablab purpureus* (dolicho) during the germination process were studied to improve its chemical composition. Three experiments were conducted and grains germinated for 96 h under different light conditions: 1 (12-h light intervals), 2 (total light) and 3 (total darkness). A one-way classification design was applied to each experiment, with four repetitions. The treatments were: 1 control (unprocessed grain), 2 (grain soaked for 6 h + 24-h germination), 3 (soaked grain + 48-h germination), 4 (soaked grain + 72-h germination) and 5 (soaked grain + 96-h germination). As the germination process moved forward, there was an increase in the weight of the germinated grains, the root longitude and the percentage of the germinated grains in the different germination assays. In all the experiments, the percentage of the germinated grains was stabilized from 72 h on. The variant of conducting the germination process under the condition of total darkness had the superior transformations, with increases in CP (30.08 vs. 26.67 %), TP (21.37 vs. 19.76 %), ADF (16.37 vs. 14.42 %) and cellulose (14.67 vs. 11.23 %), compared to the control without germination. The profile of the mineral elements showed that ash and K increased with the germination time in the three germination variants. The rest of the minerals, except phosphorous that decreased in total darkness and light, did not vary significantly. The results indicated that the procedure applied in the germination is feasible for obtaining new products in *Lablab purpureus*. Modifications during germination imply changes on the physico-chemical properties of this legume. Therefore, it can be considered as an effective and promising method as it enhances the function and the quality of the product. Although 72 h are enough for germination percentages superior to 80 %, germination under total darkness conditions and for 96 h is recommended for guaranteeing the bromatological quality of the product.

Key words: *legumes, germination, bromatology, dolicho.*

Germination is a simple and economic treatment resulting in a natural product. It allows eliminating or inactivating certain anti-nutritional factors and increases proteins and starch digestibility in legumes. Legumes germination may improve the properties of these plants as animal feeding (Martín- Cabrejas *et al*. 2007, Roy *et al*. 2010 and Aguilera *et al*. 2011a).

Different changes in the distribution of secondary metabolites occur during germination, reserve proteins stored in the protein bodies of cotyledons are used and modifications on the composition of soluble amino acids take place (Zyla *et al*. 2004 and Urbano *et al*. 2005). Time and germination conditions, as light and temperature, are determining factors on the smell and taste of the germinated seeds and their humidity content (Blázquez 1999 and Díaz *et al*. 2004). Likewise, humidity determines physical and chemical changes, such as the composition of soluble carbohydrates, the amount of phytate and alkaloids, and the levels of vitamin C. These changes modify the nutritive value and, therefore, the character of functional feed of legumes (Vidal-Valverde *et al*. 1998, Khatoon and Prakash 2006 and Benítez *et al*. 2011a).

There is abundant germplasm of grain legumes in the tropics that could be a promising alternative to animal feeding. The results obtained in Cuba by Díaz *et al*. (2007 and 2010) and Aguilera *et al*. (2011 b) in *Vigna unguiculata* (cowpea) allowed obtaining a germination methodology of grain legumes with changes in their physico-chemical properties. This method may be considered effective as it increases the grains functions and improves the quality of the resulting product.

Previous studies showed the important agronomic and nutritional potential of the species *Lablab purpureus* (dolicho) as non-conventional feeding source. However, the presence of toxic or anti-nutritional compounds (ANF) in these plants is the main limitation to their use in animal feeding, especially in non-ruminants (Díaz 2003, Rivas-Vega *et al*. 2006 and Benítez *et al*. 2011b).

Out of the experiences on cowpea assays, the objective of this paper was to study some changes during the germination of *Lablab purpureus*, in order to improve its chemical composition.

Materials and Methods

Three experiments were conducted and *Lablab purpureus* grains germinated for 96 h under different light conditions: experiment 1 (light intervals of 12 h), 2 (total light) and 3 (total darkness).

*Treatment and desig*n. A one-way classification design was applied to each experiment, with four repetitions. The treatments were: 1 control (unprocessed grain), 2 (grain soaked for 6 h + 24-h germination), 3 (grain soaked for 6 h + 48-h germination), 4 (soaked grain for 6 h + 72-h germination) and 5 (grain soaked

for 6 h + 96-h germination).

Experimental Procedure. The legume was planted on a typical red ferralic soil (Anon 1999) during the rainy season in 2006. It was harvested after seed maturation, when 95% of the pods were dried. After threshing, the grains were sun-dried for one or two days to reduce humidity up to 12 - 14 %. Later, they were stored at 6 - 10 °C, with relative humidity lower than 85 % up to their use.

The grains were carefully cleaned, removing strange material. For the analysis of the control grains, they were ground in a hammer mil Culatti Typs MFC, with 1mm-diameter sieve and the corresponding analyses were conducted.

The germination process was performed by the method of Blázquez (1999), modified by Díaz *et al*. (2004). Before the grains were subject to different processes, their disinfection was conducted with sodium hypochlorite at 0.07 % for half an hour. Later, consecutive washings with distilled water were conducted until reaching neutral pH.

Four portions of 150 g were weighed in a 1-L Erlenmeyer, adding 450 mL of distilled water. They were kept soaked for 6 h at room temperature. After this time, the soaking water was removed and the grains were drawn off and put to rest for 24 h. At 24 h, the samples were obtained for the treatment 2. The grains in germination forming the rest of the treatments were placed in plastic trays with capacity for four repetitions.

The germination was performed under normal laboratory conditions, at room temperature between 30 and 35 ºC. The treatments were sprinkled with water three times a day (morning, noon and afternoon) to keep grains wet. After the time for each treatment, the germinated seeds were dried in an oven at 60 ºC for 48 h and ground for their later analysis. The indicators under study were germinated grains weight, longitude of the roots after germination and germination percentage.

In order to determine the bromatological composition, dry matter (DM), crude protein (CP), ash, calcium (C), phosphorous (P), potassium (K) and magnesium (Mg) were analyzed according to AOAC (1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (CEL) and lignin (LIG) were determined according to van Soest *et al*. (1991). True protein (TP) was calculated by the method of Berstein *et al*. (1983), cited by Meir (1986).

The analysis of variance was applied for the statistical interpretation of the results, according to selected design and through the statistical software SPSS (Visauta 1998).

Results and Discussion

The germination process changed the morphological characteristics of the dolicho grains (table 1). There was an increase of the germinated grains, root longitude and percentage of the germinated grains in the different germination assays. Frías (1992) and Górecki (2000) stated that the germination process begins when the dried seed is in contact with water, with intensive movement of the reserve substances inducing morphological changes and consequent diminishing of the DM percentage as germination goes forward in the three experiments.

As well as cowpea (Díaz *et al*. 2007), the percentage of germinated grains was stabilized in all the experiments from 72 h on because this time was enough for achieving germination percentages superior to 80 % in the assays conducted (figure 1). The germination percentages achieved in cowpea were over 90 %. This indicator is closely related with the intrinsic characteristics of the each species seeds. The 80 % values corresponds with that reported for a group of quality seeds in this species (Matías *et al*. 1990 and Moussa 1991).

Although morphological variations were significant, the bromatological transformations were less marked (table 2). The germination under total darkness conditions coincided with the results in cowpea (Díaz *et al*. 2007), having better biotransformations with increases in CP (13 %), TP (8 %), ADF (7 %) and cellulose (30 %), compared to the control without germination.

The low variability in the bromatological composition during germination was in accordance with Martín-Cabrejas *et al*. (2003), Cuadrado *et al*. (2004) and Torres *et al*. (2007) in *Pisum sativum*, *Vicia faba* and *Cajanus cajan*, when applying different light alternatives. There were no changes in the content of total protein during the first six days of germination. In respect to the variations of nitrogen content during the germination process, there was no stable performance pattern per species; in many instances, it could even diminish the protein content. The studies of Sangronis *et al*. (2004) in *Phaseolus vulgaris* and *Cajanus cajan* corroborated that the variations in the protein content during germination depended on the plant species, the seed variety and the germination conditions.

The most complete researches prove that germination periods up to 6 d, with or without light, increase non-protein nitrogen, substantially reducing the protein nitrogen. This is due to the hydrolysis of the reserve proteins releases peptides and free amino acids, increasing non-protein nitrogen which can be solubilized in NaOH, and does not precipitate with trichloroacetic acid in the non-protein nitrogen determinations (Urbano *et al*. 2005). These authors proved in biological assays that this pre-digestion of the legume proteins during germination may improve the digestive utilization of these legumes.

The studies of protein quality in rats with grains of germinated and non germinated dolicho (Savón *et al*.

Table 1. Processing effect on the morphological characterisitics of the germinated grains.

Treatments	Weight of the germinated grains, g	Root longitude, cm	DM, %	Germination, %
Light intervals of 12 h				
Control	-	-	89.88[c]	-
24 h	257.50[a]	0.90[a]	51.48[b]	47.17[a] (53.78)
48 h	321.25[b]	2.55[b]	42.43[a]	59.66[b] (74.44)
72 h	317.50[b]	4.15[c]	41.73[a]	62.62[bc] (78.69)
96 h	307.50[b]	5.20[d]	41.35[a]	65.65[c] (82.97)
SE ±	13.53***	0.31***	1.19***	1.75***
Total light				
Control	-	-	89.88[c]	-
24 h	263.75[a]	1.23[a]	49.80[b]	44.90[a] (49.84)
48 h	267.50[a]	2.10[a]	47.68[b]	58.25[b] (72.28)
72 h	335.00[b]	3.15[a]	41.24[a]	63.86[c] (80.58)
96 h	318.75[b]	4.40[d]	40.06[a]	64.25[c] (81.08)
SE ±	14.33**	0.21***	0.72***	1.53***
Total darkness				
Control	-	-	89.88[d]	-
24 h	287.50[a]	0.89[a]	47.38[c]	50.97[a] (61.01)
48 h	277.50[a]	2.20[b]	46.86[c]	51.37[a] (60.33)
72 h	325.00[b]	4.36[c]	40.30[b]	64.49[b] (81.43)
96 h	351.25[c]	5.86[d]	35.10[a]	66.70[b] (84.34)
SE ±	5.34***	0.22***	0.74***	1.30***

[abc] Means with different letters within each column differ at $P < 0.05$ (Duncan 1955) * $P < 0.05$ **$P < 0.01$ ***$P < 0.001$. ( ) real values transformed by arc sin x

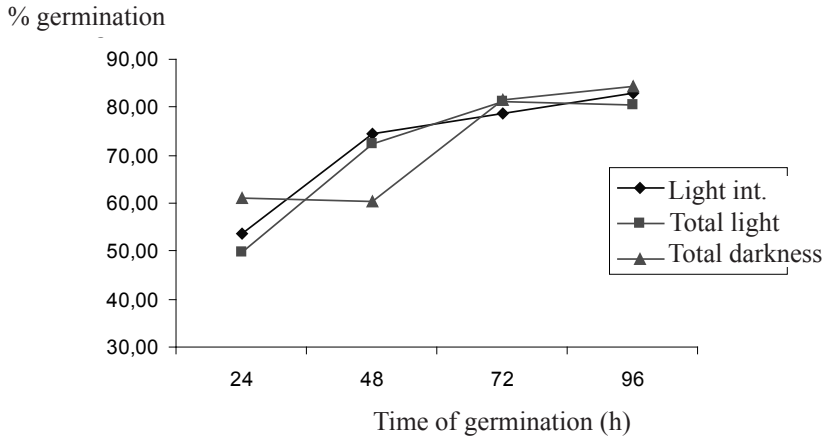

Figure 1. Graphic representation of the germination process on the percentage of germinated dolicho grains.

Table 2. Effect of the germination process on the protein and fiber content of the grains (%)

Treatments	Ash	TP	FND	FAD	LIG	CEL
Light intervals of 12 h						
Control	26.67	19.76^{ab}	30.04	14.42	1.11^{a}	11.23
24 h	24.02	19.14^{a}	30.07	13.70	1.78^{b}	11.79
48 h	23.63	20.29^{b}	30.14	13.52	1.37^{ab}	11.77
72 h	24.10	21.48^{c}	30.31	13.99	1.72^{ab}	12.16
96 h	22.90	19.72^{ab}	30.98	13.59	1.47^{ab}	12.00
SE±	1.32	0.27***	0.31	0.49	0.20*	0.39
Total light						
Control	26.67^{ab}	19.76	30.31^{b}	14.42^{ab}	1.11	11.23^{a}
24 h	24.41^{a}	19.30	27.99^{a}	13.01^{a}	1.14	11.75^{ab}
48 h	26.91^{ab}	18.88	32.24^{bc}	12.58^{a}	1.58	10.87^{a}
72 h	27.87^{b}	19.53	34.69^{d}	15.89^{b}	1.48	14.32^{c}
96 h	27.97^{b}	19.10	34.12^{dc}	13.97^{ab}	1.55	13.66^{bc}
SE±	0.90*	0.26	0.68***	0.83*	0.22	0.62*
Total darkness						
Control	26.67^{a}	19.76^{a}	30.31a	14.42^{a}	1.11	11.23^{a}
24 h	27.89^{a}	18.28^{a}	31.34^{ab}	13.63^{a}	1.33	12.61^{bc}
48 h	26.84^{a}	19.32^{a}	31.25^{ab}	13.53^{a}	1.29	12.10^{ab}
72 h	27.70^{a}	19.38^{a}	30.63^{a}	14.82^{a}	1.22	13.80^{cd}
96 h	30.08^{b}	21.37^{b}	32.65^{b}	16.37^{b}	1.61	14.67^{d}
SE±	0.46**	0.50**	0.50*	0.49***	0.22	0.38***

^{abc} Means with different letters within each column differ at P< 0.05 (Duncan 1955) * P< 0.05 **P< 0.01 ***P< 0.001

2008 and Díaz *et al*. 2010) showed that the animals did not survive with non-germinated dolicho. However, when germination took place, although the protein quality of the germinated dolicho was low (51 % of the biological value and 63 % of true digestibility), the effectiveness of the process was proved as the animals did not die and the experimental assessment was conducted. This corroborates the results of Urbano *et al*. (2005) in *Pisum sativum*, L.

In respect to the performance of the fibrous indicators, deeper studies on feed fiber through enzymatic methods (Martín- Cabrejas *et al*. 2007) indicated that dolicho had a slight decrease of 10 % (396.6 g/kg DM) in the content of total feed fiber (TF), and of 6 % (379.0 g/kg) when germinated in intervals and total light, respectively, compared to the control without germination (420.2 g/kg). However, in germination under total darkness conditions, TF increased 10 % (461.2 g/kg) compared to the control. These changes are in correspondence with the content of insoluble feeding fiber (celluloses, some hemicelluloses and lignin), constituting 95 % of the TF in this legume. This information corroborates the results of this experiment, determining the fiber components by the method of van Soest *et al*. (1991).

The profile of the mineral elements showed that ash and K increased in the germination time of the three variants used, while the rest of the minerals, except phosphorous that decreases in total darkness and light, did not vary significantly (table 3). Some authors refer that germination influences erratically on the mineral content; some others (Kavas and Nehir 1992 and Oloyo 2004) have reported significant increase on the mineral content. Generally, the variations of nutrients and anti-nutrients are attributed to the combined effect of germination and the previous soaking process, where great amount of components are solubilized. (Sangronis and Machado 2007).

It has been proved that the mineral availability in the germinated seeds increases phosphorous, potassium, magnesium, zinc and copper availability, as direct consequence of the phytase activation. This diminishes the phosphate insitoles hexa and penta phosphorylated to less phosphorylated shapes (Vidal-Valverde *et al*. 1998, Khalil 2001 y Zyla *et al*. 2004).

The results indicated that the procedure used in germination is feasible for obtaining new products in *Lablab purpureus*. As germination went forward, there was an increase of the germinated grains, root longitude and germination percentage. This conditioned bromatological changes in the germinated grains. The bromatological transformations were less marked than in cowpea. However, the variant of conducting germination under total darkness conditions coincided with superior biotransformations in CP, TP, ADF and cellulose.

The modifications occurred during the germination

Table 3. Effect of the germination process on the mineral content of the grains (%)

Treatments	Ceniza	Ca	P	K	Mg
		Light intervals of 12 h			
Control	3.52[a]	0.31[ab]	0.41	1.13[a]	0.27
24 h	4.54[b]	0.27[a]	0.44	1.22[a]	0.25
48 h	4.43[b]	0.34[ab]	0.44	1.30[a]	0.25
72 h	4.64[bc]	0.36[b]	0.41	1.45[a]	0.26
96 h	5.00[c]	0.30[ab]	0.41	1.94b	0.28
SE ±	0.13***	0.02*	0.02	0.14*	0.017
		Total light			
Control	3.52[a]	0.31	0.41[c]	1.13[a]	0.27
24 h	4.15[ab]	0.34	0.22[ab]	1.88[b]	0.28
48 h	4.27[b]	0.38	0.17[a]	1.41[ab]	0.27
72 h	4.17[ab]	0.36	0.23[ab]	1.77[b]	0.26
96 h	4.27[b]	0.32	0.24[b]	2.42[c]	0.26
SE ±	0.21*	0.03	0.02***	0.16**	0.02
		Total darkness			
Control	3.52[a]	0.31	0.41[c]	1.13[a]	0.27[ab]
24 h	4.40[bc]	0.34	0.26[ab]	1.39[a]	0.27[ab]
48 h	4.15[b]	0.32	0.24[ab]	1.45[a]	0.24[a]
72 h	4.49[bc]	0.31	0.29[b]	2.25[b]	0.33[c]
96 h	4.57[c]	0.35	0.23[a]	2.16[b]	0.30[bc]
SE ±	0.11**	0.02	0.01***	0.20**	0.01

abc Means with different letters within each column differ at P< 0.05 (Duncan 1955) * P < 0.05 ***P < 0.001

imply changes on the physico-chemical properties of this legumes, depending on the light conditions of the process. Therefore, it can be considered as an effective and promising method, as increasing its function and improves its quality. Although 72 h are enough for reaching germination percentages superior to 80 %, germination under total darkness conditions is recommended in all assays, as well as extend process to 96 h for guaranteeing the bromatological quality of the product. Besides, deeper studies on antinutritional factors and the nutritive value of the grains germinated is recommended, so the results of this study are complemented.

References

Aguilera, Y., Benítez, V., Díaz, M. F., Martín-Cabrejas, M. A, 2011a. Mejora de la fracción de carbohidratos prebióticos en leguminosas temporales germinadas. VII Congreso Internacional de Nutrición, Alimentación y Dietética. Madrid, España. [CD ROM]

Aguilera Y., V. Benítez, T. Jiménez, S. Calvo, M. F. Díaz, M. A. Martín-Cabrejas, 2011b. Improvement of nutrient bioavailability by germination process on *Vigna unguiculata*. : IV Congreso Internacional "Salud y Calidad de Vida". Holguín, Cuba. [CD ROM]

Anon. 1999. Nueva versión de la clasificación genética de los suelos en Cuba. Ministerio de la Agricultura, Ciudad de la Habana, Cuba. 64 pp.

AOAC 1995. Official methods of analysis of AOAC 16. Ed Assoc. Official Agriculture Chemists, Washington DC.

Benítez V., Y. Aguilera, T. Jiménez, S. Calvo, M. F. Díaz, M.A. Martín-Cabrejas, 2011 a. Utilization of processed legume flours as potential source of bioactive carbohydrates: IV Congreso Internacional "Salud y Calidad de Vida". Holguín, Cuba. [CD ROM]

Benítez V., Y. Aguilera, T. Jiménez, S. Calvo, M. F. Díaz, M.A. Martín-Cabrejas, 2011b. Evaluación de factores antinutricionales en legumbres no-convencionales: Efecto del proceso de germinación. VII Congreso Internacional de Nutrición, Alimentación y Dietética. Madrid, España. [CD ROM]

Blázquez, I. 1999. Contenido de vitamina B1 y vitamina B2 en guisantes y lentejas. Efecto de los procesos de germinación y extracción alcohólico. Graduate Thesis. Universidad Autónoma de Madrid. Madrid. 75 pp.

Cuadrado, C., Gillamón, E., Goyoaga, C., Pedrosa, M.M., Altares, P., Urbano, C. & Muzquiz, M. & Romero, C. 2004. Modification of seed storage proteins during germination and seedling growth of faba bean cotyledons. Recent advances of research in antinutritional factors in legume seeds and oilseeds. Fourth International Workshop on Antinutritional Factors in Legume Seed and Oilseeds. Eds. G.D. Muzquiz, C. Cuadrado Hill, M.M. Pedrosa y C. Urbano. EAAP. Publication no. 110. Toledo, España. 307 pp.

Díaz, M.F., Torres, V., González, A. & Noda, A. 2004. Biotransformations in the germination of *Vigna unguiculata*. Cuban J. Agric. Sci. 38:87

Díaz, M.F., Martín-Cabrejas, M.Á., González, A., Torres, V.

& Noda, A. 2007. Biotransformation of *Vigna unguiculata* during the gemination process. Cuban J. Agric. Sci. 41:161

Díaz M. F., L. Savón, V. Torres, G. Coto, M. Á. Martín-Cabrejas, A. González, A. Noda I. Scull & M. Orta, 2010. Obtención y caracterización química de germinados de leguminosas temporales para la alimentación animal en cuba. II Simposio Internacional de Producción de Monogástrico. III Congreso Producción Animal Tropical, La Habana, Cuba. [CD ROM]

Díaz, M. F., Padilla, C., Torres, V., González, A. & Noda, A. 2003. Bromatological characterization of seasonal legume species and varieties with possibilities in animal feeding. Cuban J. Agric. Sci. 37:443

Duncan, D.B. 1955. Multiple range and multiple F. test. Biometrics 11:1

Frías, J. 1992. Eliminación de α-galactósidos en lentejas (*Lens culinaris*) mediante procesado. Efecto en el contenido de almidón y en fibra alimentaria. Graduate Thesis. Universidad de Alcalá de Henares. Facultad de Farmacia. Departamento de Nutrición y Bromatología. Madrid. 102 pp.

Górecki, R.J. 2000. Seed Physiology and Biochemistry. In: Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics. Eds. C.L. Hedley, John Innes Centre, Norwich, UK. 117 pp.

Kavas, A. & Nehir, S. 1992. Changes in nutritive value of lentils and mung beans during germination. Chemie, Mikrobiologie, Technologie der Lebensmittel 14:3

Khalil, M. 2001. Effect of soaking, germination, autoclaving and cooking on chemical and biological value of guar compared with faba bean. Nahrung. 45: 246

Khatoon, N. & Prakash, J. 2006. Nutrient retention in microwave cooked germinated legumes. Food Chem. 97:115

Martin-Cabrejas, M. A., Ariza, N., Esteban, R., Mollá, E., Waldron, K. & López-Andreu, F. 2003. Effect of germination on the carbohydrate composition of the dietary fiber of peas (*Pisum sativum* L.). J. Agric. Food Chem. 51:1254

Martín-Cabrejas, M.A., Díaz, M.F., Aguilera, Y., Benítez, V., Mollá, E., Esteban, R.M. 2007. Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. Food Chem. 107: 1045

Matías, C., Esperance, M. & Ruíz, V. 1990. Efecto de los momentos y la distancia de siembra en la producción de semilla de la *Lablab purpureus* cv Rongai. Pastos y Forrajes 13:4

Meir, H. 1986. Laboraptaktibure. Tierernahrung und Futtermitterkum der fur Tiererproduzenten. Verlag. Alemania

Moussa, B. 1991. Aspectos de la agrotecnia y el manejo para la producción de forraje *Lablab purpureus* cv. Rongai. PhD Thesis. Universidad Agraria de La Habana "Fructuoso Rodríguez Pérez", La Habana, Cuba

Oloyo, R.A. 2004. Chemical and nutritional quality changes in germinating seeds of *Cajanus cajan* L. Food Chem. 85:497

Rivas-Vega, M.E., Goytortúa-Bores, J.M., Ezquerra-Brauer, M.G., Salazar-García, L.E., Cruz-Suárez, H., Nolasco, R. & Civera-Cerecedo, L. 2006. Nutritional value of cowpea (*Vigna unguiculata* L. Walp) meals as ingredients in diets for Pacific white shrimp (*Litopenaeus vannamei* Boone). Food Chem. 97:41

Roy, F., Boye, J.I. & Simpson, B.K. 2010. Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. Food Res. International 43:432

Sangronis, E. & Machado, C.J. 2007. Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. Food Sci. Tech. 40:116

Sangronis, E., Machado, C.J. & Cava, R. 2004. Propiedades funcionales de las harinas de leguminosas (*Phaseolus vulgaris* y *Cajanus cajan*) germinadas. Interciencia 29:80

Savón, L., González, B., Arteaga, M. E., Forte, C., Orta, M., Torres, V. & Díaz, M. F. 2008. Avances en la evaluación biológica de harina de granos germinados de leguminosas temporales utilizando la rata como modelo experimental. Porcinocultura 2008. Instituto de Investigaciones Porcina, La Habana, Cuba. CD-ROM

Torres, A., Frías, J., Granito, M. & Vidal-Valverde, C. 2007. Germinated *Cajanus cajan* seeds as ingredients in pasta products: Chemical, biological and sensory evaluation. Food Chem. 101:202

Urbano, G., Aranda, P., Vilchez, A., Aranda, C., Cabrera, L., Porres J. M. & López-Jurado, M. 2005. Effects of germination on the composition and nutritive value of proteins in *Pisum sativum*, L. Food Chem. 93:671

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition L. Dairy Sci. 74:3583

Vidal-Valverde, C., Pascual-Montaner, M., Diáz-Pollán, C. & Vicente, G. 1998. Changes of antinutritional factors during germination of peas. In: 3rd European Conference on Grain Legumes. Valladolid, España. 388 pp.

Visauta, B.V. 1998. Análisis estadístico con SPSS para Windows. Estadística multivariante. Volumen II. Ed. Concepción Fernández. Madrid. 24 pp.

Zyla, K., Fortuna, M., Mika, M. & Czubak, M. 2004. Limited phytate hydrolysis by endogenous phytase of sesame seeds during germination. Recent advances of research in antinutritional factors in legume seeds and oilseeds. Fourth International Workshop on Antinutritional Factors in Legume Seed and Oilseeds. Eds. G.D. Muzquiz, C. Hill, M.M. Cuadrado, Pedroza, U. EAAP Publication no. 110. Toledo, España. 347 pp.

Received: february 2, 2009