Research Paper Biological Science

Protein Hydrolysis Degree in Vitro and in Vivo Protein Digestibility of Four Autoclaved Tropical Legume Seeds

Itza Can P.E.	Campus de Ciencias Biológicas y Agropecuarias- Universidad Autónoma de Yucatán.
Santos-Ricalde R.H.	Campus de Ciencias Biológicas y Agropecuarias- Universidad Autónoma de Yucatán.
Sarmiento-Franco L.	Campus de Ciencias Biológicas y Agropecuarias- Universidad Autónoma de Yucatán.
Segura-Correa J.	Campus de Ciencias Biológicas y Agropecuarias- Universidad Autónoma de Yucatán.

ABSTRACT

The objective of this study was to evaluate the digestibility of crude protein in vivo (DCP) and the protein hydrolysis degree (PHD) of Mucuna pruriens (MP), Cajanus cajan (CC), Phaseolus lunatus (PL) and Vigna unguiculata (VU) seeds, which were autoclaved at different times. The difference between the PHD and DCP of the evaluated legumes was significant (P<0.05). Mucuna pruriens had the highest PHD (5.74%) followed by VU (4.55%), CC (4.42%) and PL (2.45%). In addition, VU (80.46%) had the greatest DCP followed by CC (69.92%), PL (67.14%) and MP (61.51%). For all legumes, the PHD and DCP significantly increased (P<0.05) with an increase in autoclaving time. The results indicate that an increase in autoclaving time correlated to an increase in DCP and PHD for the evaluated legumes. However, the PHD of MP and PL did not correspond to the DCP results.

KEYWORDS : legumes, digestibility in vitro, digestibility in vivo, degree of hydrolysis

INTRODUCTION

An extensive variety of legumes are grown by farmers in the rural areas of Mexico, especially southeastern Mexico. Many varieties are local, such as cowpea (*Vigna Unguiculata*), pigeon pea *(Cajanus cajan)* and Hib *(Phaseolos lunatus*). Other varieties, such as Velvet bean (*Mucuna prureiens*), have been well accepted by farmers.

Legume seeds have several nutritional qualities. They are a great alternative for feeding due their high nutritional content of protein and carbohydrates (Satya *et al*., 2010). Despite these qualities, the nutritional value of certain legumes are significantly lower than expected due to antinutritional factors that limit and reduce the efficient use of their nutrients when consumed. The presence of antinutritional factors reduces the bioavailability and digestibility of protein.

However, many of these antinutritional factors can be inactivated by an appropriate application of various methods, such as heating, autoclaving, cooking and roasting (Adeparusi, 2001; Satya *et al.,* 2010; Mugendi and Njagi, 2010; Udensi *et al.,* 2007).

The objective of this study was to determine the digestibility in vitro and the digestibility in vivo of *Cajanus cajan* (Pigeon pea), *Mucuna pruriens* (Velvet bean), *Phaseolus lunatus* (Hib) and *Vigna unguiculata* (Cowpea), which were autoclaved at different times.

MATERIALS AND METHODS

Legumes and autoclaving process

Seeds from four species of legumes *Cajanus cajan* (CC), *Mucuna pruriens* (MP) *Phaseolus lunatus* (PL) and *Vigna unguiculata* (VU) were evaluated. The chemical compositions are listed in table 1. The legumes were autoclaved at 120° C with 1.5 kg/cm2 pressure for each of the scheduled times (0, 5, 10, 15, 20, 30 and 60 minutes).

Table 1. Chemical analysis of the evaluated legumes seeds.

Legume	Dry matter (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Fat (%)
Cajanus cajan	90.2	23.2	8.8	4.4	5.6
Mucuna pruriens	92.9	25.6	7.6	3.6	0.8
Phaseolus lunatus	96.3	22.2	5.4	3.7	1.2
Vigna unguiculata	87.9	26.7	5.5	4.2	4.1

Protein hydrolysis experiment

Protein hydrolysis degree was estimated using a technique based on the pH stat method. The procedure was performed according to the methodology described by Salgo *et al.* (983), using a solution of trypsin and pancreatin (Sigma T0303 Trypsin-1G, Pancreatin P-1750 from porcine pancreas). An aqueous suspension of each legume protein (200 mg protein per 25 ml of bidistilled water), which was equilibrated to a temperature of 37° C at a pH of 8.0, was prepared with constant agitation. The solution of both enzymes (0.8 mg/mL of trypsin and 20 mg/mL of pancreatin) was adjusted to a pH 8.0 at a temperature of 37° C. Subsequently, 0.5 ml of enzyme solution was added to the protein suspension and automatically added to the protein suspension in an alkaline solution of 0.1 N NaOH, which was sufficient to maintain a pH 8.0. The enzymatic activity was measured after 900 seconds of incubation.

The protein hydrolysis degree (PHD) was determined from the volume of NaOH 0.1 N utilised to maintain a pH 8.0 and was expressed as the percentage of hydrolysed (h) peptide linkages, with respect to the total peptide bonds of the protein. The value of 7.8 (htot) was employed as the total peptide linkages in the legumes, according to Adler-Nissen (1986).

The following equations were applied to estimate the protein hydrolysis degree.

$$PHD\ (\%) = (h/htot) \times 100$$

where PHD is the protein hydrolysis degree, h is the number of hydrolysed peptide linkages, and htot is the total number of peptide linkages in the legumes.

The number of hydrolysed peptide linkages was estimated using the followed equation.

$$h = Vb \times Nb \times 1/\alpha \times 1/PM$$

where Vb is the NaOH consumption in ml; Nb is the normality of the base; α is the dissociation constant of the groups α-NH 2, which is 1.3 at 37°C and pH 8.0 (Adler-Nissen 1983); PM is the protein mass in the reaction mixture; and h is the number of hydrolysed peptide linkages.

In vivo digestibility experiment

To evaluate the digestibility of crude protein (DCP), an in vivo bioassay was performed using 65 *Mus musculus* male mice that were three months old. They were identified and randomly housed in individual metabolic cages. The legume seeds were autoclaved for 0, 30 and 60 minutes. Five mice were randomly assigned to each treatment. Additionally, five mice were only fed corn. The experimental period has two stages: six days for adaptation to the diets and six days of faecal collection. The offered feed, the refused feed and the collected faeces were weighed daily.

The experimental diets were elaborated mixing the legume flour with corn (50:50%). Then, the mixture was pelleted.

The DCP was estimated according to the difference method cited by Burch *et al.* (1975). The following formula was utilised to calculate the protein digestibility of the legume seeds.

$$D_t = D_{T+B}(\%) - D_B(\%) \times (N_{B+T})/(N_T) \times (N_{B+T})$$

where

D = digestibility; T = evaluated food; N = fraction of the nutrient; and B = basal food (corn)

Experimental design and statistical analysis.

A completely randomised design with the factorial arrangements 4 × 7 and 4 × 3 for digestibility in vitro and in vivo, respectively, was employed. The legume seeds that were evaluated correspond to the first factor and autoclaving time correspond to second factor. The differences between the means were subjected to Tukey's test when necessary. The data from the in vitro experiment were analysed as orthogonal polynomials to evaluate the linear and quadratic effect of the autoclaving time.

RESULTS AND DISCUSSION

The results listed in Table 2 show the average PHD of legumes seeds that were autoclaved at 0, 5, 10, 15, 20, 30 and 60 min. The PHD values for MP were significantly higher (P<0.05) than the PHD values for VU, CC and PL from 0 to 60 minutes.

Table 2. Protein hydrolysis degree (%) of legume seeds autoclaved during 0, 5, 10, 15, 20, 30 and 60 minutes.

	Autoclaving time (min)								Anova*		
Legume	0	5	10	15	20	30	60	Mean	Factor	SE[1]	P Value
Cajanus cajan	2.6	2.3	2.9	3.0	3.2	7.5	9.3	4.4	LEG[2]	0.06	0.0001
Mucuna pruriens	3.7	4.3	4.7	4.9	5.1	6.6	11.0	5.7	AT[3]	0.08	0.0001
Phaseolus lunatus	1.8	2.0	2.1	2.0	2.1	2.6	4.6	2.5	LEG x AT	0.16	0.0001
Vigna unguiculata	3.3	2.8	2.9	2.7	2.9	7.3	10.0	4.6	Contrast of AT		
Mean	2.9	2.9	3.1	3.1	3.3	6.0	8.8		Lineal		0.0001
									Quadratic		0.0001

[1]SE: standard error, [2]LEG: legume, [3]AT: autoclaving time.

The differences between the PHD of the legumes may be attributed to factors related to protein structure (tertiary and quaternary), which renders them more or less resistant to attack by proteolytic enzymes (Vadivel *et al.,* 2008). Some investigations report that PL seeds have a high amount of albumin (62.3%) compared with MP (48%), VU (44.7%) and CC (10.2%) (Singh and Eggum, 1984, Gallegos *et al.,* 2004; Thangadurai, 2005). These findings explain the low PHD of PL in the in vitro experiment compared with other legumes.

A significant linear increase in PHD (P <0.05) was observed as an effect of autoclaving time. According to various researchers the thermal process dissociates the protein structure, which facilitates the access of digestive enzymes to the nutrients and accelerates proteolysis and also, reduces the activity of the antinutritive factors (Udensi *et al.,* 2007; Radha *et al.*, 2008; Vadivel *et al.,* 2008; Mugendi *et al.,* 2010).

Concerning the DCP (Table 3), the autoclaving time has a positive effect on DCP (P<0.05). Differences between legume seeds on DCP were observed (P<0.05).

Table 3. Protein apparent digestibility *in vivo* (%) of the legume seeds autoclaved 0, 30 and 60 minutes.

	Autoclaving time (min)				Anova		
Legume	0	30	60	Mean	Factor	SE[1]	P Value
Cajanus cajan	58.7	72.6	78.4	69.9	LEG[2]	0.65	0.0001
Mucuna pruriens	48.2	67.2	69.2	61.5	TA[3]	0.56	0.0001
Phaseolus lunatus	50.8	69.5	81.1	67.1	LEG x TA	1.12	0.0001
Vigna unguiculata	75.9	82.1	83.4	80.5			
Mean	58.7	72.6	78.0				

[1]SE: standard error, [2]LEG: legume, [3]AT: autoclaving time.

Note that MP had the highest value of PHD and the lowest DCP (P<0.05). The lowest DCP of MP compared with a greater PHD may be attributed to physical, chemical or biological factors that are not considered in the in vitro assays and overestimate the values obtained in vivo. These seeds contain a toxic nonprotein amino acid L-DOPA, (Mugendi and Njagi, 2010) which induces antiphysiological effects in animals. The removal of L-DOPA is difficult using heat treatments (Josephine and Janadharnan, 1992).

CONCLUSIONS

The autoclaved treatment increased the PHD and DCP in the evaluated legumes. However, the lowest DCP in MP was not consistent with the highest PHD observed. Other antinutrive factors from the MP that were not inactivated by autoclaving reduced in vivo protein digestibility.

REFERENCES

1. Adeparusi E. 2002. Effect of processing on the nutrients and anti-nutrients of lima bean (*Phaseolus lunatus L.*) flour. Nahrung/Food. 45:94–96.
2. Adler-Nissen J. 1986. Enzymic Hydrolysis of Food Proteins. Elsevier Applied Science Publishers. London, 427 pp.
3. Burch H., Schneider W., Flatt P. 1975. The evaluation of feeds through digestibility experiments. Ed. The University of Georgia press Athens. pp. 143-168.
4. Gallegos S., Pacheco J., Betancur D., Chel L. 2004. Extracción y caracterización de las fracciones proteínicas solubles del grano de *Phaseolus lunatus L.* Arch. Latinoam. Nutr. 54:81-88.
5. Josephine R.M., Janadharnan K. 1992. Studies of chemical composition and antinutritional factors in three germoplasm seed material s of the tribal pulse, M*ucuna pruriens.* Food Chem. 43:13-18
6. Mugendi J, Njagi E. 2010. Effects of Processing Mucuna Bean *(Mucuna pruriens L.)* on Protein Quality and Antinutrients Content. In: Conference on International Research on Food Security, Natural Resource Management and Rural Development. Tropentag ETH Zurich.
7. Radha Ch., Kumar P., Prakash V. 2008. Enzymatic modification as a toolto improve the functional properties of heat-processed soy flour. J. Sci. Food Agric. 88:336–343.
8. Salgo A., Ganzler K., Jecsai J. 1983. Simple enzymic methods for prediction of plant protein digestibility. In: Proc. Int. Assoc. cereal chem. Symp. Amino Acid Comp. and Biol. Value of Cereal Proteins, Budapest, Hungary.
9. Satya S., Kaushik G., Naik S. 2010. Processing of food legumes: a boon to human nutrition. Mediterr J. Nutr. Metab. 3:183–195.
10. Singh U., Eggum B. 1984. Factors affecting the protein quality of pigeon pea *(Cajanus eajan*L.). Plant Foods Hum. Nutr. 34:273-283.
11. Thangadurai D. 2005. Chemical composition and nutritional potential of *Vigna unguiculata* subsp. *Cylindrica* (fabaceae). J. Food biochem. 29:88–98.
12. Udensi E., Ekwu F., Isinguzo F. 2007. Antinutrient Factors of Vegetable Cowpea (*Sesquipedalis*) seeds during thermal processing. Pak. J. Nutr. 6:194-197.
13. Vadivel V., Pugalenthi M. 2008. Effect of various processing methods on the levels, of antinutritional constituents and protein digestibility of *mucuna pruriens* (l.) Dc. Var. *Utilis*wall. Ex wight) baker ex burck (velvet bean) seeds. J. Food Biochem. 32:795–812.