Comparative Effects of Processing Methods on Hemagglutinating and Antitryptic Activities of *Canavalia ensiformis* and *Canavalia braziliensis* Seeds

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The effects of various processing methods on the hemagglutinating and antitryptic activities of the seeds of two species of the genus *Canavalia, C. ensiformis* and *C. braziliensis*, were investigated. Raw *C. ensiformis* and *C. braziliensis* contained 13 532 and 10 204 hemagglutinating units (HU), respectively, and also 1682 and 1662 trypsin inhibitor units (TIU) g^{-1} of seed, respectively. Moist heat proved more effective than dry heat as a method of inactivating the two antinutritional factors in the seeds. Whereas 90 min of cooking at 96 °C was required to completely eliminate antitryptic activities of both species, 3 h of cooking was required to completely eliminate their hemagglutinating activities. Pressure cooking eliminated their antitryptic activities in 30 min and their hemagglutinating activities in 45 min. Soaking in water for even up to 96 h before cooking had no effect on hemagglutinating and antitryptic activities of *C. braziliensis.* Soaking in water for 72 h, however, completely eliminated the antitryptic activity of *C. ensiformis* in 30 min of cooking and the hemagglutinating activity in 60 min of cooking. Breaking the seeds before cooking proved to be most effective, resulting in complete elimination of antitryptic and hemagglutinating activities of both species in 30 and 60 min of cooking, respectively, and is therefore most highly recommended.

Keywords: *Canavalia ensiformis; Canavalia braziliensis; antitryptic/hemagglutinating activities; processing methods*

INTRODUCTION

One of the serious problems facing most developing tropical countries is the scarcity of food for the teeming human population and feed for the dwindling livestock industry. The cowpea (*Vigna unguiculata*), soybean (*Glycine max*), groundnut (*Arachis hypogaea*), and a few other legumes have been playing key roles as foodstuffs and feedstuffs in most of these countries. There is need, however, for exploitation of hitherto neglected novel legumes that abound in the region in view of the pressure on the conventional ones arising from increasing multiple uses.

The genus *Canavalia* comprises a small group of some 48 species that are distributed throughout the tropics. Among them are *Canavalia ensiformis*, commonly known as jackbean, and *Canavalia braziliensis*, commonly known as feijão bravo do Ceara in Brazil, haba in Colombia and Guatemala, choncho in El Salvador, and "barbicou bean" in the Leeward and Windward Islands (Howard, 1988). These are New World plants that were grown in the drought-ridden regions of Arizona and Mexico in ancient times and utilized as high-protein food and forage crops for many centuries by natives of the southwestern United States, Mexico, Central American countries, Brazil, Peru, Ecuador, the West Indies, Bolivia, Paraguay, and Argentina (Sauer and Kaplan, 1969). Under optimal agronomic management conditions, total yields of their forage can reach up to 10 tonnes of dry matter (DM)/ha and dry seed yields of up to 2.5 t/ha have been reported in various regions (Addison, 1957; Mora and Parra, 1980, unpublished data; Pound et al., 1982; Okonkwo and Udedibie, 1991). A record seed yield of 4.32−5.40 t/ha had been reported for *C. ensiformis* at the U.S. Federal Agricultural Experimental Station in Mayagüez, Puerto Rico (FAO, 1959).

Raw unprocessed *Canavalia* seeds contain about 300 g kg^{-1} crude proteins and 600 g kg^{-1} carbohydrates (Udedibie, 1990). Although they are low in methionine, like most proteins of plant origin, they are relatively high in lysine (Molina et al., 1974; D'Mello et al., 1985; Bressani et al., 1987; Rajaram and Janardhanam, 1992). However, as with other tropical legumes, the raw unprocessed seeds contain toxic factors that limit their use as human food or animal feed (Orru and Demel, 1941; Borchers and Ackerson, 1950; Herrera et al., 1981; Montilla et al., 1981; Carlini and Guimaraes, 1981; D'Mello et al., 1985; Babar et al., 1988; Udedibie, 1990; Nakatsu et al., 1996), the most important being trypsin inhibitors and concanavalin A (Con A).

Trypsin inhibitors have been implicated in reducing protein digestibility and in pancreatic hypertrophy (Liener, 1976). They are polypeptides that form well-characterized stable complexes with trypsin on a one-to-one molar ratio, obstructing the binding sites and disrupting the enzymatic action.

Con A is a lectin, a carbohydrate-binding protein that is resistant to digestion (Pusztai, 1989). In addition to its ability to agglutinate the erythrocytes of numerous animal species, clump certain bacteria, and precipitate glycogen and starch from solutions, Con A negatively affects nutrient utilization by various mechanisms. It binds to the glycoprotein and glycolipids of the digestive tract mucosa (Hague, 1975; Jaffe, 1980), inhibits the activity of the enzymes of the brush border of the enterocytes (Rosenthal, 1972), and interferes with the adherence of enterobacteria to the intestinal wall (Jayne-Williams, 1973). It has been implicated in the patho-

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genesis of coeliac disease (Kolberg and Sollid, 1985) and possibly has several side effects on immune functions, protein metabolism, enzyme activities, and hormonal regulation (Pusztai, 1989). It induces severe reduction in feed intake of nonruminants (Liener, 1953; Larue-Achagiotis, 1992). Con A ordinarily is highly heat-labile but somehow seems to be protected against heat destruction as a component of the seed (Carlini and Udedibie, 1996, unpublished data). It appears therefore that for the *Canavalia* seeds to be rendered acceptable as human food or animal feed, method(s) must be devised that can completely and economically inactivate the trypsin inhibitors and Con A in them.

This paper reports the results of successive studies carried out to determine the responses of the two antinutritional factors to processing methods previously applied to the seeds and to new methods considered simple and feasible for rendering the *Canavalia* seeds completely trypsin inhibitor/Con A-free.

MATERIALS AND METHODS

Sources of Materials. The *Canavalia* seeds used for the studies were obtained from Agrodora in São Paulo, Brazil, a store that specializes in agricultural products. The seeds were harvested in the summer of 1996 by farmers who use the crop as cover crop and bought for the store. L-BAPNA and bovine trypsin were obtained from Sigma Chemical Co. (St. Louis, MO).

Dry Heat (Toasting). Raw seeds of *C. ensiformis* and *C. braziliensis* were ground using a 2 mm screen. Part of each meal was kept raw. The other part was thinly spread in a pan and placed in the oven (120 °C). It was stirred from time to time to maintain uniform heating. The heating (toasting) was considered adequate when the meal changed from whitish to light brown and became crispy to the touch. The process took 20−25 min.

Protein Extraction. Protein extraction was done by stirring 10 g of sample in 50 mL of phosphate-buffered saline (PBS, pH 7.00) for 2 h at 40 °C. Thereafter, the mixture was centrifuged (2000*g*) and the supernatant dialyzed for 20 h, also at 40 °C. Protein concentration was determined using a spectrophotometer by absorbance at 280 nm (Carlini and Guimaraes, 1981).

Hemagglutinating Activity. Con A content was measured by hemagglutination of fresh rabbit erythrocytes according to the method of Coffey et al. (1985) with some modifications. Fresh rabbit blood was collected in 5 mM EDTA and centrifuged to remove soluble blood constituents. The suspended erythrocytes were then washed three times with PBS. Protein samples (25 μL) in duplicate were serially (2-fold) diluted in PBS in 96-microwell plates, and 25 μL of a 2% (v/v) erythrocyte suspension was then added to each well. Hemagglutination titers were examined after 2 h at room temperature, and hemagglutinating activity was expressed as minimal concentration (mg/mL) of protein inducing hemagglutination of about 10^6 cells (hemagglutination unit, HU).

Trypsin Inhibitor Activity. Trypsin inhibitor activity was determined in duplicate according to the method of Erlanger et al. (1961) with some modifications. To measure 100% trypsin activity, 20 μL of 1 mg/mL trypsin was mixed with 100 μL of 0.1 mM of Tris buffer (pH 8.5) and made up to 450 μL with distilled water. This was then mixed with 50 μL of 4 mM L-BAPNA (benzyol-L-arginyl-*p*-nitronilide), and the rate of liberation (per minute) of *p*-nitroaniline from the l-BAPNA by trypsin was determined with a spectrophotometer at 405 nm absorbance. Trypsin inhibitor activity was measured by mixing 100 μL of the buffer, 20 μg in 20 μL of trypsin, 310 μL of distilled water, and 20 μL of protein extract to inhibit up to 60−70% of the trypsin activity. The mixture was then allowed to incubate for 15 min before 50 μL of L-BAPNA was added. Rate of liberation of *p*-nitroaniline was determined as earlier stated. One unit of trypsin inhibitor (TIU) was the amount of material, mg/mL, inhibiting 1 mg of trypsin under the experimental conditions.

Urea Solution Treatment. Samples were processed according to the method of Montilla et al. (1981) as modified by Udedibie (1990). Briefly, the *Canavalia* seeds were soaked in a 4% solution of urea and allowed to stand for 1 week. Thereafter, they were rinsed in tap water, cooked for 1 h, dried, and milled as described under Dry Heat (Toasting). Protein extraction and hemagglutinating and antitryptic activities were done as described above.

Dry Urea Treatment. Samples were processed according to the method of Udedibie et al. (1994). A batch of the raw seed meal was mixed with 2.5% of its weight of dry milled urea; another batch was treated with 5% of its weight of the dry milled urea. The samples were stored for a week and then toasted as described under Dry Heat (Toasting). Protein extraction and hemagglutinating and antitryptic activities were done as earlier described.

Sequential Cooking. The seeds were cooked in stages at 96 °C as follows. First, the seeds were cooked for 1 h. Cooking time was taken as from boiling. At the end of the hour, the cooking water was removed and changed with fresh water. Seeds removed at the end of the 1 h were referred to as first-stage cooked beans. The cooking was continued for another 40 min, at the end of which time the second-stage cooked beans were removed, the cooking water was changed, and the cooking continued for another 40 min. The third-stage cooked beans were removed, the cooking water was changed, and the cooking was continued again for the final 40 min; the fourth-stage cooked beans were produced. The beans were partly dried in the sun and then in the oven at 80 °C and then milled. Protein extraction and determination of hemagglutinating and antitryptic activities were done as earlier described.

3-h Nonstop Cooking. Since it took a total of 3 h to do the four stages of cooking, it became necessary to do a 3-h nonstop cooking of the beans for comparison. Consequently, some of the beans were cooked for 3 h at 96 °C, with the cooking water replenished as necessary. The products were further processed as described under Sequential Cooking.

Results. The data on the effects of toasting, urea tretments, and sequential and 3-h nonstop cooking on hemagglutinating and antitryptic activities of *C. ensiformis* and *C. braziliensis* seeds are presented in Table 1. Of the five processing methods, toasting alone appeared to be the least efficient as a method for inactivating the Con A and trypsin inhibitors in the seeds of the two *Canavalia* species. This reaffirms the earlier observations that dry heat is not an effective method of processing the *Canavalia* seeds for appreciable dietary inclusion (D'Mello et al., 1985; Babar et al., 1988; Udedibie et al., 1994). Although dry urea treatment prior to toasting drastically reduced the two antinutritional factors in the seeds, the effects could not compare with any of the moist heat treatments. The use of urea was based on the fact that urea is a strong protein denaturing agent and *Canavalia* seeds have urease activity (Sumner, 1926). The exact mode of action of urea on the antinutritional factors in the seeds is, however, not clear (Udedibie et al., 1994). It took 3 h of nonstop cooking at 96 °C to completely inactivate Con A in both species. Trypsin inhibitor activities were, however, completely eliminated within 1 h 40 min in both species, indicating higher susceptibility of trypsin inhibitors to heat treatment compared with Con A. The use of multiple-stage cooking appeared not to be necessary since it conveyed no advantage over continuous cooking.

Generally, the data showed that none of the methods investigated and which had been employed in various feeding trials (Montilla et al., 1981; D'Mello et al., 1985; Udedibie, 1990; Udedibie et al., 1994, 1996) is effective enough as to completely inactivate Con A in the seeds. This perhaps explains the poor performance of experimental animals fed high dietary levels of such processed *Canavalia* seed meals. It therefore seems that even minute amounts of Con A left in processed *Canavalia* seeds can still constitute a problem to animals due to its resistance to proteolytic digestion in the gut and the tendency to accumulate in the animals under ad

Table 1. Effects of Toasting, Urea Solution and Dry Urea Treatments, and Multiple-Stage and 3-h Nonstop Cooking on Hemagglutinating and Antitryptic Activities of *C. ensiformis* and *C. braziliensis* Seeds

	C. ensiformis				*C. braziliensis*			
treatment	HU[a] (g^{-1} of seed)	% recov[b]	TIU[c] (g^{-1} of seed)	% recov[d]	HU (g^{-1} of seed)	% recov	TIU (g^{-1} of seed)	% recov
raw seed	13531.8	100.00	1681.5	100.0	10204.1	100.00	1662.2	100.0
toasting	4545.5	33.36	1404.2	83.5	1428.6	14.00	1423.6	85.4
urea solution	32.3	0.24	NI[f]	0.0	216.7	2.12	464.5	27.9
2.5% dry urea	1086.9	8.04	851.3	51.1	1076.3	0.54	915.1	59.9
5% dry urea	526.3	3.89	413.4	24.8	434.2	4.26	582.0	34.9
1st stage cooked	102.0	0.76	353.8	21.2	285.7	2.80	473.3	28.4
2nd stage cooked	12.1	0.09	NI	0.0	71.4	0.70	NI	0.0
3rd stage cooked	9.2	0.07	NI	0.0	32.3	0.32	NI	0.0
4th stage cooked	5.6	0.04	NI	0.0	17.5	0.17	NI	0.0
3-h cook	<3.6[e]	<0.03	NI	0.0	<3.6	<0.03	NI	0.0

[a] 1.0 HU is the minimal concentration of Con A expressed in mg/mL present in the sample that was able to agglutinate about 10^6 cells as described in the text. [b] Percent of Con A recovered in sample after treatment. [c] Trypsin inhibitor unit: the amount of material [μunits (mg of protein)$^{-1}$] that was able to inhibit 1 mg of trypsin under the experimental conditions. [d] Percent of trypsin inhibitors recovered in sample after treament. [e] Limit of detection of hemagglutinating activity. [f] NI, no inhibition.

Table 2. Effects of Length of Pressure Cooking on Hemagglutinating and Antitryptic Activities of *C. ensiformis* and *C. braziliesis* Seeds

	C. ensiformis				*C. braziliensis*			
treatment	HU[a] (g^{-1} of seed)	% recov[b]	TIU[c] (g^{-1} of seed)	% recov[d]	HU (g^{-1} of seed)	% recov	TIU (g^{-1} of seed)	% recov
raw seed	13531.8	100.00	1681.5	100.00	10204.1	100.00	1662.2	100.00
15 min cook	16.7	0.12	585.0	35.10	12.5	0.12	785.3	47.12
30 min cook	4.0	0.03	NI[f]	0.00	3.7	0.04	NI	0.00
45 min cook	<3.6[e]	<0.03	NI	0.00	<3.7	<0.04	NI	0.00
60 min cook	<3.9	<0.03	NI	0.00	<3.6	<0.04	NI	0.00

[a–f] As in Table 1.

libitum feeding systems by binding to the intestinal wall. The data have, however, established that for the *Canavalia* seeds to be rendered lectin- and trypsin inhibitor-free, and therefore acceptable as human food or animal feed, moist heat treatment is the approach to follow. This is in agreement with the findings of Babar et al. (1988) and Bressani and Sosa (1990), who had earlier reported the superiority of moist heat over dry heat as method for processing the *C. ensiformis* seeds. Although the data have shown that moist heat treatment is the direction to go if the *Canavalia* seeds are to be made edible, the 3 h of cooking required to completely inactivate Con A in them is obviously not an economically viable option.

There is need, therefore, for quicker, commercially applicable, moist heat based alternatives.

Pressure Cooking. Since it is a common practice to use a pressure cooker (panela de pressao) to cook most legume grains to save time and cost, we decided to determine how long it would take the pressure cooker to completely inactivate the Con A and trypsin inhibitors in the two *Canavalia* species.

The beans were subjected to four different pressure cooking times: 15, 30, 45, and 60 min, respectively, using the common pressure cooker (panela de pressao) bought from the supermarket and the same heat intensity used in the ordinary cooking above. The beans so cooked were dried, milled, extracted, and analyzed for hemagglutinating and antitryptic activities as earlier described.

Results. The data on the effects of pressure cooking on the hemagglutinating and antitryptic activities of the seeds of the two *Canavalia* species are presented in Table 2. The Con A and the trypsin inhibitors in the seeds of the two species responded similarly to pressure cooking. Whereas it took 30 min of pressure cooking to completely inactivate the trypsin inhibitors in the two species, Con A required 45 min for complete inactivation, reaffirming the stronger resistance of Con A to heat treatments than trypsin inhibitors.

The analytical data seem to support observations in the literature. Nonruminants have been reported to show some negative response to 200−300 g kg^{-1} dietary inclusion of *C. ensiformis* that had been pressure-cooked for 30 min (Jayne-Williams, 1973; D'Mello et al., 1985; Udedibie and Nwaiwu, 1988; Udedibie and Madubuike, 1988). This also tends to show that in the absence of trypsin inhibitors, minute amounts of Con A in the diet can still constitute a problem to the animals in ad libitum feeding system.

Although pressure cooking of the *Canavalia* seeds can be useful for some domestic purposes, its application in large-scale commercial operations cannot be recommended with enthusiasm in view of difficulties relating to equipment.

Soaking in Water/Cooking. The assumption that soaking legume grains in water, mostly overnight, facilitates their detoxification during cooking has not proved for the *C. ensiformis* seeds (D'Mello et al., 1985; Esonu, 1996). Perhaps the seeds require a prolonged period of soaking. We therefore decided to investigate the effect of length of soaking in water coupled with length of cooking on the two antinutritional factors in the seeds.

The seeds were soaked in water for five different periods: 12, 24, 48, 72, and 96 h. At the end of each soaking time, the seeds were subjected to four different cooking times: 30, 60, 90, and 120 min at 96 °C. Drying, milling, extraction, and determination of hemagglutinating and antitryptic activities were done as above.

Results. The data on the effects of length of soaking the seeds in water prior to cooking on their hemagglutinating and antitryptic activities are presented in Table 3. Soaking the seeds of *C. braziliensis* in water for up to 96 h had practically no effect on the response of Con A or trypsin inhibitors to moist heat treatment. This was in contrast to the response of the seeds of *C. ensiformis*. Soaking *C. ensiformis* in water for 12 h did not have any effect on Con A content but was able to reduce the time required to completely inactivate trypsin inhibitors to 60 min of cooking. It took 48 h of soaking in water to completely inactivate the trypsin inhibitors in 30 min of cooking but 2 h of cooking to completely inactivate Con A. The optimal length of soaking time of *C. ensiformis* in water appeared to be 72 h, which required only subsequent 1 h of cooking to completely eliminate the hemagglutinating activity of the seeds. Soaking in water for 96 h did not have any advantage over 72 h. The seeds started to germinate when left in water for 72 h. The germination process was believed to have facilitated the inactivation process of Con A in the seeds since hemagglutinating activity has been reported to decrease with germination (Liener, 1986). The germination of the seeds failed to occur when the seeds were soaked in urea

Table 3. Effects of Length of Soaking in Water and Cooking Time on Hemagglutinating and Antitryptic Activities of *C. ensiformis* and *C. braziliensis* Seeds

	C. ensiformis				*C. braziliensis*			
treatment	HU[a] (g^{-1} of seed)	% recov[b]	TIU[c] (g^{-1} of seed)	% recov[d]	HU (g^{-1} of seed)	% recov	TIU (g^{-1} of seed)	% recov
raw seed	13531.8	100.00	1681.5	100.0	10204.1	100.00	1662.2	100.0
12 h soak								
30 min cook	62.5	0.46	697.1	41.8	9005.3	88.25	1121.9	67.3
60 min cook	14.3	0.11	NI[f]	0.0	238.6	2.32	473.3	28.4
90 min cook	8.3	0.06	NI	0.0	81.9	0.81	NI	0.0
120 min cook	4.2	0.03	NI	0.0	34.4	0.34	NI	NI
24h soak								
30 min cook	62.5	0.46	567.5	34.1	8196.2	89.32	1104.6	66.3
60 min cook	13.3	0.09	NI	0.0	241.2	2.36	473.3	28.4
90 min cook	7.7	0.06	NI	0.0	69.7	0.68	NI	0.0
120 min cook	4.0	0.03	Ni	0.0	29.8	0.29	NI	0.0
48 h soak								
30 min cook	33.3	0.25	NI	0.0	7981.0	78.22	1093.9	65.6
60 min cook	14.3	0.11	NI	0.0	298.8	2.93	522.4	31.3
90 min cook	3.7	0.03	NI	0.0	70.1	0.67	NI	0.0
120 min cook	<3.6[e]	<0.03	NI	0.0	24.2	0.24	NI	0.0
72 h soak								
30 min cook	3.2	0.02	NI	0.0	8191.4	80.32	1104.4	66.3
60 min cook	<3.7	<0.03	NI	0.0	301.9	2.96	427.7	25.7
90 min cook	<3.3	<0.03	NI	0.0	73.3	0.72	NI	0.0
120 min cook	<3.6	<0.03	NI	0.0	30.4	0.30	NI	0.0
96 h soak								
30 min cook	4.3	0.03	NI	0.0	7813.5	76.56	1072.8	64.4
60 min cook	<4.3	<0.03	NI	0.0	236.1	2.31	434.7	26.1
90 min cook	<4.3	<0.03	Ni	0.0	68.4	0.67	NI	0.0
120 min cook	<3.2	<0.02	NI	0.0	29.1	0.29	NI	0.0

[a–f] As in Table 1.

Table 4. Effects of Cracking Prior to Cooking on the Hemagglutinating and Antitryptic Activities of *C. ensiformis* and *C. braziliensis* Seeds

	C. ensiformis				*C. braziliensis*			
treatment	HU[a] (g^{-1} of seed)	% recov[b]	TIU[c] (g^{-1} of seed)	% recov[d]	HU (g^{-1} of seed)	% recov	TIU (g^{-1} of seed)	% recov
raw seed	13531.8	100.00	1681.5	100.0	10204.1	100.00	1662.2	100.0
cracked/cooked								
30 min	14.3	0.11	NI[f]	0.0	19.2	0.19	NI	0.0
60 min	<4.4[e]	<0.03	NI	0.0	<4.6	<0.04	NI	0.0
90 min	<2.4	<0.02	NI	0.0	<3.5	<0.03	NI	0.0
120 min	<3.6	<0.03	NI	0.0	<3.4	<0.03	NI	0.0
cracked/prior to cooking								
15 min	<4.0	<0.03	NI	0.0	<4.3	<0.04	NI	0.0
30 min	<3.8	<0.03	NI	0.0	<3.5	<0.03	NI	0.0
45 min	<3.8	<0.03	NI	0.0	<4.1	<0.04	NI	0.0

[a–f] As in Table 1.

solution for a week, which may explain why the seeds that were soaked in urea solution prior to cooking for an hour retained some hemagglutinating activity.

The relatively very poor response of *C. braziliensis* to soaking in water arose from its very tough seed coat that appeared impermeable to water.

These data have once again reaffirmed the stronger resistance of Con A to heat treatments relative to trypsin inhibitors. It may mean as well that any moist heat treatment that completely inactivates Con A has already eliminated trypsin inhibitors along the way.

Cracking and Cooking. *Canavalia* seeds are large seeds, about 5 times the size of soybean. They are very hard, too, and coated with thick and tough testa that constitute up to 20% of the seed total weight (Bressani et al., 1987). Most large legume grains, notably fava and kidney bean, have also been reported to take unusually longer times of cooking to become lectin-free (Coffey et al., 1985; Udedibie and Carlini, 1997, unpublished data). We therefore decided to verify the hypothesis that the physical structure of the seeds could be a limiting factor as follows.

The seeds were broken into smaller pieces (~3−6 parts) using a grinding machine. The broken seeds were then subjected to four ordinary cooking times, 30, 60, 90, and 120 min at 96 °C, and pressure cooking for 15, 30, and 45 min, respectively. The products were dried in the sun, milled, extracted, and analyzed for hemagglutinating and antitryptic activities as earlier described.

Results. The data on the effects of cracking prior to cooking of the beans on their hemagglutinating and antitryptic activities are presented in Table 4. Cracking the seeds before cooking resulted in complete inactivation of the trypsin inhibitors in both species in 30 min of ordinary cooking and of the Con A in 1 h of ordinary cooking or 15 min of pressure cooking. It is believed that the relative ease and rapidity with which the broken seeds absorbed the cooking water and hence moist heat was responsible for the unusually rapid elimination of the two antinutritional factors from the seeds. The broken seeds rapidly absorbed the cooking water at the onset of the cooking and about twice their volume of water disappeared within the first 3 min of cooking. It is perhaps important to observe too that it took the cooked material only 4 −6 h of the January summer sunshine of Rio de Janeiro to dry to 90% dry matter.

As earlier stated (Carlini and Udedibie, 1996, unpublished data), Con A ordinarily is highly heat-labile but relatively thermostable as a component of the seed, possibly protected by the characteristics of the seeds mentioned above. By breaking the seed into smaller pieces, that protection is

dissipated, thus exposing it to greater susceptibility to denaturation by moist heat.

GENERAL DISCUSSION

Canavalia seeds have been reported to contain a number of toxic factors. However, in discussing toxic and antinutritional factors in these seeds and indeed any other seeds, it should be appreciated that it is only the toxicity associated with oral ingestion of the factor that has any nutritional significance. The toxic alkaline non-protein amino acid in the seeds, canavanine (about 50 g kg^{-1} seed), a naturally occurring analog of L-arginine, has been reported to induce reduced feed intake in nonruminants but at the equivalent of about 300 g kg^{-1} dietary level of the raw seed or in the condition of inadequate dietary arginine (Rosenthal, 1972; Michelangeli and Vargas, 1994; Swaffer et al., 1994) Nutritionally speaking, a 300 g kg^{-1} dietary level of any raw legume grains for nonruminants is objectionable. Furthermore, other edible legume seeds often contain twice as much canavanine as the *Canavalia* seeds (VanEtten et al., 1963; Rosenthal, 1974). It therefore seems doubtful that the contribution of canavanine to the toxicity of the seeds is really significant. The concentrations of the specialized secondary plant biomolecules in the seeds—cyanogenic glycosides, saponins, alkaloids, terpenoids, and tannins—have also been shown to drop below detectable levels following 1 h of cooking (Udedibie and Nwaiwu, 1988; Oliveira, 1997). Urease and canatoxin, which were isolated from the seeds, were shown to be highly toxic if injected into experimental animals (Sumner, 1926; Tauber and Kleiner, 1931; Carlini and Guimaraes, 1981) but to exhibit no toxic effects if orally administered (Sumner and Howell, 1935; Carlini and Guimaraes, 1991). They therefore cannot be classified as antinutritional factors in the seeds.

Jackbean seeds have been processed in various ways by different investigators with conflicting results, indicating no more than partial detoxification. The improvement in their nutritive value through toasting was first reported by Borchers and Ackerson (1950). However, toasting alone could improve their nutritive value for broiler chickens only to the extent of 100 g kg^{-1} dietary inclusion (Udedibie et al., 1994). Partial detoxification by heat treatments of the seeds, mainly by cooking and autoclaving (D'Mello et al., 1985; Udedibie and Madubuike, 1988; Udedibie, 1990; D'Mello and Walker, 1991) and by extrusion cooking (Aguirre-Montana, 1988; Bressani and Sosa, 1990; Leon et al., 1991; Melcion et al., 1991) has also been reported. Although Melcion et al. (1991) claimed complete elimination of hemagglutinating activity of the jackbean through extrusion cooking, a feeding trial with the product indicated only partial detoxification since it still depressed growth of the experimental cockerels. It is therefore doubtful if the process actually succeeded in complete destruction of the lectin. Recent studies by Pinto et al. (1997) have shown that extrusion cooking of soybean even after malting could not completely inactivate trypsin inhibitors in the seed. Any heat process that cannot completely eliminate trypsin inhibitors from a seed will obviously have little effect on lectins.

CONCLUSION AND RECOMMENDATIONS

We believe that the two most important antinutritional factors in the *Canavalia* seeds are the trypsin inhibitors and Con A. The results of these trials have reaffirmed that for the *Canavalia* seeds to be rendered lectin- and trypsin inhibitor-negative, and therefore acceptable as human food or animal feed, moist heat treatment is the option of choice for processing them. The relative ease and rapidity with which trypsin inhibitors and Con A in the seeds were inactivated by the process of cracking the seeds before cooking, coupled with the subsequent production of very easy to dry and mill product, make that process the method of best option, particularly for the production of livestock feed from the *Canavalia* seeds. It is a process that can be started and finished in a day at any scale of operation. However, in the event of unavailability of means of cracking the seeds, soaking in water (but not for *C. braziliensis*) for 72 h prior to cooking for an hour is considered the second best option.

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