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Title: BIOCHEMICAL AND NUTRITIONAL STUDIES OF PHILIPPINE INDIGENOUS FOOD AND FORAGE LEGUMES

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Principal Investigator: Evelyn Mae T. Mendoza, Ph.D.
Institute of Plant Breeding
College of Agriculture
University of the Philippines
at Los Baños
College, Laguna, Philippines 3720

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Workers: Evelyn Mae T. Mendoza*, Project Leader
Antonio C. Laurena, Research Associate, Study Leader Jan-July 16, 1986
Felicito M. Rodriguez*, Research Associate, Study Leader
Ma. Jamela T.R. Revilleza*, Research Assistant, Study Leader
Charlene F. Barroga, Research Assistant, Jan-May 31, 1986
Study Leader, June 1-Dec. 31, 1986
Ana Gina T. Sembrano, Research Assistant
Cleotilde A. Caldo, Research Aide
Cleotilde M. Vicencio, Research Aide
Wilson W. Pacheco, Plant Propagator

* Part-time; the rest are full-time for the project.

Cooperators: Forage and Pasture Section, IPB
National Plant Genetic and Resources Laboratory, IPB c/o Dr. Nestor C. Altoveros
Vegetables Section, IPB
c/o Dr. Eufemio T. Rasco, Jr.

I. Introduction

In recent years, worldwide attention has focused on tropical legumes as a possible major source of animal and human food. Although the protein contents of legume seeds are usually high, from 20 to 40%, their nutritive values are low due to the limiting sulfur amino acids, cysteine and methionine, and the

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presence of anti-nutritional factors--trypsin inhibitors, hemagglutinins, flatulence factors and tannins. Other toxins may occur in the leaves, pods and seeds of these legumes like cyanogenic glucosides, saponins, alkaloids, non-protein amino acids like mimosine, etc.

This is a comprehensive study of the biochemical and nutritional qualities of the indigenous food and forage legumes in the Philippines, with focus on the underutilized species. Among the food legumes, the following are being studied: batao, lima bean, rice bean, jack bean, sword bean, sabawel, and sam-samping; the forage legumes include *Aeschynomene*, *Centrosema*, *Calopogonium*, *Crotolaria*, *Desmodium*, Kudzu, *P. lathyroides* and Porpogon, and a possible forage, balatong-aso.

This project consists of the following studies:

Study 1. Determination of proximate chemical composition, amino acid composition and levels of trypsin inhibitors, hemagglutinins, flatulence factors and tannins in the seeds, leaves, green pods and mature seeds of different indigenous legumes. In vitro protein digestibility and relative nutritive value will also be measured.

Study 2. Investigation of toxic substances such as cyanogenic glucosides, alkaloids, saponins, etc. in the seeds, leaves, green pods and mature seeds of the plant. Simple ways of detoxification like heat treatment, leaching and others will be tried.

Study 3. Biochemical, physicochemical and nutritional characterization of the seed proteins of selected indigenous

legumes.

These studies are expected to provide a sound basis for the utilization of these heretofore relatively unknown and underutilized legume species.

II. Accomplishments

Study 1. Determination of chemical composition, anti-nutritional factors and nutritional value.

Chemical Composition

Food legumes. Chemical analyses done in 1986 on food and forage legumes were only to complete the data gathered in the first two years of the project. In general, the protein content of mature seeds of food legumes ranged from 17 to 30%, carbohydrate (nitrogen free extract or NFE) comprised from 50 to 63%. About 80% of the NFE was polysaccharide. Fat ranged from 1.2 to 3.7% only. Moisture content ranged from 6.8 to 10.2%.

Immature pods of indigenous food legumes had high moisture content of 76.5 to 87.6%, low fat content (0.85-1.46%) and low protein content (1.66-3.89%). Carbohydrate content was also a low at 5.58-15.18%. Mature pods had similar ranges of chemical constituents.

Immature leaves contained high moisture content (76.8 to 84.11%) low fat (1.98 to 3.65%), low protein (3.61 to 5.49%). Carbohydrate content comprised of 6.34 to 10.46%. The mature leaves had lower moisture (60.2 to 81.4%) and correspondingly, slightly higher levels of the other components.

The complete tables will be given in the final report.

Forage legumes. Mature seeds of indigenous forage legumes ranged 4.5 to 9.4% moisture, 18.0 to 33% protein, 2.1 to 17% fat

and 2.4 to 5.2% ash. Carbohydrates accounted for 33.3 to 58.0%.

Immature pods of some of the forage legumes had 74.0 to 82.2% moisture, 1.2 to 2.1% fat, 2.1 to 5.5% protein, 1.1 to 1.4% ash, 2.2 to 3.7% crude fiber and 9.4 to 15.1% nitrogen-free extract. Mature pods had 64.5 to 72.5% moisture and corresponding slightly higher levels of the other constituents although of similar ranges as with the immature pods.

Immature leaves had 67.6 to 81.4% moisture, 1.0 to 3.1% fat, 4.5 to 10.41% protein, 1.0 to 2.9% ash, 1.3 to 4.9% fiber and 7.0 to 15.6% NFE. Centrosema BPI-T1 had the highest level of protein in immature leaves. These protein contents are higher than those in immature leaves of food legumes. Mature leaves had slightly lower moisture content (62.2 to 76.6%) and similar ranges of the other constituents. Centrosema, balatong aso and Aeschynomene had high levels of protein (8-10%) in the mature leaves.

Anti-nutritional Factors.

Only the data for alkaloids and saponins are presented herein as data for tannins, phytohemagglutinins, and phytic acid have been presented in the previous reports.

(1) Alkaloids. The mature seeds of all the food legumes tested had no alkaloids except for sam-samping (Table 1). Mature and immature pods of food legumes also alkalcids (Table 1). Alkaloids were detected in both mature and immature leaves of jackbean, pigeon pea and sword bean (Table 1).

In forage legumes, only *Crotolaria* seeds contained alkaloid (Table 2). Immature and mature pods also did not contain alkaloids (Table 2). However, the mature and immature leaves of

most of the forage legumes tested were positive for alkaloids, except for balatong aso, and Kudzu (Table 2). Interestingly, the immature leaves of *Crotolaria* and *P. lathyroides* were negative for alkaloids but their mature leaves were positive for alkaloids.

(2) *Saponins*. All the food and forage legumes analyzed contained saponins in both pods and seeds, with the forage group having relatively higher concentrations than the food legumes (Table 3 and 4). Saponin concentrations generally increased as the pods matured. There seems to be no correlation between the saponin concentration determined by the modified Lieberman-Burchard and froth tests.

(3) *Oligosaccharides*. A detailed study of the ontogeny of oligosaccharides in developing seeds of batao, sabawel and sam-samping is being undertaken. The mature seeds of food legumes have previously been identified to have high levels of oligosaccharides. During the last two weeks of maturity, the level of total soluble sugars decreased in the cotyledon of sam-samping and batao and increased in that of sabawel. To be noted is the low level of reducing sugars (<1.0%) and higher non-reducing sugars for batao (4%) and sam-samping (3.5%) as compared to sabawel which had 3.5% reducing sugars and 2.5% non-reducing sugars. Non-reducing sugars include sucrose and the oligosaccharides.

In forage legumes all samples had significant amounts of oligosaccharides--raffinose, stachyose and verbascose. Based on the intensity of the bands on the HPTLC chromatograms, the legumes were found to have decreasing flatus potential as

follows:

Aeschynomene > *Desmodium* > *Crotolaria* > *P. lathyroides*, *Kudzu*, *Colopogonium* > *Balatong Aso* > *Centrosema*. Fig. 1 shows the oligosaccharide profile of the raw mature seeds of some indigenous forage legumes.

Amino Acid Composition. Data on amino acid composition are reported under Study 3 in relation with protein quality.

Study 2. Investigation of toxic substances

(1) *Cyanogenic glucosides*. In general, very low levels of cyanide considered nonpoisonous (< 50 ug/g or ppm) were observed in several indigenous legumes tested (Table 5). To check the accuracy of the method used, samples of cassava were included in the analyses.

The anti-nutritional factors under study 1 can also be considered toxic, if due to excessive amounts, they cause physiological disorders or even death. So far, saponins, alkaloids and phytohemagglutinins, and oligosaccharides have been identified to be high in some of the legumes studied. Thus, simple methods of detoxifying the legumes of these anti-nutritional and/or toxic substances are being studied. Results of these studies will be reported in the final report.

Study 3. Biochemical, physicochemical and nutritional evaluation of seed proteins.

(1) Screening for High Methionine Content

From a total of 40 legume samples comprising 14 different species, 7 samples had high methionine contents ranging from 2.2 to 3.9 g met/16 g N (Table 6). A *Centrosema* spp. and *Phaseolus*

lathyroides had the highest values of 3.9% which is still lower than the reported met content of *Eragrostis africana* of 5.6% and *Digitaria exilis* of 6.1%. All the 7 samples except one (*Dolichos lablab*) had leucine as the first limiting amino acid.

However, these samples with high methionine contents had a poor amino acid balance as shown by their chemical scores (Table 7), except for *Centrosema* JS-1 and *Dolichos lablab* which had chemical scores of 88% and 70%, respectively. The value for the former is even higher than that reported for tamarind of 80%

The *in vitro* protein digestibility of these legumes is generally good (70-80%) and comparable to the values of better known and utilized legumes (Table 7). Their relative nutritive values (RNV), however, are low (<60%) indicating the presence of anti-nutritional factors and/or toxins. Again, *D. lablab* was exceptional in having consistently high (>80%) RNVs.

(2) Characterization of proteins

Aside from ricebean and balatong aso which had been reported before, characterization of sam-samping, sabawel, jackbean and swordbean are in progress. Table 8 shows the percentage of proteins fractionated according to solubility of the 4 legumes. Sabawel and sam-samping have high globulin-albumin ratios (7.46 and 9.46, respectively) typical of leguminous seeds. However, jackbean and swordbean have low globulin-albumin ratio (1.41 and 0.62, respectively), verifying our previous results.

Aside from physicochemical characterization for molecular weight, sub-units, we are searching for fraction(s) that may have

high methionine content. Thus, the albumins and globulins have been fractionated further based on (a) solubility at increasing ammonium sulfate saturation, (b) molecular size and (c) differences in net ionic changes at specific pH. These studies are now in progress.

III. Plan of Study

Based on the accomplishments in 1986 as summarized in Table 9 the following plan of study for 1987 is proposed.

Study 1. Complete chemical analyses and determination of nutritional quality. (Determination of relative nutritive value (RNV) on cooked seeds).

Study 2. Complete detoxification experiments for the following: (1) oligosaccharides in seeds of batao, sabawel and sam-samping, (2) phytohemagglutinins in seeds of jackbean and swordbean, (3) alkaloids in leaves of *Centrosema* and (4) saponins in leaves of jackbean.

Study 3. Complete characterization of proteins of sam-samping, sabawel, jackbean and swordbean with focus on determining fraction(s) with high methionine content.

IV. Awards, Papers presented

Our paper entitled "Biochemical Assessment of Five Underutilized Philippine Food Legumes:
by A.C. Laurena, C.F. Barroga, A.G.T. Sembrano, F.M. Rodriguez, Ma. J.R. Revelliza, E.T. Villena and E.M.T. Mendoza was adjudged best paper in the poster session during the Second National Chemistry Congress, Iloilo city, May 29-June 1, 1986.

We also presented a paper in the Poster Session of the Fourth Federation of Asian and Oceanian Biochemists (FAOB) Congress entitled "Screening Philippine Indigenous Legumes for High Methionine Content" by Antonio C. Laurena and Evelyn Mae T. Mendoza.

Table 1. Alkaloid content of indigenous food legumes.

Sample		Mature seed	Leaves: Immature	Leaves: Mature	Pods: Immature	Pods: Mature
Batao A-37		-			-	-
A-45		-			-	-
A-51	5-2	-	-	-	-	-
A-52	5-3	-	-	-	-	-
A-57	5-7	-	-	-	-	-
	5-14	-	-	-	-	-
Jackbean A-1		-				
A-3		-				
A-5		-				
A-6		-				
A-8		-				
8-8		-	+++	+++		
Limabean A-508		-			-	-
A-513		-				
A-525		-				
A-537		-	-	-		
A-538		-				
A-541		-				
A-544		-	-	-		
A-546		-				
A-535		-	-	-		
Sabawel A-2		-				
A-5		-				
A-8		-				
Sam-samping 7-2		+				
Swordbean A-3		-			-	-
A-4		-			-	-
A-5		-			-	-
A-9		-			-	-
A-12		-			-	-
Swordbean 8-4			+	+++		
8-6			+++	+++		
8-9			+++	+++		
Pigeon pea			+	+++		
Tapilan 26		-				
28		-				
46		-				

- negative; + present, slight turbidity; ++ definite turbidity; +++ with precipitation.

Table 2. Alkaloid content of mature seeds of indigenous forage legumes.

Sample	Mature Seed	Leaves		Pods	
		Immature	Mature	Immature	Mature
Aeschynomene IPB-1	-	+	+	-	-
IPB-2	-	+	+	-	-
Baatong aso BP	-	-	-	-	-
7	-	-	-	-	-
24	-	-	-	-	-
Centrosema BM-1	-	+++	+++		
BPI-T1	-	+++	+++		
JS-1	-	+++	+++		
BPTB	-				
Cajanus cajan BP-B	-	+	+++		
Colopogonium JS-1	-	+++	+++		
BM-1	-				
Crotolaria PC-1 (NB)	+++	-	+		
PC-1 (WB)	+++	-	+++		
BM-1	+				
Desmodium DQ-1	-	-	-		
DQ-2	-	-	-		
CT	-	-	-		
93	-				
195	-				
Kudzu DQ-1	-	-	-		
JS-1	-				
BP	-				
P. lathyroides IPB	-	-	+	-	-
158	-	-	-	-	-
Porpogon BPI	-				

- negative; + slight turbidity; ++ definite turbidity; +++ with precipitation.

Table 3. Saponin contents of raw mature seeds of some of the indigenous food and forage legumes.

Samples	Lieberman-Burchard* test (%)	Froth test
Food Legumes		
Batao		
A-51	4.85	++
A-52	4.41	+
A-57		+
Jackbean		
A-1	3.95	++
A-3	4.43	++
A-5	4.05	+
A-6	4.12	+
A-8	2.08	+
B-8	5.74	+++
Lima bean		
A-535	4.95	+
A-537	11.13	+++
A-544	4.93	+++
Sabawel		
A-2	4.95	+
A-5	4.95	+++
A-8		
Sam-samping 7-2		
Swordbean		
A-4	0.95	+
A-9	4.09	+
A-12	5.05	+
Forage Legumes		
Balatong-aso	6.83	++
Calopogonium BM-1	11.98	++
Calopogonium JS-1	10.85	-
Centrosema BM-1	16.29	++
Centrosema JS-1	9.97	+
Centrosema BPI-T1	5.55	+++
Crotolaria BM-1	15.22	++
Crotolaria WB PC-1	8.08	++
Crotolaria PC-1	4.12	+
Phas. lathyroides IPB	9.18	
Phas. lathyroides 158		+++

*- Computed on the basis of fat free and moisture free samples.

Table 4. Saponin contents of raw mature and immature pods of the indigenous food legumes.

Samples	Immature Pods Lieberman-Burchard* test (%)	Immature Pods Froth test	Mature Pods Lieberman-Burchard* test (%)	Mature Pods Forth test
Batao				
A-15	4.64	++	6.78	++
A-35	5.17	++	8.94	+
A-37	5.44	+	8.08	+
A-45	8.83	+++	8.12	+
A-51	8.40	-	11.45	+
A-52	3.58	-	5.17	+
A-57	5.17	+	5.96	+
A-59	5.70	-		
Jackbean				
A-3	2.0	+	6.23	++
A-5	10.08		n.s.	
A-6	n.s.		n.s.	
A-8	n.s.		5.88	+
8-8	n.s.		n.s.	
Lima bean				
A-508	1.45	++		
A-513	3.58	+	2.37	
A-517	2.71	++	3.19	++
A-525	1.09		2.09	-
A-535	5.15	+	4.15	+
A-537	10.98			+
A-538	n.s.		3.68	+
A-541	7.16		8.50	
A-544	4.76	++	2.79	+
A-546	n.s.		4.44	-
Sabawel				
A-2	4.11	++	1.82	+
A-5			6.76	+
A-8			5.88	+
Sam-samping 7-2	3.49	++	4.05	++
Swordbean				
A-4	2.09	++	9.77	+
A-9	0.81	++	3.39	+++
A-12	11.19	-		
8-4	n.s.		8.96	++
8-6		++	6.56	++

* - computed on the basis of fat free and moisture free samples.
ns, no samples
Other missing data, not determined

Table 5. Cyanide content of some legumes and cassava.

Samples	Cyanide, ppm
Immature whole pod	
Lima A-557	0.65
Lima A-537	1.02
Immature Leaves	
Jackbean A-5	0
Jackbean 8-8	0
Sabawel A-2	0.83
Sabawel A-5	0
Lima A-537	5.31
Lima A-544	10.35
Sam-samping	0
Mature Leaves	
Jackbean A-5	0.14
Jackbean 8-8	0
Sabawel A-2	0
Sabawel A-5	0
Lima A-537	8.88
Lima A-544	0.83
Sam-samping	0
Immature Seeds	
Lima A-537	0.08

Cassava

	ppm Cyanide			
	Immature leaves	Mature leaves	Parenchyma	Cortex
Native	169.3	108.0	52.9	423.9
Lakan	160.0	101.4	26.8	386.9
Datu	182.3	134.6	105.0	637.3

Table 6. Protein content, essential amino acids content[a] and chemical scores[b] of some Philippine indigenous legumes.

Legume/Sample	Protein Content (% N X 6.25)	Essential Amino Acids (g/16 gN)							limiting amino acid	
		Met	Thr	Phe + Tyr	Lys	Val	Leu	Ile	first	second
Dolichos lablab A-51	18.52	2.2 (>62)	5.2(130)	11.3(186)	4.6(84)	7.8(157)	4.9(70)	6.6(165)	Met + Cys	Leu
Mucuna curanii A-5	29.42	2.8 (>79)	2.4(60)	8.0(131)	4.6(84)	4.0(81)	3.9(55)	4.0(100)	Leu	Thr
Aeschynomene spp. IPB	24.77	2.3 (>65)	3.3(82)	8.3(136)	11.5(211)	4.3(87)	3.6(51)	4.5(112)	Leu	Met + Cys
Calopogonium spp. JS-1	33.28	2.5 (>71)	4.8(120)	6.3(104)	4.4(81)	3.7(74)	2.4(34)	3.8(95)	Leu	Met + Cys
Centrosema spp. JS-1	19.72	3.9 (>111)	4.0(100)	14.6(240)	6.2(114)	5.9(119)	6.2(88)	6.2(155)	Leu	Thr
Phaseolus lathyroides 158	23.24	3.9 (>111)	3.4(85)	5.7(94)	9.0(165)	7.0(141)	3.8(54)	4.3(120)	Leu	Thr
Bovine alpha-casein[c]	99.00	2.5 (>71)	3.8(95)	10.5(173)	7.7(141)	5.4(109)	8.3(118)	4.9(120)	Met	Thr

[a]Tryptophan was not determined. Met + Cys via performic acid oxidation was not done.

[b]Data in parentheses are chemical scores, calculated according to the provisional amino acid scoring pattern (4). Chemical score for Met + Cys was calculated from the data of met alone.

[c]Data from Waters Associates (2).

Table 7. Protein content and some nutritional quality parameters of raw mature seeds of high met-containing Philippine food and forage legumes.

Legume	Protein (%) Content (6.25 x % N)	IVPD (%)	RNV (%)	6-PER	Chem. Score
1. *Dolichos lablab* A-51	18.52	70.18	80.84	1.94	63 (Met)
2. *Mucuna curanii* Sabawel A-5	29.42	72.46	5.98	1.52	60 (Thr)
3. *Aeschynomene* spp. IPB	24.77	77.87	86.54	1.55	51 (Leu)
4. *Calopogonium* spp. JS-1	33.28	73.22	0.00	0.73	34 (Leu)
5. *Centrosema* spp. JS-1	19.72	73.00	0.00	2.72	88 (Leu)
6. *P. Lathyroides* 158	23.24	76.31	53.38	1.73	54 (Leu)
Bovine -Casein	Sigma Pure	97.07	100.00	2.28	

In parenthesis, limiting amino acid.

Table 8. Protein Fraction of sabawel, sam-samping, Jackbean and swordbean.

Sample	Albumin (%)	Globulin (%)	Prolamin (%)	G'utelin (%)	Residue (%)
Sabawel A-8	9.78	73.08	0.64	10.67	1.68
Sam-samping 7-2	8.00	75.7	0.60	12.48	4.41
Swordbean	48.7	30.41		20.9	
Jackbean	35.5	49.9		14.6	

Table 9. summary of accomplishments in the analyses of food forage legumes

Analyses	Sample parts	Food Legumes	Forage Legumes
1. Proximate composition	mature seeds mature leaves immature leaves mature pods immature pods	100%	100%
2. Trypsin inhibitor activity	mature seeds		
3. Hemagglutinins	mature seeds	100%	100%
4. Oligosaccharides	mature seeds	100%	100%
5. Cyanide	mature seeds leaves pods	100%	0
6. Saponins	mature seeds mature pods immature pods leaves	100% 100% 100%	100% 100% 100%
7. Alkaloids	mature seed pods leaves	100% 50% 40%	100% 50% 40%
8. In vitro Protein digstibility	mature seeds leaves	100% 100%	100%
9. Relative nutritive value (RNV)	mature seeds cooked seeds	100% 0	100% 0
10. Amino acid composition	mature seeds	80%	80%

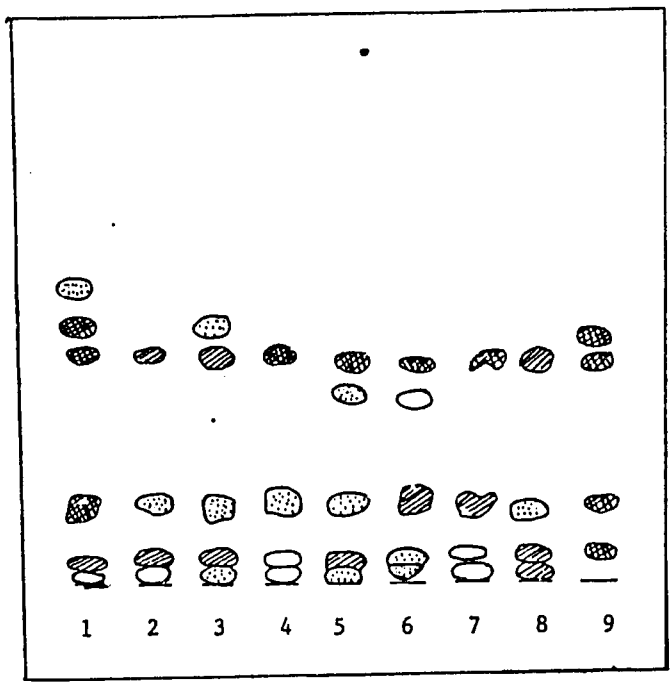

Fig. 1. Oligosaccharide profile of the raw, mature seeds of some indigenous forage legumes.

Legend:

1-Aeschynomene
2-Crotolaria
3-Phaseolus lathyroides
4-Centrosema
5-Kudzu
6-Colopogonium
7-Balatong aso
8-Desmodium
9-standards

a-glucose
b-sucrose
c-raffinose
d-stachyose
e-verbascose

· highly intense
· moderately intense
· light
· faint

Decreasing flatus potencial:
Aeschynomene> Desmodium> Crotolaria> P. lathyroides, Kudzu, Colopogonium> Balatong aso> Centrosema