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

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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 different parts and different stages of maturity of the plant. Simple ways of detoxificationn like heat treatment, leaching and others will be tried.

Study 3. Biochemical, physicochemical and nutritional characterization of the seed proteins of some of these 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

Collection of samples. The mature seeds obtained from various sources like the National Plant Genetic Resources Laboratory, Vegetables and Forage and Pasture Sections of IPB and different places in Laguna, Batangas and Quezon have been planted. Samples of mature and immature leaves and pods have been or being harvested and prepared for various analyses.

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

Food Legumes

Chemical analyses. Table 1 summarizes the chemical analyses of raw mature seeds of several Philippines indigenous food legumes. Proteins (17-31%) and polysaccharides (37-49%) were the predominant constituents. Fat content was only 1.7-3.5%.

At first, samples of leaves from 3,4,5 and 6 month old plants were collected. The leaves from 3 and 4 mo old plants were relatively immature compared to those from 5 and 6 mo old plants. Moisture content of leaves ranged from 72-84%, protein from 3.3-8.0%, fat from 0.7-3.0% and crude fiber from 1.8-4.0% (fresh weight basis) (Table 2a-d).

Immature pods of lima, swordbean and jackbean contained about 84-90% moisture, 0.4-1.1% fat, 1.3-2.4% protein and 0.4-1.1% ash. Mature pods of lima and swordbean had 62.6-71.8% moisture, 2.0-2.8% fat, 3.1-4.6% protein and 1.6-8.5% ash.

Anti-nutritional factors, relative nutritive value (RNV) and in vitro protein digestibility (IVPD). Table 3 summarizes results obtained for raw mature seeds of indigenous food legumes. Rice

bean exhibited low TIA (5.0-7.0 units/mg) and tannin content (1.52-18.2% catechin eq) and high RNV (120-132%) and IVPD (74%).Batao also had high RNV (78-113%) and IVPD (72%), low tannin (0.9-1.2% catechin eq) but relatively high TIA (26-44 units/mg). Sam-samping and swordbean had very low RNV (40-54%) which indicate the presence of toxic factor(s) in these legumes.

TIA of immature green pods had relatively low TIA ranging from 5-17 units/mg compared to 26-44 units/mg in mature seeds.

IVPD of leaves from 1 to 6 mo old plants ranged from 74-80% (Table 4).

The raw mature seeds of all the accessions of jackbean and swordbean were found to be highly positive for hemagglutinins (Table 5). Sabawel, batao and wild lima were also positive for hemagglutinins and the rest, negative.

The analysis for oligosaccharides which account for the flatulence in legumes has been started by optimizing conditions for their analysis by gas liquid chromatography.

Forage Legumes

Chemical analyses. Raw mature seeds of several indigenous forage legumes had 4.6-9.1% moisture, 5.4-12.5% fat, 18.5-33.3% protein and 2.4-4.3% ash, with Colopogonium having the highest protein content (Table 6). Immature leaves contained 64-81% moisture, 1.0-3.8% fat, 5.3-10.4% protein, 1.3-2.0% ash, with Centrosema and C. occidentalis having the highest protein levels. Mature leaves had 60-89% moisture, 1.5-7.3% fat, 5.2-10.3% protein, 1.8-3.6% ash and 1.9-17.7% crude fiber. Centrosema and P. lathyroides had the highest protein levels (8.8-10.3%). Colopogonium had high crude fiber.

The immature pods of 3 species had 74-82% moisture, 1.2-1.7% fat, 2.1-4.9% protein and 1.2-1.4% ash. Mature pods had 51-63% moisture, 1.6-3.6% fat, 4.5-6.9% protein, 1.2-2.5% ash and 8.4-12.8% crude fiber.

Study 2. Investigation of toxic substances.

Total hydrocyanic acid (HCN) potential was determined using an enzymatic method to hydrolyze the cyanogenic glucosides and subsequent trapping of the released cyanide in alkali which is then measured colorimetrically. Using various preparations of enzyme (A-D) from the samples being analyzed, the total HCN (bound and free) was found to be low (<50 mg/kg or ppm) in all samples (Table 7). The level of toxicity has been arbitrarily defined by Bolhuis (1954) as: <50 mg HCN/kg, harmless; 50-100 mg HCN/kg, moderately toxic; and >100 mg HCN/kg, highly toxic or poisonous.

Alkaloids were present in immature and mature leaves of Centrosema and Colopogonium, and in mature leaves of Crotolaria and P. lathyroides. Among food legumes, swordbean and jackbean mature leaves contained alkaloids (Table 8).

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

In addition to Vigna umbellata (rice bean), the following species will be subjected to this study: swordbean, jackbean, sam-samping, sabawel and balatong aso. Preliminary fractionation experiments showed that the albumins were the major protein fraction in swordbean, jackbean and balatong aso, in contrast to the general observation that globulins are the major protein fraction in legumes. The fractionation of the proteins is being repeated in the laboratory; characterization studies on isolated proteins are underway.

III. Plan of Activities

Table 9 summarizes the accomplishments in 1984 and on this basis, the following activities are planned for 1985:

Study 1. a) Proximate chemical analyses of both food and forage legumes will be completed.

b) Determination of anti-nutritional factors in forage legumes will be done and on food legumes completed.

c) RNV and IVPD of forage legumes will be started.

d) Study on the flatulence factors (oligosaccharides will be continued.

e) Amino acid analysis using the HPLC for amino acid analysis just acquired through the grant will be started.

Study 2. a) Determination of cyanogenic glucosides will be completed.

b) Determination of saponins, alkaloids and phytic acid will be continued.

c) Detoxification studies will be started.

Study 3. Isolation and characterization of seed proteins of swordbean, jackbean, sabawel, sam-samping and balatong aso will be continued.

IV. Others

The equipment, chemicals and other laboratory supplies requested arrived in November 1984 and are now being used for the project.

V. LITERATURE CITED

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Table 1. Proximate analysis of raw mature seeds of several Philippine indigenous legumes.

Sample	Moisture (%)	Protein (%N x 6.25)	Fat (%)	Starch (%)	Total Sugars (%)	Crude fiber (%)	Ash (%)
Batao (15)	6.5-8.4	19.5-23.4	1.66-2.27	38.8-42.8	3.51-5.86	4.12-8.76	4.74-6.38
Sabawel (2)	7.7-8.4	29.3-28.2	2.25-2.50	38.4-38.5	2.13-3.62	6.32-7.25	4.66-4.69
San-sanping (1)	6.2	30.9	2.20	38.6	8.72	10.40	5.41
Sword bean (3)	8.2-8.7	24.7-31.4	2.20-2.44	36.6-39.5	2.77-8.72	3.26-8.74	4.13-4.57
Jack bean (1)	8.4	28.7	2.50	37.5	2.88	5.76	3.90
Tapilan (2)	8.9-9.3	17.0-17.4	3.23-3.54	48.6-49.2	6.60-8.20	5.41-6.90	4.33-4.55

Except for moisture, all other results are expressed on dry weight basis.

Table 2a. Proximate analyses of leaves from 3 mo old plants of Philippine indigenous food legumes.

Sample	Moisture (%)	Fat (%)	Protein (%)	Crude fiber (%)	Ash (%)	CHO (%)
Batao (4)	77.5-80.8	1.2-1.6	5.4-6.0	2.4-2.9	2.0-2.2	8.0-9.4
Jackbean (2)	79.0	1.3-1.9	3.6-4.7	2.0-3.6	4.0-4.4	8.1-8.3
Sabawel (1)	74.0	1.4	8.0	3.6	2.7	10.1
San-sanping (1)	73.0	1.5	6.9	3.9	2.8	11.2
Swordbean (4)	77.3-79.8	1.2-1.6	3.3-4.2	2.5-3.9	3.6-4.5	8.8-14.4

Number in parentheses refers to number of accessions analyzed.

Table 2b. Proximate analyses of leaves from 4 mo old plants of Philippine indigenous food legumes.

Sample	Moisture (%)	Fat (%)	Protein (%)	Crude fiber (%)	Ash (%)	CHO (%)
Batao (4)	79.2-81.4	0.9-1.1	5.4-6.1	2.2-2.9	1.7-2.0	7.0-10.0
Jackbean (1)	75.6	1.1	4.9	3.6	3.7	11.1
Sabawel (2)	72.4-75.8	1.3	6.9-7.8	3.7-4.0	2.0-3.1	10.3-12.4
San-sanping (1)	81.7	0.7	5.4	3.2	2.0	7.1
Swordbean (3)	77.4-81.9	0.9-1.0	4.4-6.6	3.2-3.7	2.2-3.3	7.4- 8.4

Number in parentheses refers to number of accessions analyzed.

Table 2c. Leaves from 5 mo plants of Philippine indigenous food legumes.

Sample	Moisture (%)	Fat (%)	Protein (%)	Crude fiber (%)	Ash (%)	CHO (%)
Batao (4)[a]	80.6-82.9	1.0-1.4	5.0-5.4	2.1-3.2	1.4-1.8	7.5- 8.0
Jackbean (1)	81.1	1.1	4.0	2.6	2.8	11.1
Sabawel (2)	75.1-81.6	1.3-1.5	5.6-6.7	2.5-2.8	1.6-2.3	7.4-11.7
Sam-samping (1)	84.5	0.9	4.7	1.8	1.4	6.7
Swordbean (3)	79.5-82.3	1.0-1.4	3.6-4.6	2.7-3.3	1.7-2.6	8.7- 8.9

[a]Number in parentheses refers to number of accessions analyzed.

Table 2d. Leaves from 6 mo old plants of Philippine indigenous food legumes.

Sample	Moisture (%)	Fat (%)	Protein (%)	Crude fiber (%)	Ash (%)	CHO (%)
Batao (4)[a]	80.3-83.6	1.4-2.7	4.5-5.0	2.1-2.6	1.4-1.9	7.0-8.0
Jackbean (1)	80.4	2.7	4.9	2.3	2.4	7.4
Sabawel (2)	75.8-78.3	2.1-2.5	5.9-6.4	3.9-3.6	1.7-1.9	8.0-9.8
Sam-samping (1)	80.3	2.9	5.7	3.1	1.6	6.4
Swordbean	78.8-81.2	2.7-3.0	4.6-5.3	3.0-3.3	2.0-2.5	5.7-6.7

[a]Number in parentheses refers to number of accessions analyzed.

Table 3. Trypsin inhibitor activity, tannin content, *in vitro* digestibility and relative nutritive value of raw mature seeds of some Philippine indigenous legumes (dry weight basis).

Sample	No. of cultivars tested	Trypsin inhibitor[a] activity (TIA) (units/mg sample)	Tannin Content (Expressed as % catechin)[b]	In Vitro Digestibility[c] (%)	Relative Nutritive Value[d] (%)
Batao	3	26.2-43.6	0.90-1.22	71.19-72.76	78.3-113.3
Sabawel	2	16.5-19.8	5.77-8.81	68.71-70.50	39.3- 70.0
Sam-samping	1	ND[e]	8.29	76.19	53.6
Sword bean	2	5.8-6.5	4.08-5.00	71.38-71.95	39.6- 49.2
Jack bean	1	1.9	1.17	73.21	69.7
Tapilan (Rice bean)	3	5.0-7.0	1.53-1.82	73.48-74.30	128.5-132.0

[a]According to the method of Kakade et al. (1974).

[b]According to the method of Price et al. (1978).

[c]According to the method of Hsu et al. (1977).

[d]According to the method of Landers (1975).

[e]Not Determined.

Table 4. *In vitro* protein digestibility (IVPD) (%) of leaves from developing plants of several Philippine indigenous food legumes[a].

Sample		In vitro protein digestibility (%) Age of plant (mo) 1	2	3	4	5	6
D. lablab	5-2	74.88	76.25	78.00	76.18	76.37	77.33
	5-3	75.94	77.18	77.96	76.11	76.49	nd
	5-7	75.27	74.93	79.96	75.62	78.48	78.40
	5-14	76.36	75.14	74.58	78.30	78.61	74.88
Sabawel	6-1	77.49	80.29	78.19	75.7[illegible]	79.82	76.18
	6-2	76.43	76.38	76.24	77.05	80.67	77.14
Swordbean	8-4	n.d.	74.33	76.81	76.38	77.42	74.10
	8-6	n.d.	74.40	74.06	77.33	77.74	75.48
	8-9	n.d.	77.81	74.66	77.89	77.11	76.37
Jackbean	8-8	n.d.	75.39	76.29	73.53	76.38	74.02
Sam-samping	7-2	n.d.	76.74	75.50	7[illegible].59	n.d.	76.91

[a]Measured according to the method of Hsu et al. (1977).

n.d., not determined.

Table 5. Hemagglutinin activity in raw mature seeds of several Philippine indigenous food and forage legumes.

Sample		RBC Rabbit	Bovine
Food Legumes			
Jack bean	8-6	++	++
	8-3	++	++
	44	++	++
	45	++	++
Sword bean	8-4	++	++
	8-9	++	++
Sabawel	6-1	+	+
	6-2	+	+
Batao 5-7		+	+
Wild lima		+	+
V. umbellata 28		-	-
Sam-samping		-	-
Forage Legumes			
Balatong Aso		-	-
Centrosema		++	++
Forpogen		++	++
Crotolaria		++	++
Phaseolus lethyroides		-	-
Calopogonium		-	-
Madre Cacao		+	+

Legend:

+ - mild/small degree of agglutination

++ - large amounts of agglutinated RBC - clearly visible as clumps.

- negative reaction

Table 6. Proximate analysis of raw mature seeds of several Philippine forage legumes.

Sample	Moisture (%)	Fat (%)	Protein (% Nx6.25)	Ash (%)
Aeschynomene (1)	9.1	11.9	24.8	3.6
Centrosema (3)	4.8-6.3	5.4- 7.2	18.5-22.7	2.4-2.9
Colopogonium (2)	4.5-4.7	10.7-12.5	30.4-33.3	3.9-4.3
Crotolaria (3)	4.6-6.4	6.6- 7.8	28.1-30.8	3.3-3.7
P. lathyroides (2)	6.5-8.7	5.7- 6.3	22.5-23.2	3.3-3.4
C. occidentalis (3)	5.9-6.5	2.0- 2.9	17.9-21.4	3.8-3.9

Table 7. Total HCN potential of some Philippine indigenous legumes.

Sample	HCN[a], mg/kg			
Trials	A	B	C	D
Batao, dry seeds	15	42		33
green pods	13	25	0.6	
green seeds	0	18	32	
Subawel, dry seeds	24	0		44
green seeds	20	6	13	
green pods	19	44	23	
Sword bean, green pods	32	0.6	12	
green seeds	21	0	0	
dry seeds	22			
Tapilan				23
Jack bean, dry seeds				46
Sam-samping				20
Balatong Aso				33

[a]Analyzed according to the method of Reay and Conn (1970) and modified for this experiment.

In trials A-D, different enzyme preparations from the samples tested were used.

Table 8. Partial results of tests for the presence of alkaloids in several Philippine indigenous food and forage legumes.

Sample	Immature leaves					Mature leaves				
	V	M	D	W	H	V	M	D	W	H
Forage Legumes										
Balatong Aso A_7	-	-	-	-	-	-	-	-	-	-
Balatong Aso A_{24}	-	-	-	-	-	-	-	-	-	-
Balatong Aso BP	-	-	-	-	-	-	-	-	-	-
Centrosema JS-1	+3	+2	+2	+3	+1	+3	+1	+1	+3	+1
Colopogonium JS-1	+2	+1	+1	+2	+1	+2	+2	+1	+3	+1
Crotolaria	-	-	-	-	-	+3	+2	+2	+3	+1
P. Lathyroides IPB	-	-	-	-	-	+1	-	-	+1	-
Food Legumes										
Batao 5-2						-	-	-	-	-
Batao 5-3						-	-	-	-	-
Batao 5-7						-	-	-	-	-
Batao 5-14						-	-	-	-	-
Cajanus cajan						+1	+1	+1	+2	-
Jack bean 8-8						+2	+2	+2	+3	+2
Sam-samping 7-2						-	-	-	-	-
Swordbean 8-4						+2	+1	+1	+2	+1
Swordbean 8-6						+3	+2	+2	+3	+1
Swordbean 8-9						+2	+2	+2	+2	+1

Legend: - not turbid; +1 turbid; +2 highly turbid; +3 with precipitation Turbidity and precipitation indicate presence of alkaloids. V-Valser; M-Mayer; D- Dragendorff; W-Wagner; H-Hager; these are reagents for testing for alkaloids. Reference: Hultin and Torsell (1965).

Table 9. Summary of accomplishment in the analyses in food and forage legumes.

Analyses	Sample Parts[a]	Food Legumes	Forage Legumes
1. Trypsin Inhibitor activity	mature seeds leaves	95% 30%	90% 0
2. Tannin content (vanillin and protein precipitation method)	mature seeds;	95%	
3. Hemagglutinins	mature seeds	90%	0
Oligosaccharides	mature seeds	10%	0
4. Cyanide	mature seeds; leaves pods	90% 80% 50%	
5. Saponins	mature seeds pods leaves	0	0
6. General test for alkaloids	mature seeds pods leaves	30% 20% 30%	50% 30% 30%
7. Phytic acid	mature seeds pods leaves	10%	0
8. a) *In vitro* protein digestibility	mature seeds leaves	95% 75%	0 0
b) *In vitro* rumen digestibility		0	0
9. relative nutritive	mature seed	95%	0
10. amino acid composition	mature seeds	5%	0
11. proximate composition	mature seeds leaves pods	95%	95%

[a]The concentration of the different anti-nutritional factors will be determined in various plant parts.