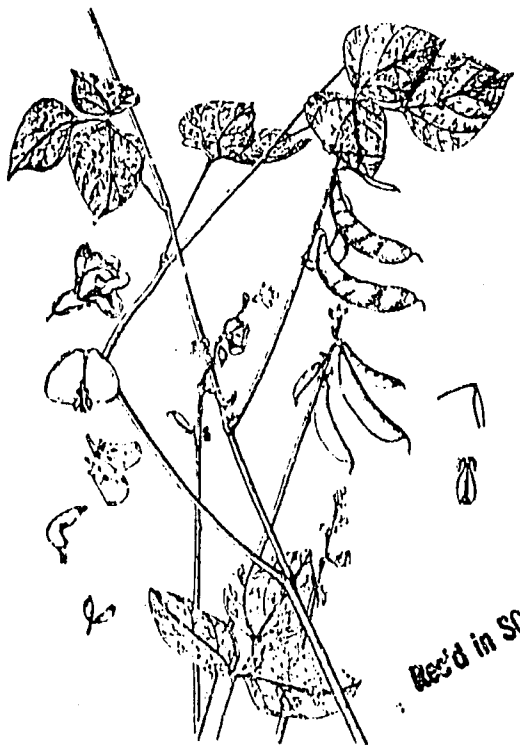
# BIOCHEMICAL AND NUTRITIONAL STUDIES OF PHILIPPINE INDIGENOUS FOOD AND FORAGE LEGUMES

(USAID Grant No. 936-5542; AID/SCI 2N-04)

## Terminal Report





Submitted by

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College, Laguna, Philippines

Terminal Report

The papers in this report will be submitted for publication and may still need some revision. Please refer to/ cite published articles.

# BIOCHEMICAL AND NUTRITIONAL STUDIES OF PHILIPPINE INDIGENOUS FOOD AND FORAGE LEGUMES

USAID Grant No. 936-5542-G-00-1374-00
(AID/SCI 2N-04)

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Program in Science and Technology Cooperation

Project Leader:
**Evelyn Mae T. Mendoza**

August 1989

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## TABLE CONTENTS


| | | Page |
|---|---|---|
| | ACKNOWLEDGEMENT | i |
| | TECHNICAL STAFF | iv |
| I. | Introduction | 1 |
| II. | Proximate Chemical Composition of Several Philippine Indigenous Food Legumes | 21 |
| III. | Amino Acid Composition, Relative Nutritive Values and In vitro Protein Digestibility of Several Philippine Indigenous Food Legumes | 31 |
| IV. | Polyphenols, Phytate, Cyanogenic Glucosides and Trypsin Inhibitor Activity of Several Philippine Indigenous Food Legumes | 45 |
| V. | Oligosaccharides in Several Philippine Indigenous Food Legumes: Determination, Localization and Removal | 61 |
| VI. | Changes in Oligosaccharides, Soluble Sugars and Hydrolyzable Polysaccharides in Developing Seeds of Batao (*Dolichos lablab*) and Sabawel (*Mucuna curanii*) | 81 |
| VII. | Determination of Saponins in Philippine Indigenous Food Legumes | 91 |
| VIII. | Alkaloids of Several Philippine Indigenous Food Legumes: Determination and Removal | 99 |
| IX. | Lectins in Philippine Indigenous Fodod Legumes. I. Screening | 109 |
| X. | Lectins in Philippine Indigenous Food Legumes. II. Sword bean Lectins: Removal and/or Inactivation | 117 |
| XI. | Isolation and Characterization of Soluble Proteins from Rice Bean [*Vigna umbellata* (Thumb. Ohwi Ohashi)] | 131 |
| XII. | Characterization of Seed Proteins from Sabawel (*Mucuna curanii*) and Sam-samping (*Clitoria ternatea*) | 155 |
| XIII. | Isolation, Fractionation and Characterization of Seed Proteins from Sword bean (*Canavalia gladiata*) and Jack bean (*Canavalia ensiformis*) | 167 |

XIV. Proximate Analyses and In Vitro Dry Matter Digestibility of Philippine Indigenous Forage Legumes 187

XV. Saponins, Phytate, Polyphenols, Oligosaccharides, Lictisns and Cyanogenic Glycosides in Philippine Indigenous Forage Legumes 202

XVI. Alkaloids in Several Philippine Indigenous Forage Legumes: Determination and Removal 215

XVII. Amino Acid Composition, In Vitro Protein Digestibility and Relative Nutritive Values of Seeds of Philippine Indigenous Forage Legumes 222

XVIII. Survey on Uses of Seven Philippine Indigenous Legumes: (A Preliminary Report) 231

Summaries and Highlights of Research Accomplishments 243

Possible Research Projects Arising from Philippine Indigenous Legumes Project 255

Comments and Constraints/Problems on Project Implementation 255

UNIVERSITY OF THE PHILIPPINES AT LOS BAÑOS
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INSTITUTE OF PLANT BREEDING

August 24, 1989

US Agency for International Development
Program in Science and Technology Cooperation
Washington DC 20523

Through Mr. Kevin A. Rushing
Agricultural Development Officer
ORAD
USAID, Manila

Ref: Philippine Indigenous Legumes Project
Grant No. 936-5542-G-00-1347-00 (AID/SCI 2N-04)

Dear Mr. Rushing:

I am very pleased to submit to USAID ten (10) copies of the terminal report for our above referenced AID/SCI project.

I thank USAID for the grant that enabled us to work on this project. Many of the papers contained in this report are ready for submission for publication while a few need refinement and, perhaps, a few more data. We hope that the results of this work will be helpful to plant breeders, food scientists, nutritionists, other researchers, students and others, and will aid in the further utilization of these indigenous legumes which constitute a major potential source of food for many.

Instead of an executive summary, we have compiled the abstracts of the 17 papers at the end of the report. We have also included a list of constraints and problems that we encountered during the implementation of the project.

In addition, since documentation on the utilization of the legumes under study is nil, we conducted a survey on their food and nonfood uses. This is part XVIII of this report.

We have prepared a rather detailed report in the form of journal papers so that the results of the research may be better and clearly understood and utilized.

Again, our sincerest thanks.

Truly yours,

EVELYN MAE T. MENDOZA, PhD
Project Leader
Researcher (Biochemist)

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Direcor of Research

## ACKNOWLEDGEMENT

This project "Biochemical and Nutritional Studies of Philippine Indigenous Food and Forage Legumes" started when a young researcher from the Vegetable Crops Division, Mrs. Sheilachu Paje-Gomez, excitedly asked us to analyze the chemical composition of her samples of several indigenous legumes in 1981. We were also asked that a similar study be done on indigenous forage legumes by Mr. Reynaldo C. Mendoza, then of the Forage and Pastures Section of IPB. A year later, our energetic Dr. Ruben L. Villareal, at that time the deputy director of IPB, asked us to submit a pre-proposal for submission to USAID in response to an invitation made by some visiting USAID officials. By June 1983, approval of the project had been obtained. Being the first project under the USAID Program in Science and Technology Cooperation (PSTC) at UPLB, the project went through rough sailing before the grant's contract was approved in December 1983. The project officially started on January 1, 1984.

This report on "The Biochemical and Nutritional Studies of Philippine Indigenous Food and Forage Legumes" is a culmination of a 5-year study by the team efforts of the researchers and support staff of the Biochemistry and Analytical Services Laboratories of the Institute of Plant Breeding (IPB). In addition, researchers from other units within and outside IPB contributed their expertise to the conduct of this study.

I am pleased to record herein our team's gratitude to the US Agency for International Development (USAID) Program in Science and Technology Cooperation (PSTC) for this grant,

and the following for their help and support in many different ways to our project:

Mrs. Sheilachu Paje-Gomez and Mr. Reynaldo C. Mendoza--they gave us the initial idea and samples for this project;

Dr. Ruben L. Villareal, former deputy director and Director of IPB, now Dean of the College of Agriculture, UPLB--His infectious enthusiasm catalyzed the making of this project;

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Ms. Abel de la Viña and Dr. Liwayway M. Engle of the Forage and Pastures Section--They generously shared their samples and expertise;

Mr. Freddie Fajardo, office of the Director of Research-- He relentlessly worked on the signing of the project's memo of agreement from July to December 1983;

Dr. Camilo Opeña, Executive Director, UPLB Foundation Inc., and his staff, for the efficient administration of the project funds.

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Dr. Edward J. Rice--His favorable evaluation and endorsement of the project proposal helped in securing the grant;

Mr. Lawrence J. Ervin, Energy Advisor and first project adviser--His encouragement and support got us through the difficult initial phase of the project;

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Ms. Baby Juico, Dr. Baby Silva, Ms. Fely Juanillo and Ms. Precy Rubio--for their kindness and their efficient handling of the project needs;

Mr. Robert Doucette and Mr. Robert B. Stader--for their kind help in the initial phase of the project.

Other USAID Staff--for the efficient administration of the grant on the USAID side.

Lastly to

Dr. Emil Q. Javier--His vision keeps us moving.

EVELYN MAE T. MENDOZA, PhD
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August 1989

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National Plant Genetic Resources Laboratory (NPGRL), IPB
Mr. Nestor C. Altoveros and Dr. Noel G. Mamicpic

Vegetable Crops Section, now Division, IPB
Mrs. Sheilachu Paje (1981); Dr. Eufemio T. Rasco, Jr.

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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 (National Acad. of Sci., 1979; Kay, 1979; National Acad. of Sci., 1975). The publication "Tropical Legumes: Resources for the Future" very ably delineated the potentials and problems of these relatively underutilized plants.

Although the protein contents of legume seeds are usually high, from 20 to 40%, and higher for a few types, 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, tannins and others. Other toxins may occur in the leaves, pods, and seeds of these legumes like cyanogenic glucosides (Conn, 1979), saponins (Applebaum et al, 1970), alkaloids, nonprotein amino acids like mimosine in Leucaena leucocephala. However, these toxins may have a protective role in the plant against pests and diseases (Applebaum et al, 1970; Mendoza and Diag, 1982; Prasad and Weigle, 1976; Nienstaedt, 1953). Many tropical legumes described in the aforementioned books were observed/reported to be relatively resistant to pests and diseases. The role of the toxins in tropical legumes resistance to pests and diseases is not yet well established.

In the early 1980's, the Vegetable Crops Section of the Institute of Paint Breeding began the collection and study of indigenous tropical vegetables including legumes. In cooperation

with this group, we initiated the analysis of the legumes (both mature seeds and green pods) for proximate chemical composition and some anti-nutritional factors (IPB Annual Report 198:).

The Forage and Pasture Section also started the collection of indigenous forage legumes. However, no biochemical evaluation of these forage legumes was done due to lack of financial support.

This study therefore was undertaken, under the USAID Program for Science and Technology Cooperation, to establish the nutritional quality of the seeds, leaves and pods of food and forage legumes indigenous to the Philippines and undertake relevant biochemical studies. The food legumes are: pigeon pea (Cajanus cajan), lake bean (Canavalia ensiformis), sword bean (Canavalia gladiata) sam-samping (Clitoria ternatea), patac (Dolichos lablab), sabawel (Mucuna pruriens or M. cochinchinensis), lima bean (Phaseolus lunatus) and rice bean (Vigna umbellata). The forage legumes include: joint vetch (Aeschynomene), Calopo or Brisolilla (Calopogonium), Centrosema, Crotolaria, Desmodium, Phaseolus lunatus and Pueraria. These legumes and their sources are listed in Table 1 and their photographs shown in Fig. 1.

This research aimed to accomplish its goals through the following studies:

Study 1. Determination of proximate chemical composition, amino acid composition, and levels of trypsin inhibitors, hemagglutinins, flatulence factors, phytates, and tannins in the seeds, leaves and pods of different indigenous legumes at various stages of growth.

Study 2. Investigation of toxic substances such as cyanogenic glucosides, alkaloids, saponins, etc. in different parts and different stages of growth. Simple ways of possible detoxification like heat treatment (roasting, boiling), leaching, and ash/bicarbonate treatment were investigated.

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

The results of this research are expected to aid our plant breeding program in expanding the genetic resources for improvement of pest- and disease-resistance, nutritional quality, yield and tolerance to stresses of our traditional legumes.

These legumes also contribute a major potential source of food and livelihood for our people. Thus, these studies are expected to provide a sound basis for the utilization of these relatively unknown and underutilized legume species.

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PRASAD, K. and WEIGLE, J.L. 1976. Association of the seed coat factors with the resistance to Rhizoctonia solani in Phaseolus vulgaris. Phytopath. 66: 342.

Table 1. List of indigenous legumes and their sources.

| Name | Sources |
|---|---|
| Pigeon pea | |
| (Cajanus cajan) | |
| BP-8 | |
| Jack bean | |
| (Canavalia ensiformis) | |
| A-1 | Sual, Pangasinan |
| A-3 | Daraga, Albay |
| A-5 | Mulanay, Quezon |
| A-8 | |
| B-8 | |
| Sword bean | |
| (Canavalia gladiata) | |
| A-3 | Daraga, Albay |
| A-4 | Romblon |
| A-9 | Mulanay, Quezon |
| A-12 | Tacloban, Leyte |
| Sam-samping | |
| (Clitoria ternatea) | |
| 7-2 | IPB, UPLB |
| Batao | |
| (Dolichos lablab) | |
| A-37 | Pagudpod, Ilocos Norte |
| A-45 | Banaue, Ifugao |
| A-51 | Lezo, Aklan |
| A-52 | Baybay, Leyte |
| Sabawel | |
| (Mucuna pruriens or cochinchinensis) | |
| A-2 | Cagayan Valley |
| A-5 | Cagayan Valley |
| A-8 | Cagayan Valley |
| Lima bean | |
| (Phaseolus lunatus) | |
| A-513 | Narra, Palawan |
| A-517 | |
| A-508 | Tabuk, K. Apayao |
| A-525 | Buenavista, Guimaras |
| A-535 | Panitan, Capiz |
| A-537 | Tinigao, Aklan |
| A-541 | Alcala, Romblon |
| A-544 | Mabo, Masbate |
| A-546 | |

| Name | Sources |
|---|---|
| Rice bean (*Vigna umbellata*) | |
| 28 | |
| 46 | |
| 26 | |
| **Forage Legumes** | |
| Joint vetch (Aeschynomene) | |
| IPB-1 | Bo. Masaya, Los Banos, Lag. |
| Calopo, Frisolilla (Calopogonium) | |
| BM-1 | Bo. Masaya, Los Banos, Lag. |
| JS-1 | Jamboree Site, Los Banos |
| Balatong aso (*Cassia occidentalis*) | |
| BP-1 | Bo. Putho, Los Banos |
| Centrosema | |
| BM-1 | Bo. Masaya, Los Banos |
| BPI-T1 | BPI, Tiaong, Quezon |
| JS-1 | Jamboree Site, Los Banos Laguna |
| Crotolaria | |
| BM-1 | Bo. Masaya, Los Banos, Laguna |
| PC-1 NB | Bo. Palisa/Cabay, Tiaong Quezon |
| PC-1 WB | Bo. Palisa/Cabay, Tiaong Quezon |
| Desmodium | |
| DQ-1 | Dolores, Quezon |
| Acc 93 | |
| Acc 195 | |
| *Phaseolus lunatus* | |
| A-535 | Panitan, Quezon |
| A-537 | Bo. Putho, Los Banos, Laguna |
| A-544 | Jamboree Site, Los Banos, Lag. |
| Pueraria | |
| DQ-1 | Dolores, Quezon |
| BP-1 | Bo. Putho, Los Banos, Laguna |
| JS-1 | Jamboree Site, Los Banos Laguna |

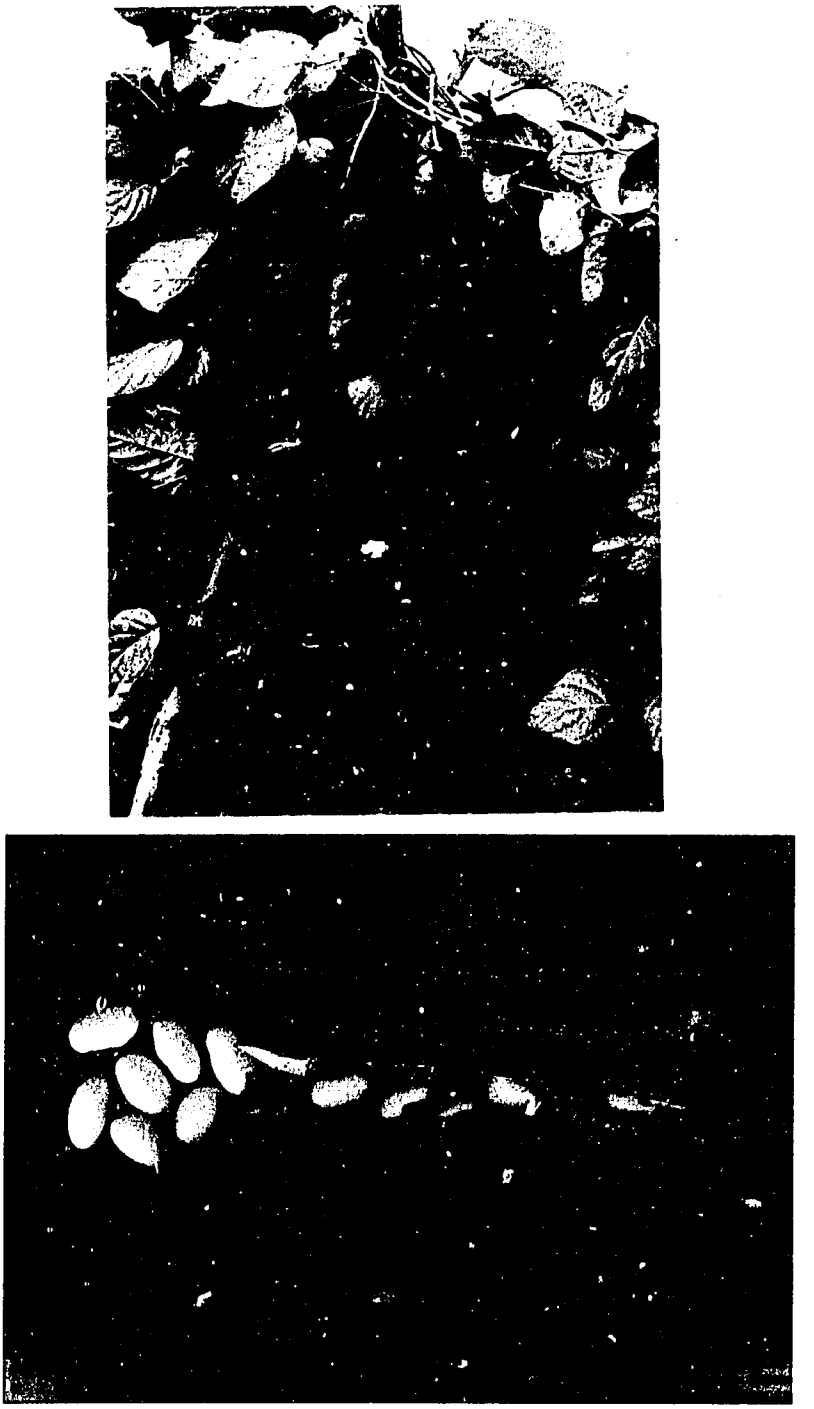

Fig. 1a. Jack bean (Canavalia ensiformis)

Fig. 1b. Sword bean (Canavalia gladiata)

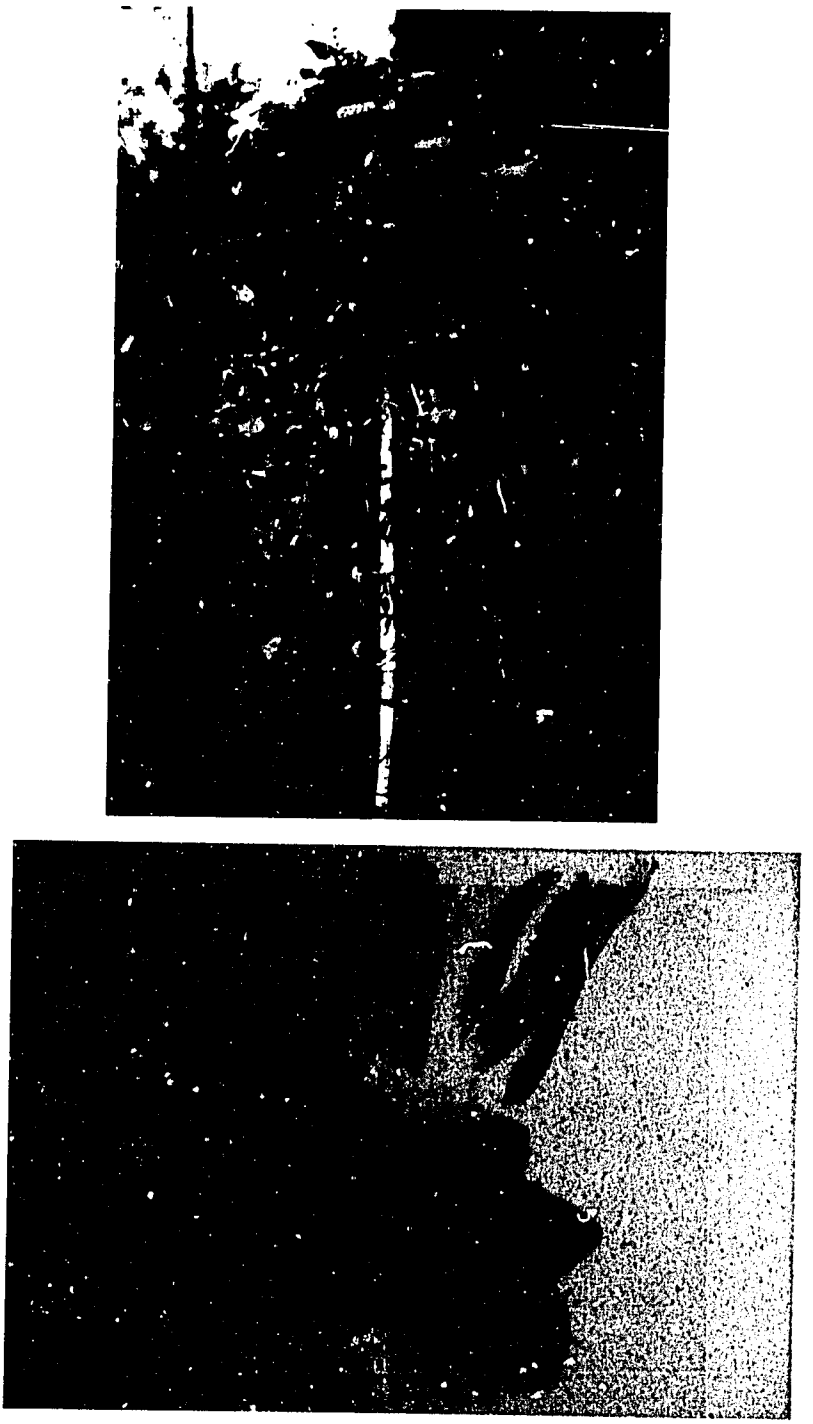

Fig. 1c. Sam-samping (Clitoria ternatea)

Fig. 1d. Batao (Dolichos lablab)

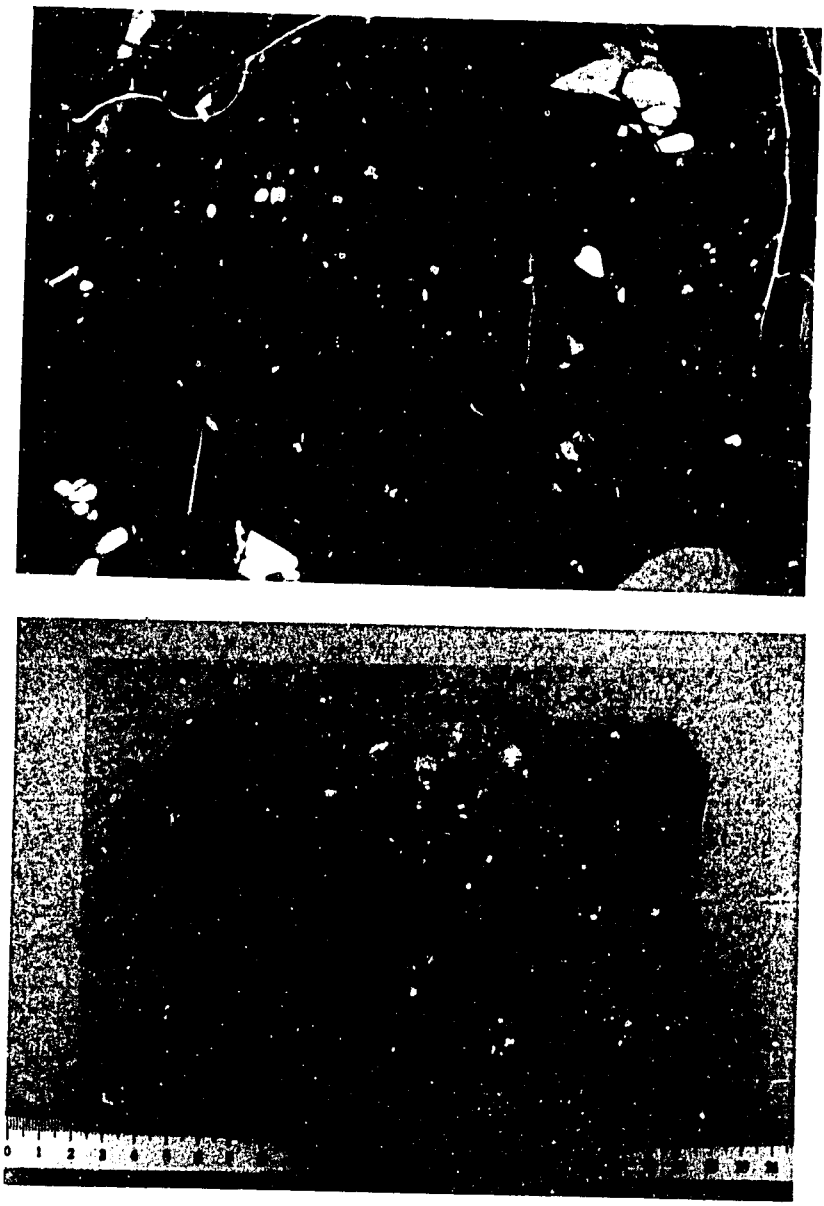

Fig. 1e. Sabawel (Mucuna pruriens or cochinchinensis)

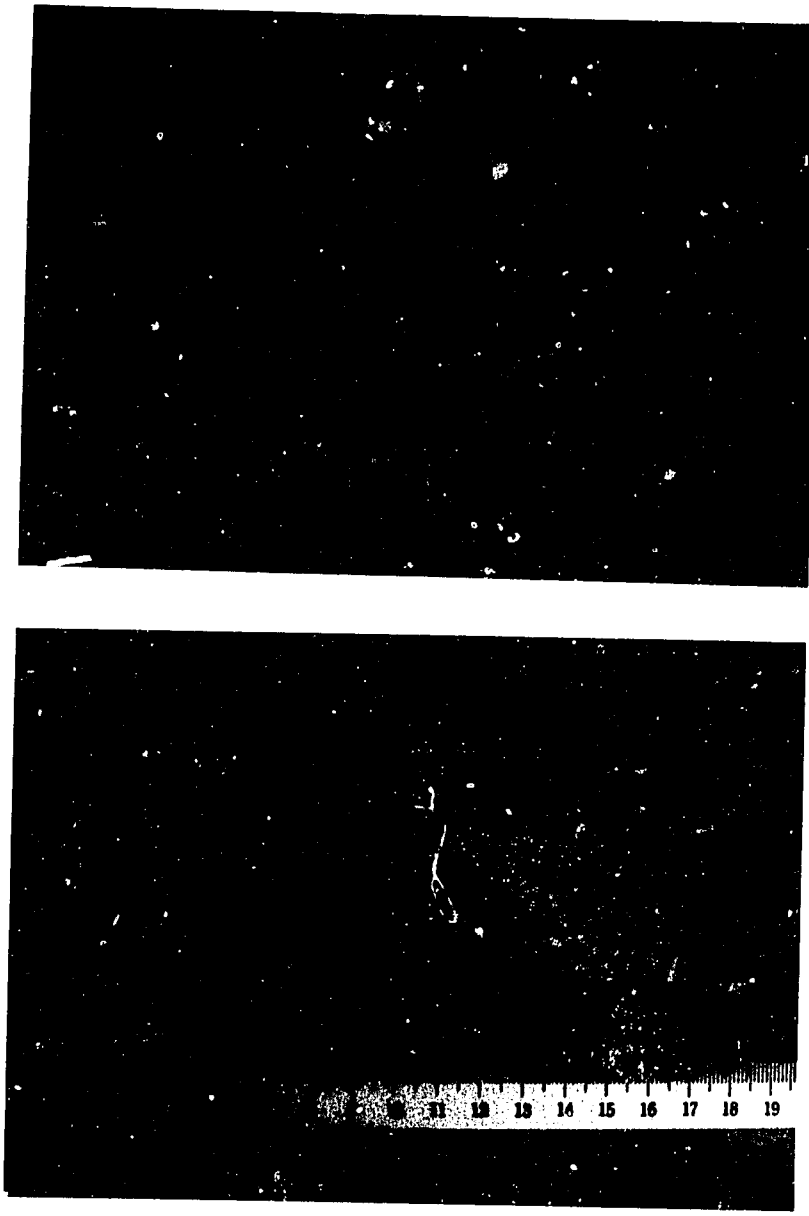


Fig. 1f. Lima bean (Phaseolus lunatus)

Fig. 1g. Rice bean (Vigna umbellata)

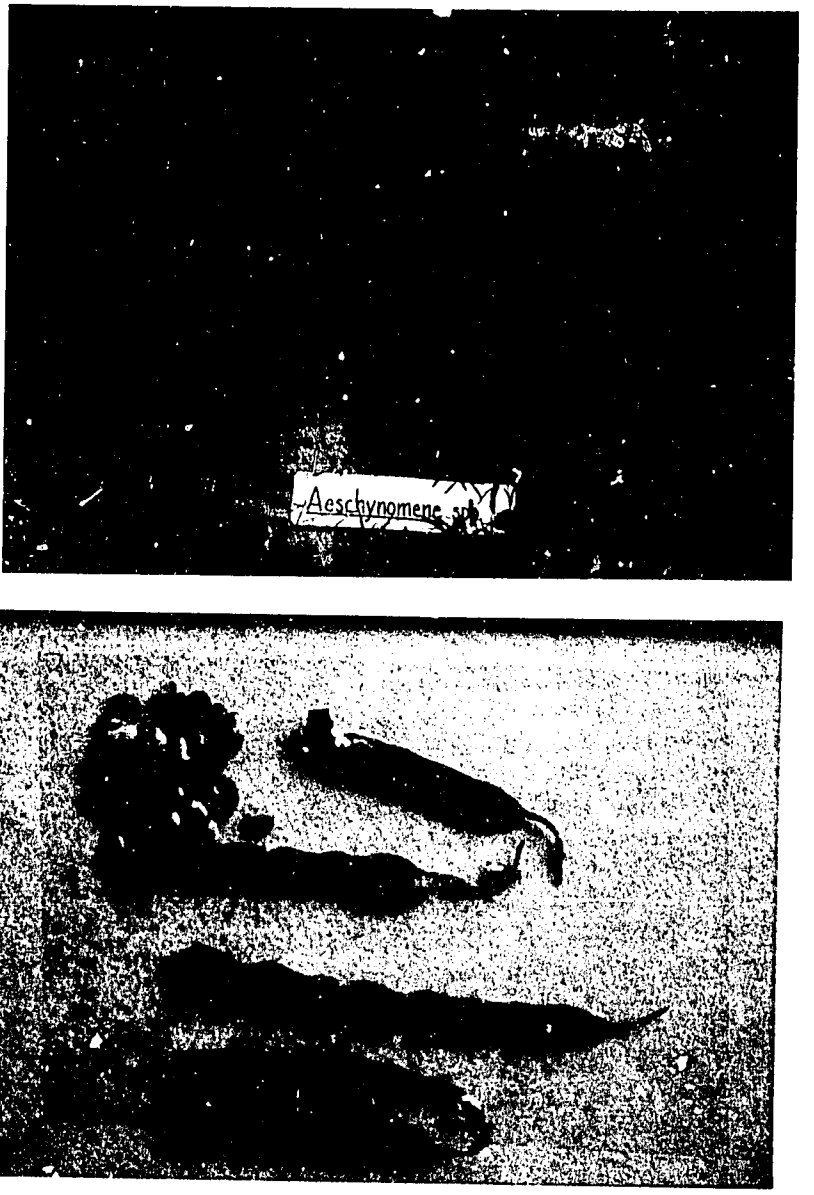


Fig. 1h. Joint vetch (Aeschynomene)

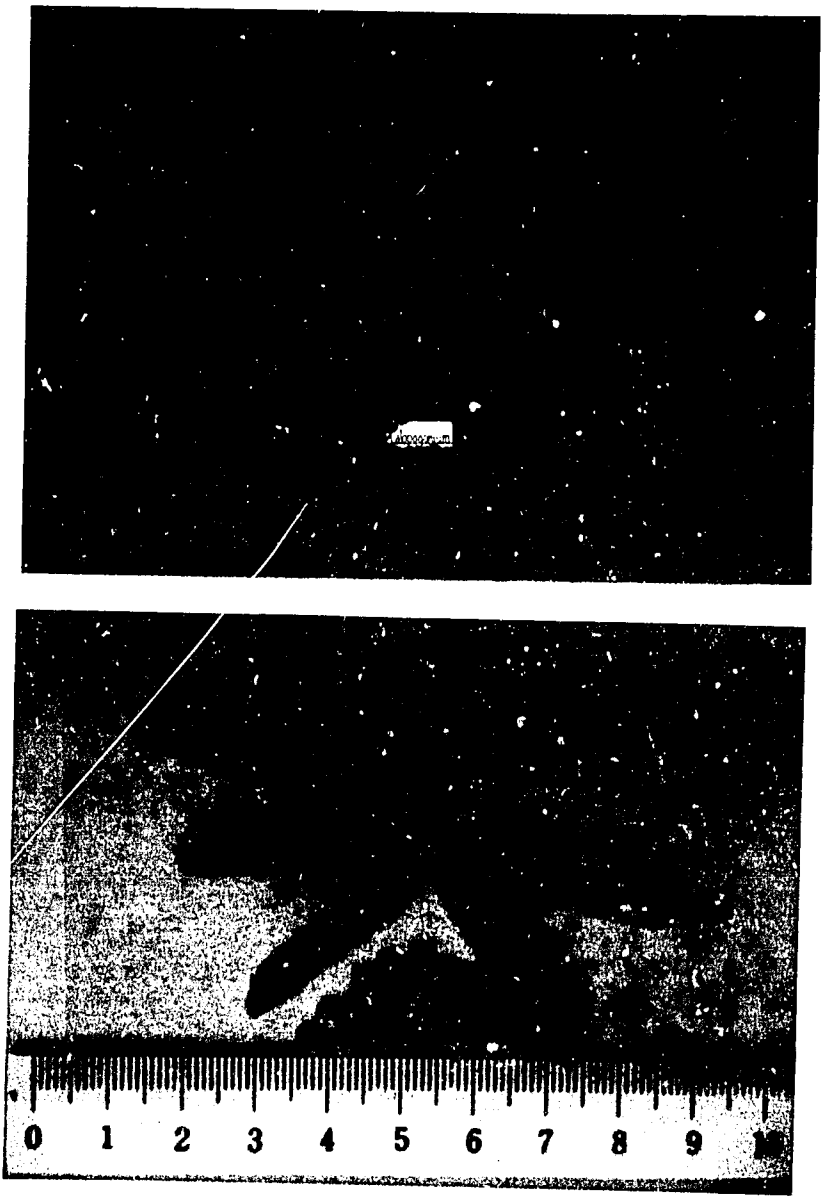


Fig. 11. Calopo, Frisolilla (Calopogonium)

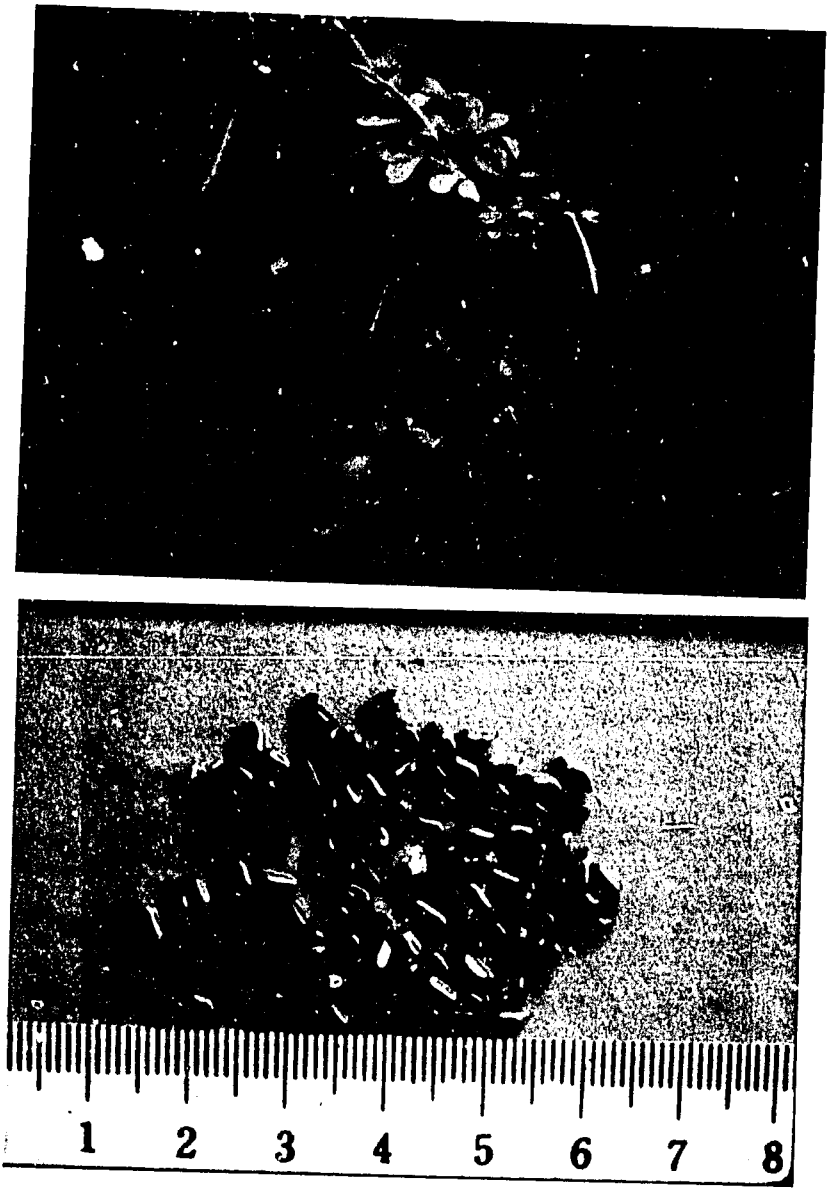


Fig. 1j. Balatong Aso (Cassia occidentalis)

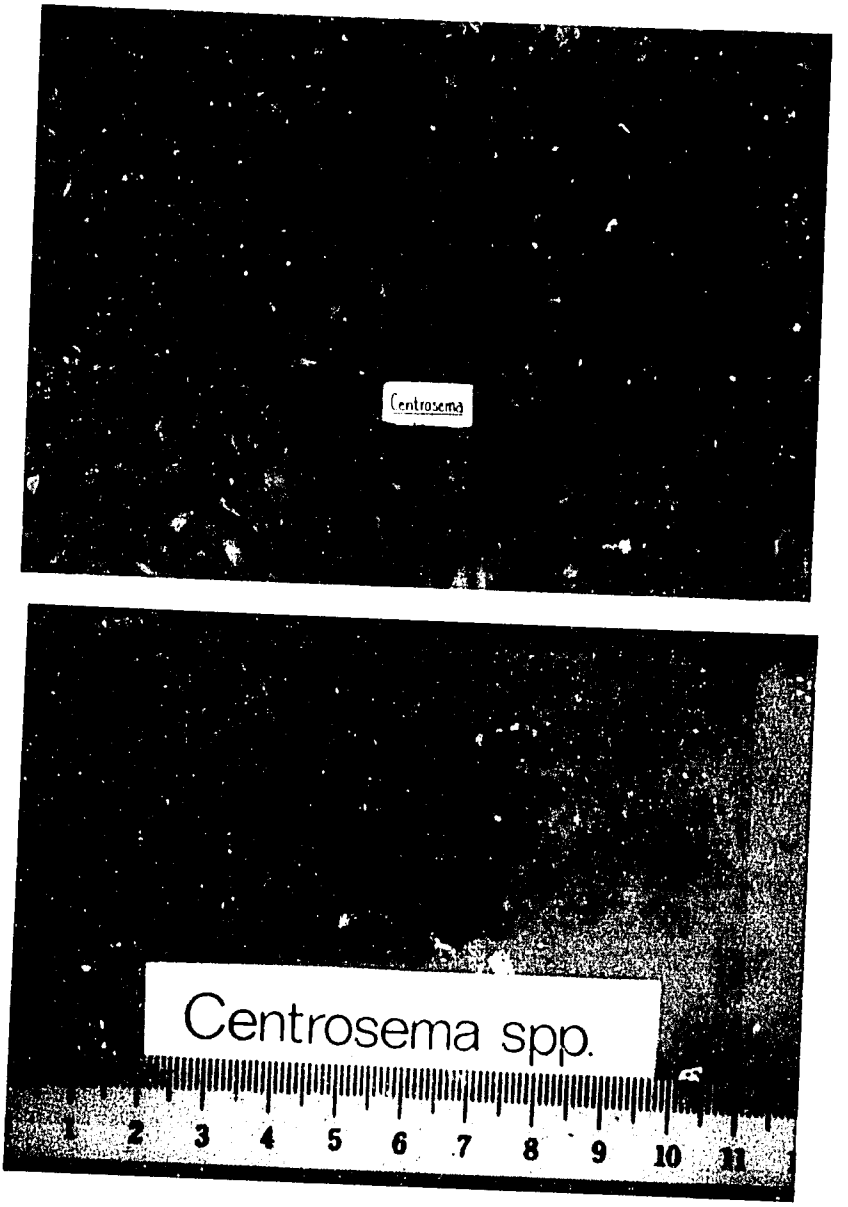


Fig. 1k. Centrosema

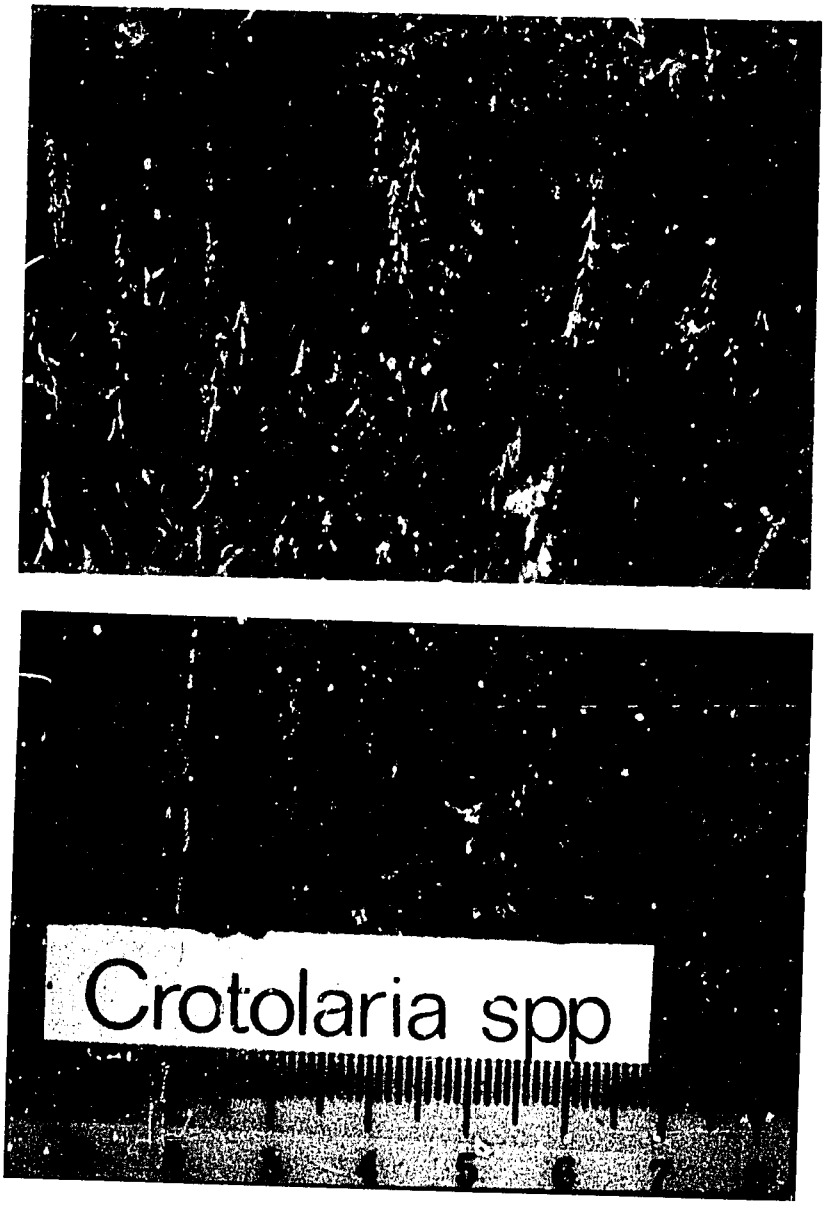


Fig. 11. Crotolaria

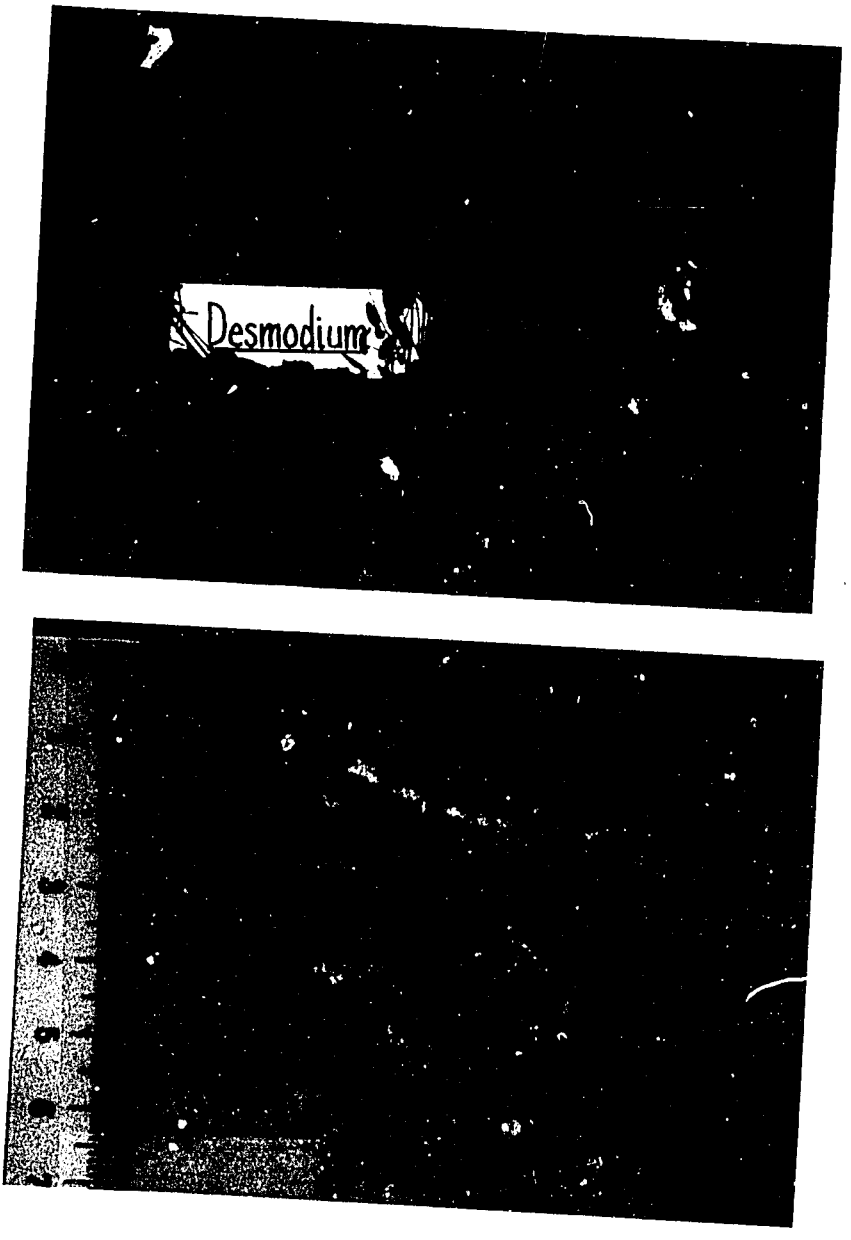


Fig. 1m. Desmodium

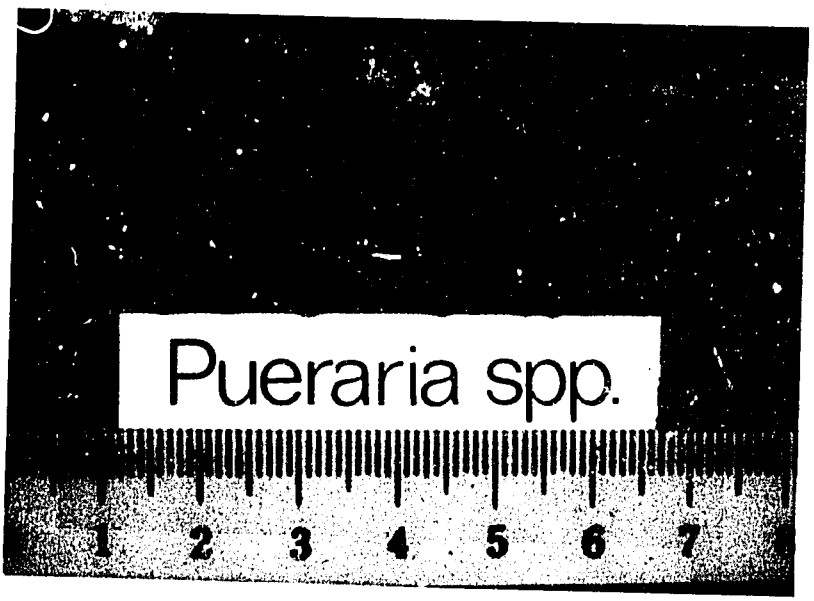


Fig. 1n. Pueraria

II

# Proximate Chemical Composition of Several Phillipine Indigenous Food Legumes

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## Abstract

Protein (18 to 30%) and carbohydrates (50 to 60%) are the major constituents of the mature seeds of 33 samples comprising seven legume species. Vigna umbellata had the lowest protein content of 17.42 to 17.56% while Canavalia ensiformis, Canavalia gladiata, Mucuna pruriens or M. cochinchinensis and Clitoria ternatea had similar protein contents of 29 to 30%. Fat content ranged for 1.2 to 3.7% Dolichos lablab, Phaseolus lunatus and Canavalia gladiata had the largest (5-6%) difference in protein and carbohydrate contents.

The proximate compositions of the immature and mature leaves and pods were also obtained: Moisture (70-90%), carbohydrates (15-18%) proteins (3-10%), fibers (2%), fat (2-4%) ash (0.5-4%).

## Introduction

Legumes are major sources of proteins and nutrients for people of the developing countries. In the Philippines and other countries in South East Asia, only 4 legumes, namely, mungbean, peanut, soybean and to a lesser extent, cowpea, are considered of economic significance. However, there are several more legume species which are up to now underutilized and could be potential food sources. As with the more popular legumes, the various parts of the legume plant can be utilized for food, such as the young immature leaves and pods, as well as the mature seeds. The seeds can also be germinated to prepare sprouts for vegetable dishes. Understandably, the utilization of these legumes is limited by the lack of knowledge about them.

This study deals with the chemical composition of the different edible parts of seven legume species, namely, batao or hyacinth bean (*Dolichos lablab*), pigeon pea (*Cajanus cajan*), lima bean (*Phaseolus lunatus*), jack bean (*Canavalia ensiformis*), sword bean (*Canavalia gladiata*), sabawel (*Mucuna pruriens* or *M. cochinchinensis*), sam-samping (*Clitoria ternatea*) and rice bean or tapilan (*Vigna umbellata*).

## Materials and Methods

Legume samples. Seeds of the aforementioned seven legume species were obtained from the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding (IPB) and were grown in the IPB experimental field. Different parts

of the plants were obtained and used in the study.

Chemical analysis. Seeds, mature and immature leaves, mature and immature pods were dried in a 110 C draft oven overnight and ground to pass through 60 mesh. Proximate analyses were performed according to official AOAC procedures (1980).

## Results and Discussion

The proximate chemical composition of different parts and accessions of the seven indigenous legumes studied is given in Table 1. It represents the most comprehensive study of such kind. Compilation of data for some of these legumes can be found in several publications (Duke, 1981, FNRI, 1980).

Mature seeds. Protein (18 to 30%) and carbohydrates (50 to 60%) are the major constituents of the matur. seeds of the 33 samples comprising seven legume species. U.umbellata had the lowest protein content of 17.42 to 17.56% while C.ensiformis, C. gladiata, M. pruriens or corbinchinensis and C.ternatea had similar protein content of 28 to 30%. Fat content ranged from 1.2 to 3.7%. These values are comparable to those for mung bean and cowpea and other "starchy" legume grains in contrast to the oil type such as soybean, peanut and winged bean.

D.lablab, P. lunatus, and C.gladiata had the largest (5-6%) difference in protein and carbohydrate contents among the accessions studied. The other constituents had small variations.

Immature and mature leaves. The protein content of immature leaves ranged from 3.4 - 6.5% for the seven species with lima bean and pigeon pea having the lowest and highest levels,

respectively (Table 1). Pigeon pea immature leaves also had the highest fat (4.22%), fibers (2.44%) and carbohydrates (15.51%). Fat content (1.98 - 3.15%) of the immature leaves was similar to the seed content. Moisture ranged from 70 - 83%.

The mature leaves had 60 - 81% moisture, Protein and carbohydrate fibers and ash contents were higher by 10% and 20% respectively in the mature leaves than in immature.

Immature and mature pods. Moisture (79 to 90%) and carbohydrates (9-18%) are the major constituents of pods. Protein contents ranged from 1.81 - 3.89% with sword bean and sabawel having the lowest and highest levels, respectively. Fiber and ash contents ranged from 0.88 - 2.10%, and 0.68-1.40%, respectively.

Moisture decreased in the mature pods (69.13 - 83.50%) Table 5). In general, there was an increase in the other constituents during maturation.

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**Table 1. Proximate composition of different parts of several Philippine indigenous food legumes.**

| Variety | Moisture (%) | Fats (%) | % Protein (% N x 6.25) | Ash (%) | Fibers (%) | NFE (%) |
|---|---|---|---|---|---|---|
| **Batao** (*Dolichos lablab*) | | | | | | |
| A-37 | | | | | | |
| immature leaves | 81.86 | 3.65 | 4.22 | 1.36 | 1.39 | 7.52 |
| mature leaves | 64.76 | 4.51 | 8.36 | 3.65 | 3.24 | 15.57 |
| immature pods | 89.26 | 0.85 | 2.37 | 0.68 | 1.26 | 5.58 |
| mature pods | 83.50 | 1.08 | 2.19 | 1.01 | 1.96 | 10.26 |
| mature seeds | 6.94 | 1.89 | 21.34 | 4.24 | 5.36 | 60.23 |
| A-45 | | | | | | |
| immature leaves | 82.10 | 2.18 | 4.23 | 1.07 | 1.27 | 7.62 |
| mature leaves | 65.37 | 4.62 | 8.42 | 3.87 | 3.56 | 14.16 |
| immature pods | 86.04 | 1.00 | 3.45 | 0.86 | 1.46 | 7.19 |
| mature pods | 80.10 | 1.27 | 2.69 | 1.11 | 2.26 | 12.57 |
| mature seeds | 6.78 | 1.70 | 21.46 | 4.05 | 4.80 | 61.21 |
| A-51 | | | | | | |
| immature leaves | 80.78 | 2.74 | 4.36 | 1.25 | 1.40 | 9.47 |
| mature leaves | 62.36 | 3.88 | 8.12 | 3.25 | 3.18 | 19.21 |
| immature pods | 87.65 | 0.92 | 3.11 | 0.78 | 1.41 | 6.13 |
| mature pods | 80.74 | 1.53 | 2.59 | 1.14 | 2.04 | 11.97 |
| mature seeds | 7.56 | 1.95 | 18.52 | 4.13 | 5.05 | 62.79 |
| A-52 | | | | | | |
| immature leaves | 81.15 | 2.56 | 4.40 | 1.48 | 1.18 | 9.23 |
| mature leaves | 60.24 | 4.66 | 8.59 | 3.82 | 3.29 | 19.40 |
| immature pods | 88.18 | 0.86 | 2.67 | 0.70 | 1.04 | 6.55 |
| mature pods | 84.21 | 1.15 | 2.10 | 1.02 | 1.62 | 9.90 |
| mature seeds | 7.40 | 1.98 | 21.12 | 4.05 | 5.05 | 60.40 |
| A-57 | | | | | | |
| immature leaves | 80.56 | 2.18 | 4.04 | 1.86 | 1.55 | 9.81 |
| mature leaves | 57.84 | 4.55 | 9.92 | 4.44 | 3.18 | 20.07 |
| immature pods | 87.42 | 0.98 | 3.08 | 0.81 | 1.30 | 6.41 |
| mature pods | 80.97 | 1.49 | 2.55 | 1.25 | 1.98 | 11.76 |
| mature seeds | 7.10 | 2.06 | 23.24 | 4.20 | 5.50 | 57.90 |
| **Pigeon Pea** (*Cajanus cajan*) | | | | | | |
| SP-3 | | | | | | |
| immature leaves | 69.75 | 4.22 | 6.50 | 1.58 | 2.44 | 15.51 |
| mature leaves | 67.37 | 4.89 | 6.75 | 2.71 | 3.71 | 14.57 |

| Variety | Moisture (%) | Fats (%) | % Protein (% N x 6.25) | Ash (%) | Fibers (%) | NFE (%) |
|---|---|---|---|---|---|---|
| **Lima bean** (Phaseolus lunatus) | | | | | | |
| A-513 | | | | | | |
| immature leaves | 82.23 | 2.48 | 3.66 | 1.62 | 1.04 | 8.97 |
| mature leaves | 79.86 | 3.22 | 3.15 | 1.68 | 1.44 | 10.65 |
| immature pods | 83.87 | 1.24 | 2.42 | 1.10 | 1.39 | 9.98 |
| mature pods | 81.82 | 1.23 | 2.33 | 1.31 | 1.74 | 11.57 |
| mature seeds | 9.29 | 1.57 | 21.38 | 4.49 | 3.70 | 59.57 |
| A-517 | | | | | | |
| immature leaves | 83.36 | 2.55 | 3.74 | 1.44 | 0.98 | 7.99 |
| mature leaves | 79.86 | 3.22 | 3.15 | 1.68 | 1.44 | 10.65 |
| immature pods | 79.31 | 1.46 | 2.87 | 1.36 | 1.90 | 13.10 |
| mature pods | 80.97 | 1.12 | 2.25 | 1.20 | 1.70 | 12.76 |
| mature seeds | 9.26 | 1.44 | 20.24 | 4.56 | 3.62 | 60.72 |
| A-508 | | | | | | |
| mature seeds | 9.71 | 1.46 | 22.45 | 4.36 | 3.42 | 58.60 |
| A-525 | | | | | | |
| immature leaves | 82.51 | 2.12 | 3.43 | 1.71 | 1.12 | 9.11 |
| mature leaves | 80.10 | 3.69 | 3.45 | 1.74 | 1.86 | 9.16 |
| immature pods | 83.10 | 1.37 | 2.15 | 1.40 | 2.10 | 9.88 |
| mature pods | 80.95 | 1.29 | 1.94 | 1.30 | 1.83 | 12.69 |
| mature seeds | 10.06 | 1.26 | 21.39 | 4.78 | 3.54 | 58.97 |
| A-535 | | | | | | |
| immature leaves | 84.39 | 2.97 | 3.38 | 1.62 | 1.16 | 5.99 |
| mature leaves | 80.35 | 3.97 | 3.32 | 1.72 | 1.32 | 9.32 |
| immature pods | | | | | | |
| mature pods | 80.24 | 1.28 | 2.38 | 1.52 | 1.95 | 12.63 |
| mature seeds | 9.47 | 1.32 | 20.35 | 4.73 | 3.47 | 60.66 |
| A-537 | | | | | | |
| immature leaves | 84.21 | 2.70 | 3.33 | 1.42 | 0.95 | 7.39 |
| mature leaves | 81.45 | 3.36 | 4.02 | 1.86 | 1.22 | 8.09 |
| immature pods | 83.85 | 1.18 | 2.56 | 1.25 | 1.94 | 9.22 |
| mature pods | 80.36 | 1.20 | 2.28 | 1.39 | 1.81 | 12.96 |
| mature seeds | 10.20 | 1.25 | 24.65 | 4.54 | 3.20 | 56.16 |
| A-541 | | | | | | |
| imamture leaves | 83.58 | 2.86 | 3.61 | 1.53 | 1.20 | 7.22 |
| mature leaves | 80.24 | 3.48 | 3.74 | 1.28 | 1.61 | 9.65 |
| immature pods | 84.43 | 1.07 | 2.20 | 1.09 | 1.75 | 9.46 |
| mature pods | 75.97 | 1.53 | 2.97 | 1.54 | 2.19 | 15.80 |
| mature seeds | 9.87 | 1.37 | 20.16 | 4.81 | 3.25 | 60.54 |

| Variety | Moisture (%) | Fats (%) | % Protein (% N x 6.25) | Ash (%) | Fibers (%) | NFE (%) |
|---|---|---|---|---|---|---|
| A-544 | | | | | | |
| immature leaves | 84.11 | 2.80 | 4.15 | 1.44 | 1.11 | 6.34 |
| mature leaves | 80.07 | 2.78 | 3.20 | 1.85 | 1.09 | 11.01 |
| immature pods | 84.43 | 1.07 | 2.20 | 1.09 | 1.75 | 9.46 |
| mature 75.55 | 1.56 | 2.84 | 1.63 | 2.26 | 16.16 | |
| mature seeds | 9.54 | 1.18 | 26.12 | 4.61 | 3.45 | 55.10 |
| A-546 | | | | | | |
| immature leaves | 82.47 | 2.39 | 3.82 | 1.32 | 1.07 | 8.93 |
| mature leaves | 79.87 | 3.61 | 3.55 | 1.71 | 1.20 | 10.06 |
| immature pods | 87.77 | 1.36 | 2.31 | 1.31 | 1.65 | 5.60 |
| mature pods | 79.97 | 1.39 | 2.46 | 1.32 | 1.82 | 13.04 |
| mature seeds | 10.11 | 1.19 | 21.14 | 4.37 | 3.28 | 59.87 |
| **Jackbean** (Canavalia ensiformis) | | | | | | |
| A-3 | | | | | | |
| immature leaves | 76.85 | 2.91 | 5.60 | 2.65 | 1.55 | 10.44 |
| mature leaves | 76.75 | 3.12 | 5.74 | 2.75 | 1.65 | 9.99 |
| immature pods | 74.56 | 1.57 | 3.14 | 1.15 | 1.57 | 19.01 |
| mature pods | 71.87 | 1.97 | 2.97 | 1.79 | 3.24 | 18.16 |
| mature seeds | 8.20 | 2.46 | 28.10 | 4.24 | 5.15 | 51.73 |
| A-1 | | | | | | |
| mature seeds | 8.40 | 1.23 | 27.82 | 3.95 | 5.50 | 51.37 |
| A-5 | | | | | | |
| immature leaves | 77.95 | 2.47 | 5.26 | 2.42 | 1.94 | 9.96 |
| mature leaves | 75.45 | 3.02 | 6.19 | 2.85 | 1.95 | 10.54 |
| immature pods | 78.62 | 1.84 | 3.45 | 1.27 | 1.62 | 13.10 |
| mature pods | 70.66 | 1.65 | 3.15 | 1.86 | 3.06 | 19.62 |
| mature seeds | 7.71 | 2.58 | 28.17 | 4.10 | 4.30 | 53.07 |
| A-8 | | | | | | |
| immature leaves | 79.83 | 2.39 | 5.73 | 2.36 | 1.35 | 8.84 |
| mature leaves | 77.80 | 2.75 | 5.77 | 2.33 | 1.58 | 9.77 |
| immature pods | 79.12 | 1.92 | 3.26 | 1.29 | 1.74 | 12.67 |
| mature pods | 74.12 | 1.84 | 3.42 | 1.94 | 2.88 | 15.88 |
| mature seeds | 7.87 | 2.60 | 30.75 | 4.07 | 4.40 | 50.56 |
| S-8 | | | | | | |
| mature seeds | 8.56 | 2.35 | 29.41 | 4.59 | 5.30 | 49.49 |

| Variety | Moisture (%) | Fats (%) | % Protein (% N x 6.25) | Ash (%) | Fibers (%) | NFE (%) |
|---|---|---|---|---|---|---|
| **Sabawel** (*Mucuna pruriens*) | | | | | | |
| A-2 | | | | | | |
| immature leaves | 78.92 | 2.50 | 5.70 | 1.60 | 1.79 | 9.59 |
| mature leaves | 62.05 | 4.01 | 8.96 | 3.58 | 3.68 | 17.82 |
| immature pods | 78.46 | 1.66 | 3.89 | 1.21 | 1.47 | 13.32 |
| mature pods | 72.44 | 1.87 | 5.21 | 1.78 | 3.18 | 15.52 |
| mature seeds | 7.76 | 2.66 | 29.36 | 4.00 | 4.40 | 51.81 |
| A-5 | | | | | | |
| immature leaves | 78.32 | 3.64 | 5.99 | 1.84 | 1.40 | 8.41 |
| mature leaves | 65.85 | 4.75 | 9.21 | 3.28 | 2.90 | 14.01 |
| immature pods | 76.51 | 1.84 | 3.81 | 1.08 | 1.59 | 15.18 |
| mature pods | 70.76 | 2.06 | 4.38 | 1.86 | 3.52 | 17.42 |
| mature seeds | 7.40 | 2.67 | 29.42 | 4.10 | 3.15 | 53.45 |
| A-8 | | | | | | |
| immature leaves | 78.94 | 3.52 | 5.29 | 1.27 | 1.52 | 9.46 |
| mature leaves | 67.36 | 3.90 | 8.14 | 3.71 | 2.28 | 14.61 |
| immature pods | 78.94 | 1.89 | 3.66 | 1.16 | 1.65 | 12.70 |
| mature pods | 73.81 | 1.98 | 4.13 | 1.75 | 3.11 | 15.22 |
| matures seeds | 7.15 | 2.48 | 28.22 | 4.11 | 4.00 | 53.78 |
| **Sam-samping** (*Clitoria ternatea*) | | | | | | |
| 7-2 | | | | | | |
| immature leaves | 80.09 | 1.98 | 6.11 | 1.50 | 1.08 | 9.24 |
| mature leaves | 72.73 | 2.48 | 7.76 | 1.87 | 1.56 | 13.60 |
| immature pods | | | | | | |
| mature pods | | | | | | |
| mature seeds | | | | | | |
| **Sword bean** (*Canavalia gladiata*) | | | | | | |
| A-3 | | | | | | |
| immature leaves | 79.67 | 2.49 | 5.61 | 1.94 | 1.52 | 8.77 |
| mature leaves | 76.58 | 2.56 | 5.40 | 3.02 | 1.80 | 10.64 |
| immature pods | 83.89 | 1.26 | 1.66 | 1.04 | 0.86 | 11.29 |
| mature pods | 69.13 | 2.56 | 3.67 | 1.74 | 3.60 | 19.30 |
| mature seeds | 8.10 | 2.20 | 26.32 | 4.16 | 5.42 | 53.63 |
| A-4 | | | | | | |
| immature leaves | 80.80 | 2.37 | 5.63 | 1.89 | 1.21 | 8.77 |
| mature leaves | 77.83 | 2.73 | 5.85 | 2.94 | 1.72 | 8.34 |
| immature pods | 84.83 | 1.07 | 1.81 | 0.95 | 0.94 | 10.40 |
| mature pods | 69.61 | 2.74 | 4.06 | 1.82 | 3.37 | 18.40 |
| mature seeds | 8.46 | 2.37 | 22.59 | 4.47 | 5.50 | 56.32 |

| Variety | Moisture (%) | Fats (%) | % Protein (% N x 6.25) | Ash (%) | Fibers (%) | NFE (%) |
|---|---|---|---|---|---|---|
| A-9 | | | | | | |
| immature leaves | 79.02 | 2.73 | 4.74 | 2.11 | 1.24 | 10.16 |
| mature leaves | 77.94 | 2.87 | 5.29 | 2.46 | 1.48 | 9.96 |
| immature pods | 84.37 | 1.15 | 1.74 | 0.92 | 0.98 | 10.84 |
| mature pods | 70.35 | 2.08 | 3.12 | 1.64 | 3.39 | 19.42 |
| mature seeds | 9.20 | 2.32 | 26.38 | 4.26 | 6.85 | 51.89 |
| A-12 | | | | | | |
| immature leaves | 78.65 | 2.66 | 5.37 | 2.63 | 1.67 | 9.02 |
| mature leaves | 76.67 | 2.63 | 5.61 | 2.74 | 1.65 | 10.70 |
| immature pods | 84.64 | 1.07 | 1.84 | 0.94 | 0.88 | 10.63 |
| mature pods | 71.82 | 2.47 | 3.31 | 1.64 | 2.92 | 17.84 |
| mature seeds | 8.76 | 2.42 | 28.05 | 4.70 | 6.35 | 49.62 |
| **Rice bean** (*Vigna umbellata*) | | | | | | |
| Tapilan 28 | | | | | | |
| immature leaves | 74.65 | 2.78 | 4.51 | 1.75 | 1.43 | 14.88 |
| mature leaves | 74.12 | 2.46 | 4.77 | 2.35 | 1.84 | 14.46 |
| immature pods | | | | | | |
| mature pods | | | | | | |
| mature seeds | 9.04 | 3.37 | 17.89 | 4.78 | 6.22 | 58.70 |
| 46 | | | | | | |
| immature leaves | 74.92 | 2.61 | 4.73 | 1.44 | 1.52 | 14.78 |
| mature leaves | 75.66 | 2.78 | 4.35 | 2.63 | 1.57 | 13.01 |
| immature pods | | | | | | |
| mature pods | | | | | | |
| mature seeds | 9.31 | 3.66 | 17.56 | 4.31 | 5.87 | 59.29 |
| 25 | | | | | | |
| mature seeds | 8.94 | 3.54 | 17.42 | 4.55 | 6.90 | 58.65 |

NFE, nitrogen-free extract

# AMINO ACID COMPOSITION, RELATIVE NUTRITIVE VALUES AND IN VITRO PROTEIN DIGESTIBILITY OF SEVERAL PHILIPPINE INDIGENOUS FOOD LEGUMES

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## ABSTRACT

This study demonstrates variability in amino acid composition among accessions of several Philippine indigenous legumes. Moreover, two accessions of D. lablab were identified to have unusually high level of methionine (> 2%) and could be possible sources of methionine rich proteins.

The IVPDs of the legumes under study ranged from > 70 to 79%. Raw mature seeds had relatively low RNVs of 11-68% which increased to 68 to 94% and 51 to 89% after boiling and roasting, respectively, suggesting the presence of heat labile toxic constituents in the raw seeds and their removal/inactivation upon cooking.

## INTRODUCTION

The nutritional quality of a protein depends primarily on its capacity to satisfy the requirements for nitrogen and essential amino acids both for maintenance of health and for net protein synthesis of a growing body. Several reviews and books on the nutritional evaluation of proteins are available (Pellet and Young, 1980; Cereal Chem, 1977). Analysis of nutritional quality usually consists of: determination of protein and amino acid composition and animal (rat) assays to determine protein effiency ratio (PER), biological value (BV), nitrogen protein utilization and true digestibility. However, because of the expense and long period of time for such animal assays, other less expensive and more rapid methods have been developed for such purpose. Two of these are used in this study, namely, relative nutritive value (RNV) and in vitro protein digestibility (IVPD).

From the amino acid composition, the chemical score of the protein is obtained. The chemical score or amino acid score is defined as the mg of essential amino acid per gram of test protein divided by mg of essential amino acid per gram of reference protein (milk or egg) as set by FAO/WHO (1973). The amino acid that shows the lowest score is termed "limiting amino acid" and the ratio obtained is the score.

Because of the limited amounts of samples available, the microbiological assay using Tetrahymena pyriformis was utilized in evaluating further the nutritional quality of the indigenous legumes instead of the rat assay. A correlation of 0.9474 for relative nutritive value (RNV) and protein efficiency ratio

(PER) has been obtained for eight foods (Landers, 1975). In contrast to chemical score, the biological tests such as the RNV are able to detect the presence of toxic factors in a test food.

Plant proteins are lower in true protein digestibility (81-96%) than animal proteins such as egg or milk proteins (PAG, 1975). Rat assays are usually utilized to determine true digestibility. However, proteolytic enzymes have been used to assay *in vitro* protein digestibility (IVPD) (Hsu et al, 1977; Saterlee et al, 1979).

It is therefore essential to subject the indigenous legumes under study to various analyses to determine their nutritional quality. For this study, the following were used: amino acid composition, relative nutritive values and in vitro protein digestibility. The indigenous legumes studied were as follows: sam-samping (*Clitoria ternatea*), batao or hyacinth bean (*Dolichos lablab*), sabawel (*Mucuna pruriens or concinchinensis*), lima bean (*Phaseolus lunatus*), sword bean (*Canavalia gladiata*), rice bean (*Vigna umbellata*), jack bean (*Canavalia ensiformis*).

## MATERIALS AND METHODS

### Materials

Seeds of the above-mentioned legumes were obtained from the National Plant Genetic Research Laboratory of the Institute of Plant Breeding. They were planted to obtain fresh seed materials for analysis.

All chemicals used were of analytical grade. *Tetrahymena pyriformis* W ATCC 10542 was obtained from the National Institutes of Biotechnology and Applied Microbiology (BIOTECH) of UPLB.

**Amino Acid Analysis**

Defatted, ground samples containing 5 mg protein were heated in 6 N HCl under vacuum for 24 h at 110 C. After sample clean up, the acid hydrolysates were dried under vacuum and redissolved in citrate buffer pH 3.2. The amino acid analysis was done using a Waters HPLC system with a cation exchange resin AAA HPLC column (P/N 80002), a post column reaction unit using orthoaldehyde and hypochlorite as derivatizing reagents and a fluorescence detector (Millipore-Waters Assoc, 1983).

For determination of cysteine and methionine, samples were subjected to performic acid oxidation.

**Determination of RNV**

The RNV assay procedure of Stott et al (1963) with minor modifications was used.

Seed samples (40 mesh) containing 15 mg N were hydrated with water to give a concentration of 3 mg N / 4 mL of test material suspension. The pH was adjusted to 8.2 and 4 mL of said suspension was placed in a series of 20 x 160 mm screw capped tubes. Two mL of solutions of minerals and nucleic acids prepared according to Stott et al were added to the test material suspension. The resulting mixtures were sterilized at 121 C for 10 min.

Vitamin solutions (Stott et al, 1963) previously diluted ten times and 15% (w/v) glucose solution were autoclaved separately at 121 C for 10 min. One mL of vitamin solution and one mL of glucose solution were added to each test material. The tubes were then inoculated with 3-day old broth culture of L.

pyriformis W, and incubated at 25 C in an inclined position and with screw caps left loose for aeration.

After 4 days of incubation, each culture tube was shaken for about 30 sec and 1 mL of the culture transferred to a 15 x 150 mm screw capped tubes containing 1 mL of 36% formaldehyde preserving fluid. Tetrahymena cells were then counted in a double cell Lumicyte haemacytometer. The organism in 8 alternate squares were counted and the mean number per 1 mL square multiplied by two gave the final population of the test cultures in units of 10⁴ organisms per mL. RNV was calculated using the formula of Holmes and Rolle (1970) as follows:

$$\%RNV = \frac{[\log (\text{count for test protein}) - \log (\text{count for inoculum})] \times 100}{\log (\text{count for casein}) - \log (\text{count for inoculum})}$$

**Determination of IVPD**

The IVPD of the samples was determined using the multienzyme technique of Hsu et al (1977). Ground samples were hydrated with enough distilled water to give a 6.25 mg N/mL mixture for at least one hr at 4 C. The IVPD was calculated according to the regression equation $Y = 210.464 - 18.103 X$ where $Y$ = in vitro digestibility (%) and $X$ = pH of the sample suspension after 10 min digestion with the multienzyme solution.

**Preparation of Samples for RNV**

1) Boiling. Samples (10 g: 200 mL) were boiled until cooked.

2) Roasting. Samples were roasted until cooked.

**Nitrogen Determination**

Nitrogen content was analyzed according to the standard AOAC method (1980) and converted to protein by multiplying % N by the factor 6.25.

## RESULTS AND DISCUSSION

**Amino Acid Composition**

Table 1a summarizes the amino acid pattern of the legumes studied. Notably, batao A-45 and A-52 had very high methionine content (2.0 and 5.8%, respectively). Such high levels of methionine content for Dolichos lablab have been reported before by our laboratory in (Laurena and Mendoza, 1986). (A study on the methionine-rich proteins in D. lablab is now underway in this laboratory in cooperation with Dr. B.O. de Lumen of University of California Berkeley). In general, performic acid oxidation of samples gave higher cys and met values for the samples since ordinary acid hydrolysis results in the destruction of said amino acids. Sabawel A-5 had higher (2.8%) met in the acid hydrolysate run; this is being re-analyzed.

As expected, most of the legumes had met as first limiting amino acid (Table 1b) with leu, lys, thr or tyr as the second limiting amino acid.

Results in Table 1a show the variability in amino acid content of various accessions of each of the legumes studied. Some of these observations on the essential amino acid are as follows: For D. lablab, met 1.3-5.8; trp, 0.05-0.21; thr, 3.1-5.2; phe, 3.6-7.2%; lys, 3.1-11.4%; val, 4.1-7.8; leu 3.9-4.0; ile, 4.2-6.6; tyr, 4.2-6.6. For C. ensiformis: met, 1.2-1.6; trp, 0.09-0.12; thr, 3.4-4.5; phe, 3.6-5.4; lys, 1.8-5.3; val, 4.3-5.8; ;leu, 3.1-4.0; ile, 3.6-4.9; tyr, 2.1-4.1. For C. gladiata: trp, 0.06-0.09; thr, 3.9-5.3; phe, 1.5-3.7; lys, 7.0-8.5; val, 3.7-6.6; leu, 2.6-5.2; ile, 2.9-6.2 and tyr, 1.5-2.3. For P. lunatus: met, 0.6-1.3; trp, 0.10-0.18, thr 2.6-5.8; phe, 3.4-7.3; lys, 6.6.-9.8; val 3.7-6.6; leu, 2.7-5.0; ile, 3.5-6.6; tyr, 1.8-4.1. For M. cumanii: met, 1.0-1.4; trp, 0.05-0.07; thr 2.4-3.9; phe, 2.7-5.0; lys, 2.4-5.2; val, 3.4-5.6; leu, 2.6-3.7; ile, 3.4-5.8 and tyr, 3.8-5.3.

**In vitro Protein Digestibility**

The IVPD of the raw mature seeds of 7 different legumes studied ranged from 70 to 78% (Table 2) for D. lablab A-51 and A-57, respectively. All three accessions of lima bean had low IVPD (70-71%) while jack bean IVPD had a wider range of 72-75%. These results for raw seeds are similar to these reported for other legumes such as mung bean (Barroga et al, 1985), cowpea (Laurena et al, 1994), horse gram and moth bean (Satwadhar et al, 1981). Cooking such as boiling was shown to increase the IVPD of these legumes suggesting the presence of heat-stable factors such as trypsin inhibitors which lower IVPD. Addition of polyvinylpyrrolidone (PVP) to mungbean and cowpea also increases

IVPD by 6-8%) (Barroga et al, 1985; Laurena et al 1984); this increase was described to the binding PVP with condensed tannins.

**Relative Nutritive Value (RNV)**

The RNV of the raw mature seeds of the seven indigenous legumes ranged from 11 to 66% (Table 3). Boiling and roasting to cooked condition resulted in large increase in RNV, 68-94% and 51-89%, respectively. Raw mature seeds of *D. lablab* had relatively higher levels of RNV (33 to 66%) compared to others. *D. gladiata* A-12 had the lowest value of 11.3%. These low levels suggest the presence of toxic constituents in the raw seed which are inactivated by heat treatment. Boiling (wet treatment) was more effective than roasting (dry treatment). The heat labile toxic constituents could be proteins such as lectins and trypsin inhibitors. Our separate studies on lectins showed that boiling and roasting decreased lectin's hemagglutinating activity (Sembrano and Mendoza, 1988a). Boiling could also remove saponins present in seeds of jack bean and sword bean (Sembrano and Mendoza, 1988b). Removal or inactivation of these substances could result in an increase in nutritional quality as measured by RNV. For jack bean, its major lectin concanavalin could account for the low RNV of the raw seeds.

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Table 1a. Amino acid composition of mature seeds of several Philippine indigenous food legumes.

| Sample | Protein Content N x 6.25 | Amino Acid ( % Amino acid or g amino acid/100 g sample) Cys* | Met* | Trp** | Met | Thr | Phe | Lys | Val | Leu | Ile | Tyr | Gly | Ala | Pro | His | Arg | Asp | Glu | Ser |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Canavalia ensiformis* | | | | | | | | | | | | | | | | | | | | |
| Jackbean A-1 | 27.82 | 1.3 | 1.2 | 0.08 | 1.9 | 4.5 | 4.7 | 5.3 | 5.8 | 4.8 | 4.8 | 2.2 | 4.5 | 6.0 | 3.9 | 2.8 | 4.7 | 12.6 | 12.8 | 6.2 |
| A-3 | 28.10 | 1.1 | 1.4 | 0.09 | 1.0 | 3.7 | 4.8 | 5.4 | 4.3 | 3.1 | 3.9 | 3.4 | 4.1 | 4.5 | 1.9 | 3.6 | 4.0 | 10.5 | 10.5 | 5.7 |
| A-5 | 24.17 | 1.7 | 1.6 | 0.10 | 1.1 | 4.3 | 4.7 | 2.7 | 5.2 | 3.4 | 4.6 | 2.3 | 4.6 | 4.9 | 2.3 | 2.9 | 4.9 | 10.4 | 11.8 | 4.4 |
| A-4 | 29.92 | 1.1 | 1.6 | 0.09 | 0.9 | 3.4 | 3.6 | 2.5 | 4.1 | 2.6 | 3.6 | 2.2 | 3.5 | 3.8 | 3.3 | 2.4 | 2.6 | 7.5 | 8.3 | 3.6 |
| A-3 | 30.75 | | | 0.12 | 0.9 | 3.5 | 4.8 | 2.2 | 5.3 | 3.1 | 4.9 | 4.1 | 5.0 | 3.5 | 2.3 | 2.2 | 7.5 | 11.7 | 12.2 | 3.5 |
| B-8 | 29.41 | 0.9 | 1.3 | 0.09 | 0.5 | 4.3 | 5.4 | 1.8 | 4.9 | 3.3 | 4.4 | 2.1 | 4.2 | 4.7 | 1.7 | 3.9 | 5.5 | 11.3 | 10.9 | 4.7 |
| *Canavalia gladiata* | | | | | | | | | | | | | | | | | | | | |
| Swordbean A-4 | 22.58 | | | 0.09 | 0.7 | 5.3 | 1.5 | 8.5 | 6.6 | 5.2 | 6.2 | 1.6 | 5.1 | 6.5 | 2.2 | 2.4 | 7.8 | 17.4 | 16.9 | 7.2 |
| A-9 | 26.36 | | | 0.06 | 0.7 | 3.3 | 3.4 | 7.4 | 3.7 | 3.5 | 3.4 | 2.3 | 2.9 | 3.4 | 1.5 | 1.6 | 5.8 | 8.4 | 9.2 | 4.6 |
| A-12 | 29.65 | | | 0.07 | 0.4 | 3.0 | 3.7 | 7.0 | 4.8 | 2.6 | 2.9 | 1.5 | 3.6 | 4.8 | 2.4 | 8.8 | 1.9 | 10.8 | 12.2 | 4.2 |
| *Dolichos lablab* | | | | | | | | | | | | | | | | | | | | |
| Batao A-45 | 27.33 | 1.5 | 2.0 | 0.05 | 2.5 | 3.1 | 3.6 | 11.4 | 4.1 | 5.0 | 4.2 | 3.7 | 4.2 | 4.4 | 5.2 | 3.1 | 7.1 | 8.5 | 11.2 | 4.7 |
| A-51 | 18.52 | | | 0.11 | 2.2 | 5.2 | 7.2 | 4.6 | 7.8 | 4.9 | 6.6 | 4.1 | 6.6 | 7.2 | 4.4 | 3.5 | 6.7 | 14.8 | 23.5 | 6.5 |
| A-52 | 21.12 | 2.3 | 5.8 | 0.11 | 0.6 | 4.0 | 5.8 | 5.1 | 4.9 | 3.9 | 4.5 | 4.3 | 4.3 | 5.1 | 3.4 | 2.3 | 4.3 | 14.0 | 18.3 | 6.5 |
| A-57 | 25.24 | 1.3 | 1.5 | 0.21 | 0.4 | 3.7 | 5.3 | 3.1 | 6.9 | 4.0 | 5.3 | 2.8 | 5.0 | 5.2 | 1.9 | 3.3 | 5.8 | 11.6 | 17.5 | 4.6 |
| *Mucuna pruriens* | | | | | | | | | | | | | | | | | | | | |
| Sabawel A-2 | 29.36 | | | 0.18 | - | 2.9 | 4.0 | 5.2 | 3.4 | 2.6 | 3.4 | 3.8 | 3.3 | 2.6 | 4.3 | 3.5 | 3.3 | 9.7 | 6.0 | 3.5 |
| A-5 | 29.42 | 1.0 | 1.0 | 0.05 | 2.8 | 2.4 | 2.7 | 4.6 | 4.0 | 3.9 | 4.0 | 5.3 | 3.9 | 2.7 | 1.1 | 6.8 | 9.5 | 7.2 | 7.4 | 2.8 |
| A-8 | 23.22 | 2.1 | 1.4 | 0.07 | 3.9 | 3.9 | 5.9 | 2.4 | 5.6 | 3.7 | 5.8 | 4.9 | 5.7 | 4.1 | 1.7 | 4.3 | 8.9 | 16.2 | 15.2 | 3.5 |
| *Phaseolus lunatus* | | | | | | | | | | | | | | | | | | | | |
| Lima A-535 | 20.35 | 3.6 | 1.3 | 0.10 | 0.6 | 3.9 | 4.9 | 6.6 | 4.6 | 4.1 | 4.5 | 2.7 | 3.2 | 4.0 | 2.4 | 1.3 | 6.5 | 12.9 | 11.7 | 6.2 |
| A-537 | 27.65 | 1.1 | 0.6 | 0.13 | 0.6 | 2.6 | 3.4 | 9.8 | 3.7 | 2.7 | 3.5 | 1.8 | 2.5 | 2.8 | 1.6 | 1.2 | 4.9 | 7.2 | 7.5 | 3.3 |
| A-544 | 26.12 | 3.8 | 0.7 | 0.18 | 0.8 | 5.8 | 7.3 | 7.9 | 6.6 | 5.0 | 6.6 | 4.1 | 5.3 | 6.4 | 2.2 | 4.2 | 7.5 | 20.4 | 18.3 | 8.9 |
| *Vigna umbellata* | | | | | | | | | | | | | | | | | | | | |
| Rice bean 26 | | 3.6 | 1.0 | | 1.43 | 3.82 | 5.94 | 7.48 | 5.08 | 8.29 | 4.14 | 3.11 | 4.30 | 4.68 | 3.79 | 3.22 | 6.45 | 12.11 | 17.16 | 5.89 |
| 28 | | 3.7 | 1.2 | | 1.20 | 3.84 | 5.97 | 7.17 | 4.54 | 8.26 | 3.85 | 3.32 | 4.18 | 4.70 | 4.16 | 3.19 | 6.35 | 11.96 | 18.25 | 5.86 |
| 46 | | 2.6 | 1.6 | | 0.96 | 3.48 | 5.43 | 7.46 | 4.75 | 7.88 | 4.19 | 3.11 | 3.93 | 4.40 | 3.75 | 3.18 | 6.02 | 11.38 | 17.15 | 4.79 |

Table 1b. Chemical score of several Philippine indigenous legumes.

| Sample | Essential amino acids (% AA or g AA/100 g sample) Met | Thr | Phe & Tyr | Ile | Lys | Leu | Val | Limiting Amino Acid First | Second |
|---|---|---|---|---|---|---|---|---|---|
| *Canavalia ensiformis* | | | | | | | | | |
| Jackbean A-1 | >54 | 112 | 115 | 120 | 96 | 57 | 116 | met | leu |
| A-3 | >28 | 92 | 137 | 97 | 98 | 44 | 86 | met | leu |
| A-5 | >31.4 | 107 | 117 | 115 | 49 | 48 | 104 | met | leu |
| A-6 | >26 | 85 | 97 | 90 | 45 | 37 | 82 | met | leu- |
| A-7 | >26 | 87 | 148 | 122 | 48 | 44 | 106 | met | lys |
| A-8 | >14 | 107 | 125 | 110 | 33 | 47 | 98 | met | lys |
| *Canavalia gladiata* | | | | | | | | | |
| Swordbean A-1 | >20 | 132 | 52 | 155 | 154 | 74 | 132 | met | phe & tyr |
| A-9 | >20 | 82 | 95 | 85 | 134 | 50 | 74 | met | leu |
| A-12 | >11 | 77 | 87 | 72 | 127 | 37 | 98 | met | leu |
| *Dolichos lablab* | | | | | | | | | |
| Batao A-45 | >48.6 | 85 | 115 | 75 | 122 | 46 | 78 | leu | met |
| A-51 | >62.8 | 130 | 188 | 165 | 84 | 70 | 156 | met | leu |
| A-52 | >17.4 | 100 | 168 | 112 | 93 | 56 | 98 | met | leu |
| A-57 | >11.43 | 92 | 135 | 132 | 56 | 57 | 120 | met | lys |
| *Mucuna cumanii* | | | | | | | | | |
| Sabawel A-2 | - | 70 | 130 | 85 | 94 | 37 | 68 | met | leu |
| A-5 | 80.0 | 60 | 133 | 100 | 84 | 56 | 80 | leu | thr |
| A-8 | 23 | 97 | 165 | 145 | 44 | 53 | 112 | met | lys |
| *Phaseolus lunatus* | | | | | | | | | |
| Lima A-535 | >17 | 97 | 127 | 112 | 120 | 58 | 92 | met | leu |
| A-537 | >17 | 65 | 87 | 87 | 178 | 38 | 74 | met | leu |
| A-544 | >23 | 145 | 190 | 165 | 127 | 71 | 132 | met | leu |
| *Vigna umbellata* | | | | | | | | | |
| Rice bean 24 | 43 | 96 | 155 | 96 | 130 | 118 | 91 | met | |
| 28 | 38 | 87 | 142 | 104 | 136 | 113 | 95 | met | thr |
| 46 | | | | | | | | | |

**Table 2. In vitro protein digestibility (IVPD) of raw mature seeds of several Philippine indigenous legumes.**

| Sample | | IVPD (%) |
|---|---|---|
| *Canavalia ensiformis* | | |
| Jack bean | A-1 | 75.44 |
| | A-3 | 75.60 |
| | A-5 | 74.61 |
| | A-6 | 75.36 |
| | A-8 | 76.61 |
| | B-8 | 72.23 |
| *Canavalia gladiata* | | |
| Sword bean | A-4 | 74.02 |
| | A-12 | 72.62 |
| *Clitoria ternatea* | | |
| Sam-samping | 7-2 | 76.19 |
| *Dolichos lablab* | | |
| Batao | A-45 | 73.71 |
| | A-51 | 70.18 |
| | A-52 | 71.19 |
| | A-57 | 78.57 |
| *Mucuna pruriens* | | |
| Sabawel | A-2 | 72.19 |
| *Phaseolus lunatus* | | |
| Lima bean | A-535 | 71.00 |
| | A-537 | 70.31 |
| | A-544 | 71.45 |
| *Vigna umbellata* | | |
| Rice bean | | 73.48 |
| | | 74.30 |

**Table 3. Relative nutritive values (RNV) of raw, boiled and roasted mature seeds of several Philippines indigenous legumes.**

| Sample | | Relative nutritive value (%) | | |
|---|---|---|---|---|
| | | Raw | Boiled | Roasted |
| *Canavalia ensiformis* | | | | |
| Jackbean | A-1 | 47.39 ± 6.40 | 85.31 ± 5.08 | 60.46 ± 5.17 |
| | A-3 | 25.31 ± 4.05 | 75.74 ± 5.64 | 76.22 ± 6.30 |
| | A-5 | 17.26 ± 0.00 | 83.32 ± 2.39 | 61.36 ± 6.48 |
| | A-6 | 40.23 ± 4.85 | 82.16 ± 4.38 | 74.10 ± 5.97 |
| | A-8 | 28.00 ± 3.02 | 78.40 ± 3.74 | 71.07 ± 5.48 |
| | B-8 | 18.54 ± 2.22 | 59.19 ± 3.05 | 50.56 ± 0 |
| *Canavalia gladiata* | | | | |
| Sword bean | A-4 | 23.19 ± 4.66 | 93.99 ± 4.85 | 69.91 ± 1.73 |
| | A-9 | 16.09 ± 2.02 | 86.38 ± 5.22 | 58.17 ± 3.13 |
| | A-12 | 11.29 ± 2.19 | 86.69 ± 3.44 | 93.18 ± 0 |
| *Clitoria ternatea* | | | | |
| Sam-samping | 7-2 | 54.09 | 90.86 ± 3.53 | 88.49 ± 4.60 |
| *Dolichos lablab* | | | | |
| Batao | A-45 | 65.97 ± 3.69 | 94.20 ± 3.40 | 82.13 ± 4.30 |
| | A-51 | 32.49 ± 3.52 | 92.3 ± 3.79 | 76.46 ± 1.63 |
| | A-52 | 42.39 ± 3.34 | 84.39 ± 3.70 | 84.93 ± 1.51 |
| | A-57 | 48.59 ± 3.11 | 87.76 ± 2.98 | 79.19 ± 4.24 |
| *Mucuna pruriens* | | | | |
| Sabawel | A-2 | 38.01 ± 1.58 | 81.87 ± 5.50 | 81.58 ± 9.30 |
| | A-5 | 34.76 ± 5.03 | 87.45 ± 0.45 | 61.59 ± 3.45 |
| | A-8 | 48.39 ± 5.7 | 67.18 ± 6.45 | 64.45 ± 5.60 |
| *Phaseolus lunatus* | | | | |
| Lima bean | A-535 | 28.99 ± 3.23 | 67.75 ± 5.72 | 51.77 ± 2.78 |
| | A-537 | 39.76 ± 1.42 | 79.97 ± 5.35 | 62.55 ± 2.30 |
| | A-542 | 51.23 ± 5.40 | 83.01 ± 4.25 | 77.17 ± 6.13 |
| *Vigna umbellata* | | | | |
| Rice bean | 28 | 22.62 ± 2.64 | 79.42 ± 1.97 | 60.40 ± 6.72 |
| | 46 | 42.42 ± 6.11 | 67.69 ± 5.80 | 55.57 ± 5.35 |

# POLYPHENOLS, PHYTATE, CYANOGENIC GLYCOSIDES AND TRYPSIN INHIBITOR ACTIVITY OF SEVERAL PHILIPPINE INDIGENOUS FOOD LEGUMES

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## ABSTRACT

Low levels of polyphenols with flavanol groups and those capable of precipitating proteins were observed in raw mature seeds of six legumes analyzed.

Seeds of batao or hyacinth bean, jack bean and lima bean had relatively high levels of phytate phosphorus ranging from 6 to 11.6 mg phytate P/g sample. Lower values (<5) were obtained for sabawel and sword bean as well as some varieties of mungbean used as control.

None of the indigenous legumes had potentially toxic levels of cyanide (<50 ppm) in the seeds, immature and mature leaves and immature pods. The lima bean samples had low HCN potential (0-10 ppm).

Among the legumes investigated, batao had the highest trypsin inhibitor activity (TIA) ranging from 14.25 to 27.05 units/mg sample for 4 accessions. Accessions 6-1 of sabawel had 19.8 units/mg sword bean and jack bean had low levels of 1.20 to 4.95 units/mg except for one jack bean accession (A-1) which had 1.45 units/mg. Rice bean also had low levels of TIA, 5-7 units/mg.

## INTRODUCTION

In addition to the low levels of sulfur-containing amino acids in legume seeds, the presence of anti-physiological and toxic factors results in the decrease in their overall nutritional quality. These anti-nutritional factors include polyphenols, trypsin inhibitors, phytate, cyanogenic glucosides, saponins, lectins and alkaloids.

Polyphenols (or tannins) are able to bind with proteins, thus, lowering their protein digestibility. This effect has been reported for cowpea (Laurena et al, 1984a), winged bean (de Lumen and Salamat, 1980), mungbean (Barroga et al, 1985). Trypsin inhibitors belong to the group of proteinase inhibitors which are polypeptides or proteins that inhibit proteolytic enzyme activity, more specifically the digestive enzyme trypsin. Tannins also have weak interactions with trypsin and thus also inhibit the latter's activity. Kakade et al (1973) reported that approximately 40% of the growth depressing effect of unheated soybeans fed to rats can be accounted for by trypsin inhibitors. Trypsin inhibitors were also observed to cause hypertrophy in rats and chicks (Rackis, 1965).

Phytic acid or myoinositol hexaphosphate is a common constituent of plant tissue which has been held responsible for the interference of plant materials in the availability of dietary minerals. The formation of insoluble phytate-metal complexes may prevent the absorption of minerals by the body. Phytate-protein complexes also result in reduced solubility of proteins which can affect the functional properties of proteins.

Cyanogenic glycosides are plant toxins which upon hydrolysis liberate hydrogen cyanide. Some examples of plants which contain cyanogenic glycosides are almonds (*Prunus amygdalus*), cassava (*Manihot esculenta* Crantz) and sorghum (*Sorghum vulgare*); they contain amygdalin, linamarin and dhurrin, respectively. The toxic effects of the free cyanide are well documented and include a wide spectrum of organisms since its site of action is inhibition of the cytochromes of the electron transport system. The exact physiological effects of the glycosides are not well established. However, oral doses of pure linamarin had been reported to produce physiological and biochemical changes in rats in the absence of linamarase activity (Philbrick et al, 1977; Barrett et al, 1978).

Because of the growing importance of legumes in the human diet, the levels of polyphenols, TIA, phytates and cyanogenic glycosides were determined in seven underutilized legumes indigenous to the Philippines. These are: sam-samping (*Clitoria ternatea*), batao or hyacinth bean (*Dolichos lablab*), sabawel (*Mucuna pruriens* or *cochinchinensis*), lima bean (*Phaseolus lunatus*), swordbean (*Canavalia gladiata*), rice bean (*Vigna umbellata*) and jack bean (*Canavalia ensiformis*). The other anti-nutritional factors-- lectins or hemagglutinins, saponins, alkaloids and oligosaccharides are the subject of other reports in this series (Barroga and Mendoza, 1988; Sembrano and Mendoza, 1988a, b; Revilleza et al, 1988).

## MATERIALS AND METHODS

### Samples

Seeds of above-mentioned indigenous legumes were obtained from the National Plant Genetic Resources Laboratory (NPGRL) and planted to obtain leaves and seeds for analysis.

### Chemicals

All chemicals used were of analytical grade.

Linamarase was partially purified from cassava cortex as previously reported (Fukuba et al, 1982).

Trypsin and benzoyl-DL-arginine-p-nitroanilide (BAPNA) used for trypsin inhibitor activity determination were obtained from SIGMA Chemicals.

### Polyphenol Content

The polyphenol content of the seed samples (ground dry mature seeds, 60 mesh) was determined using two different assays modified vanillin (Price et al, 1978) and protein precipitation (Hagerman and Butler, 1978). One % HCl in methanol and absolute methanol were used as extractants for the modified vanillin assay and protein precipitation assay, respectively. Catechin and tannic acid were used as standard in the aforementioned, respectively.

### Determination of Trypsin Inhbitor Activity (TIA)

TIA was obtained by the method of Kakade et al (1974) by the decrease in the rate in which trypsin hydrolyzed benzoyl-DL-arginine-p-nitroanilide (BAPNA). TIA is expressed as trypsin units inhibited, one trypsin unit being arbitrarily defined as an

increase of 0.01 absorbance unit at 410 nm per mL of the reaction under the conditions used.

**Determination of Phytic Acid**

Phytic acid was measured according to the method of Wheeler and Ferrel (1971). Five hundred mg of ground sample was extracted with 15 mL of 3% trichloroacetic acid for 30 min with mechanical shaking.

**Determination of Cyanogenic Glycosides**

Two methods were used in the analysis of cyanogenic glycosides, namely, enzymatic and picric acid methods. In the first, the distillation method of Reay and Conn (1970) was followed using either linamarase or crude leaf/pod extract of sample used. In the second method, toluene was added to the sample to liberate the cyanide which was trapped in picric acid.

Samples were prepared by homogenizing 10 g ground dry seeds or finely cut pods in 40 mL of 0.1 NHCl; pH was adjusted to 6.5. Total volume was adjusted to 50 mL. Crude enzyme for each legume was prepared by homogenizing 4 g of green pods or leaves in 50 mL of 0.1 M phosphate buffer pH 6.0

## RESULTS AND DISCUSSION

**Polyphenol Content**

The seeds of batao, lima bean and sabawel had low level of flavanols as measured by the vanillin assay (Table 1), about 4 to 10 times less than in mungbean (Barroga et al, 1985).

Seeds of jackbean and sword bean had no detectable flavanol groups. The values obtained for protein precipitation, 0.44 to 0.79 tannic acid equivalent g of sample (TAE) are lower than the values reported for sorghum (1.03 - 5.66 TAE) (Bullard et al, 1981), cowpea (1.44-4.77 TAE) (Laurena et al, 1984a) and common beans (1.61-5.2 TAE) (Bressani et al, 1983) but comparable with those for mungbean (0.16-0.61 TAE) (Barroga et al, 1985).

These results indicate that sword bean and jack bean have hydrolyzable tannins which are not anti-nutritional. Batao, lima bean and sabawel have low levels of polyphenols which are not large enough to precipitate proteins and thus do not seem to be a nutritional problem.

**Phytic Acid**

Among the indigenous legumes tested, batao, jack bean and lima bean had relatively high levels of phytate phosphorus, ranging for 6 to 11.6 mg phytate P/g sample (Table 7). Lower values (< 5) were obtained for sabawel and sword bean as well as some varieties of mung bean used as control. The values obtained for lima bean were higher than those for the varieties reported by Ologhobo and Fetuga (1982 and 1984) which were about 2 mg phytate P/g; this could be due to varietal differences. Horse bean gram Macrotyloma uniflorum (Lam.) Verde and moth bean [Vigna aconitifolium (Jacq.) Marechall had about 1.8 mg phytate-P/g comprising 55% of total phosphorus. Phytate-phosphorus in soy bean, cowpea and lima bean also accounted for 40 to 50% of total phosphorus (Ologhobo and Fetuga, 1984). Germination and cooking resulted in 25% decrease in phytate-phosphorus of horse gram and

moth bean (Borade et al, 1984). On the other hand, germination and soaking were very effective in removing 42-45% phytate-phosphorus from cowpea and lima bean (Dioghobo and Fetuga, 1984).

Phytic acid may be nutritionally important in batao, jack bean and lima bean because of their relatively high levels. Phytate makes about 50% of the seed's phosphorus content unavailable and also could complex with other minerals making them less available.

**Cyanogenic Glycosides**

Table 2 shows that none of the indigenous legumes had potentially toxic levels of cyanide in the seeds, immature and mature leaves and immature pods. HCN levels of 50 ppm and less are considered non-toxic.

The lima bean samples had low HCN potential (0-10 ppm) which correspond to levels measured in cultivated beans compared to 246 to 1580 ppm in wild lima beans (De la Vega and Sotelo, 1986). Canavalia gladiata and C. ensiformis samples had 22-46 ppm HCN in seeds and had none or very little HCN in other parts. Djajasukma (1977) also reported the absence of cyanide in seeds of Canavalia spp. in contrast to the reported 97 ppm of HCN in immature sword beans (Kay, 1973) and 108 ppm in jack bean seeds (Tehon, 1946). The difference could be due to different varieties/accessions and methodology used.

Mucuna pruriens had 0 to 44 ppm HCN in different parts of the plants (Table 2a and 2b). Other species of Mucuna viz, giganiea and macrophylla were also reported to be devoid of HCN

(Djajasukma, 1977). Rice bean seeds had very low (23 ppm) HCN similar to previously reported (Kay, 1972). Sam-samping or Clitoria ternatea also had very low (0-20 ppm) HCN. No reports on the HCN of sam-samping had been made before.

Unlike in cassava, the legume samples analyzed indicated lower levels in leaves than in seeds. This could be more systematically studied with lima beans especially the wild type which contain high levels of cyanogenic glycosides.

Two methods of determining cyanide content were used to ensure the accuracy of the results. The enzymatic/distillation method is considered as the most accurate (Mendoza et al, 1984) although this is limited by the need to use enzyme specific for the type of cyanogenic glycoside in the sample. In this study, crude enzyme preparation from leaves of the sample tested was used aside from partially purified linamarase which is specific for linamarin found in lima bean and cassava. The picrate method tends to overestimate cyanide content since it also gives positive test with other volatiles such as $H_2S$, aldehydes, thiocyanates and nitriles. This method also failed to detect high levels of cyanide in the samples.

**Trypsin Inhibitor Activity**

Among the legumes investigated, batao (Dolichos lablab) had the highest trypsin inhibitor activity (TIA) ranging from 14.25 to 27.05 units/mg sample for 4 accessions (Table 8). Accession 6-1 of sabawel had 19.8 units/mg sword bean and jack bean had low levels of 1.20 to 4.95 units/mg except for one jack bean accession (A-1) which had 9.45 units/mg sample. Rice bean also had low levels of

TIA, 5-7 units/mg.

In this study one lima bean accession (A-537) was found to contain 1.89 units/mg. High amounts of TIA had been reported for lima bean (Del Rosario et al, 1980). Ologhobo and Fetuga (1983) reported levels of 29.43 to 36.65 units/mg protein. However, it is difficult to directly compare the results as the methodology and units used were different.

Furusawa et al (1974) had purified a trypsin inhibitor from D. lablab which had a molecular weight of 9,500. It contained 20% carbohydrate as galactose and 10% hexosamine as glucosamine. This inhibitor had inhibitory activity against α-chymotrypsin aside from that against trypsin.

Trypsin inhibitors have been shown by various studies to cause pancreatic hypertrophy and growth depression (Rackis, 1965; Kakade et al, 1973; Chan and de Lumen, 1982), thus underlining the importance of inactivating them before ingestion of food.

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Table 1. Polyphenols and phytate phosphorus of dry mature seeds of several indigenous food legumes.

| Samples | | Vanillin assay (mg catechin) | Protein precipitation (mg tannic acid/g) | Phytate phospnorus (mg P/g) |
|---|---|---|---|---|
| Batao | A-45 | 0.73 | 0.22 | 9.62 |
| | A-51 | 0.50 | 0.27 | 10.10 |
| | A-52 | 0.59 | 0.35 | 8.47 |
| | A-57 | 0.36 | 0.29 | - |
| Jack bean | A-1 | 0 | 0.29 | 6.62 |
| | A-3 | 0 | 0.16 | 9.42 |
| | A-5 | 0 | 0.46 | 7.08 |
| | A-6 | 0 | 0.62 | 9.64 |
| | A-8 | 0 | 0.69 | 7.59 |
| Lima bean | A-535 | 2.48 | 0.54 | 7.98 |
| | A-537 | 2.15 | 0.38 | 11.62 |
| | A-544 | 1.41 | 0.55 | 9.91 |
| Sabawel | A-2 | 0.14 | 0.24 | 3.08 |
| | A-5 | 0.12 | 0.77 | 3.79 |
| | A-8 | 0.21 | 0.77 | 4.61 |
| Sword bean | A-4 | 0 | 2.34 | 4.94 |
| | A-9 | 0 | 0.59 | 4.83 |
| | A-12 | 0 | 0.53 | 4.37 |
| Rice bean | 28 | 0.32 | - | |
| | 46 | 0.84 | - | |

For vanillin assay and phytic acid determination, average of two values.

For protein precipitation, average of three values.

Table 2a. Cyanide content of pods and leaves of several Philippine indigenous food legumes.

| Sample | | HCN, ppm | |
|---|---|---|---|
| | | Enzymatic method | Picric acid method |
| Immature pods | | | |
| Lima bean | A-557 | 0.65 | 0 |
| | A-537 | 1.02 | 0 |
| Sword bean | A-4 | 0 | 0 |
| | A-9 | 0 | 0 |
| Jack bean | A-3 | 0.20 | 0 |
| | A-5 | 0.38 | 0 |
| | A-8 | 0.34 | 0 |
| Immature leaves | | | |
| Sword bean | A-4 | 0 | 0 |
| | A-9 | 0 | 0 |
| Jack bean | A-3 | 0.10 | 0 |
| | A-5 | 0.22 | 0 |
| | A-8 | 0.34 | 0 |
| | 8-8 | 0 | 0 |
| Sabawel | A-2 | 0.83 | 0 |
| | A-5 | 0 | 0 |
| Lima bean | A-537 | 3.31 | 0 |
| | A-544 | 10.35 | 0 |
| Sam-samping | | 0 | 0 |
| mature leaves | | | |
| Jack bean | A-3 | 0.10 | 0 |
| | A-5 | 0.20 | 0 |
| | A-8 | 0.27 | 0 |
| | 8-8 | 0 | 0 |
| Sword bean | A-4 | 0 | 0 |
| | A-9 | 0 | 0 |
| Sabawel | A-2 | 0 | 0 |
| | A-5 | 0 | 0 |

| Sample | HCN, ppm | |
|---|---|---|
| | Enzymatic method | Picric acid method |
| Lima bean A-537 | 8.88 | 0 |
| A-544 | 0.83 | 0 |
| Sam-samping | 0 | 0 |
| Control | | |
| Cassava | | |
| Lakan (edible) | | |
| immature leaves | 160 | |
| mature leaves | 101 | |
| parenchyma | 27 | |
| cortex | 387 | |
| Datu (poisonous) | | |
| immature leaves | 182 | |
| mature leaves | 135 | |
| parenchyma | 105 | |
| cortex | 637 | |

Table 2b. Total cyanide potential of mature seeds of several Philippine indigenous for legumes.

| Sample | HCN, ppm |
|---|---|
| Batao | 15-42 |
| Sabawel | 24-44 |
| Sword bean | 22 |
| Rice bean | 23 |
| Jackbean | 46 |
| Sam-samping | 20 |

Table 3. Trypsin inhibitor activity of raw mature seeds of several Philippine indigenous legumes.

| Samples | | TIU/mg sample |
|---|---|---|
| Food Legumes | | |
| Jack bean | A-1 | 9.45 |
| | A-3 | 1.44 |
| | A-5 | 3.56 |
| | A-6 | 4.95 |
| Batao | A-45 | 27.05 |
| | A-51 | 14.25 |
| | A-52 | 25.44 |
| | A-57 | 24.90 |
| Lima Bean | A-535 | - |
| | A-537 | 1.89 |
| Sword bean | A-4 | 1.20 |
| | A-9 | 2.20 |
| | A-12 | 3.40 |
| Sam-samping | 6-2 | 16.50 |
| Rice bean | 26 | 7.0 |
| | 28 | 5.0 |
| Sabawel | 6-1 | 19.8 |

# Oligosaccharides in Several Philippine Indigenous Food Legumes: Determination, Localization and Removal[a]

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**Key words:** oligosaccharides, raffinose, stachyose, verbascose, legumes, lima bean (*Phaseolus lunatus* L.), sword bean (*Canavalia gladiata* (Jacq.) DC.), jack bean (*Canavalia ensiformis* (L.) DC.), sabawel (*Mucuna pruriens* or *cochinchinensis*), batao or hyacinth bean (*Dolichos lablab* L.) and rice bean (*Vigna umbellata* Ohashi.)).

**Abstract.** The oligosaccharide profile of raw mature seeds of seven different legumes indigenous to the Philippines was measured in 70% ethanol extracts of the seeds by thin layer chromatography using HPTLC plates and quantified by a densitometer. Based on the results, the legumes could be ranked according to decreasing oligosaccharide content or flatulence potential as follows: Sam-samping (*Clitoria ternatea*) > Batao (*Dolichos lablab*) > Sabawel (*Mucuna pruriens*) > Lima (*Phaseolus lunatus*) > Swordbean (*Canavalia gladiata*) > rice bean (*Vigna umbellata*) > Jack bean (*Canavalia ensiformis*). Sam-samping had 4.79% total oligosaccharides and batao, 3.78%. A jack bean accession had 1.79% oligosaccharides.

Simple processing methods were tested to detoxify the oligosaccharides. Soaking of batao seeds had no effect while boiling even resulted in a net 23-31% increase in the levels of raffinose, stachyose and verbascose. On the other hand, two min

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[b] Part of MS thesis of the senior author.

of dry roasting resulted in complete removal of oligosaccharides whereas germination resulted in about 30-40% decrease after 1 and 2 days, respectively.

**Introduction**

Mature seeds of legumes contain oligosaccharides of the raffinose family, namely, raffinose, stachyose and verbascose in which galactose is present in -linkage (Calloway et al, 1971; Shallenberg and Moyer, 1961). These oligosaccharides have been shown to be responsible for flatulence manifested by rectal gas expulsions, abdominal rumbling, clamps, diarrhea and nausea, following consumption of these beans (Steggerda, 1968).

Many studies have been made on determining the oligosaccharide content and composition of various legumes such as soybean (Glycine max) (Hardinge et al, 1965; Murphy, 1973; Cristofaro et al, 1974), cowpea (Vigna umbellata) (Hernandez et al, 1981; Hardinge et al, 1965, Upadhay and Garcia, 1987, Cristofaro et al, 1974), red gram (Cajanus cajan) Bengal gram or chick pea (Cicer arietinum) (Rao and Belavady, 1978; Hardinge et al, 1965; Cristofaro et al, 1974), black gram (Phaseolus mungo), field bean (Phaseolus vulgaris), horse gram (Dolichos uniflorus Lam.), lentils (Lens esculenta L.), lima bean (Phaseolus lunatus L.), green peas (Pisum sativum) (Hardinge et al, 1965; Cristofaro et al, 1974) and winged bean (Garcia and Palmer, 1980).

Several legumes indigenous to the Philippines are potential sources of protein-rich foods. This study focused on (a) determination of the oligosaccharide profile and content in these underutilized legumes, (b) ontogeny and localization of the

oligosaccharide profile in selected legumes and (c) removal or detoxification of oligosaccharides using simple pre-cooking and cooking methods. The legumes used in this study were as follows: lima bean [Phaseolus lunatus (L.)], sword bean [Canavalia gladiata (Jacq.) DC], jack bean [Canavalia ensiformis (L.) DC.], sabawel [Mucuna pruriens or cochinchinensis], batao or hyacinth bean [Dolichos lablab (L.)], sam-samping [Clitoria ternatea], and rice bean [Vigna umbellata (Ohashi)]. The results of this study could aid in widening the utilization of these legumes.

## Materials and Methods

### Samples

Seeds of several accessions of the species/genera mentioned above were obtained from the National Plant Genetic Resources Laboratory and Vegetable Crops Division of the Institute of Plant Breeding. These seeds were further multiplied following recommended agronomic practices.

### Chemicals

Raffinose and stachyose were purchased from Nakarai Chemicals Ltd. Verbascose was prepared from mung bean by ethanol extraction and gel filtration chromatography using Bio-gel P2, 100-200 mesh with distilled water as eluant. All other chemicals used were of analytical grade. HPTLC plates precoated with silica gel 60 without indicator were from Merck (West Germany).

Measurement of oligosaccharide content

The oligosaccharides of the samples were analyzed using HPTLC and quantified by a Camag densitometer interfaced to an Spectra-Physics computing integrator.

1. Extraction and Identification of Oligosaccharides

Samples (500 mg) were extracted three times with hot 5 mL of 70% ethanol with shaking for 30 min at 60 C. The supernatants were pooled and concentrated in vacuo at 45 C to 1.0 mL. Four drops of saturated lead acetate solution were added to precipitate non-carbohydrate material. After removal of precipitate by centrifugation, the excess lead was removed by adding one drop of saturated monopotassium phosphate. An aliquot containing 1 to 5 ug of sugars was spotted on an HPTLC plate (Merck Silica Gel 60) with a 10 uL microsyringe and developed for 4 h in a fresh solvent system of pyridine: n-butanol: H O [3:14:4 (v/v/v)]. Triple development enhanced separation and eliminated most of the tailing. It was carried out by drying the plates under the hood for 30 min prior to redevelopment in the same direction.

The sugars were visualized by using a modified procedure of Jeffrey et al, 1969, consisting of spraying with a mixture of 100 mg diphenylamine, 0.1 mL aniline, 0.4 mL of 80% orthophosphoric acid in 4 mL acetone and 15 mg benzidine in 32.0 mL glacial acetic acid. The plates were incubated in a forced draft oven at 100 C for 10 min.

2. Quantitative Analysis

Reference standards containing 10 mg/mL each of glucose, sucrose, raffinose and stachyose were used. Response factor for each sugar standard was calculated and plotted against the degree of polymerization or the number of monosaccharide units. Response factor for verbascose was extrapolated from the graph (Sosulski et al, 1982).

Individual sugars were quantified by comparing their response factors with that of the corresponding sugar standard. Results were expressed as g sugar/100 g dry wt of the sample.

Detoxification experiments

Several methods were used to remove oligosaccharides from Batac A-45: soaking, boiling, roasting and germination. Analysis was performed on the processed beans.

1. Soaking

Five g whole seeds were soaked in water at 1:3 and 1:10 (w/v) bean to water ratios for 6, 12, and 24 h at 25 C.

2. Boiling

Five g whole seeds were boiled in a beaker at 1:3 and 1:10 (w/v) bean to water ratios for 15, 30, 60 and 90 min.

3. Dry-roasting

Five g whole seeds were roasted on an electric stove set at high for 0.2, 0.5, 1.0, 1.5 and 2.0 min.

4. Germination

Five g whole seeds were washed first with mercuric chloride solution to reduce surface contamination. Then, these were

soaked overnight in water and allowed to germinate in sterile Petri dishes lined with wet filter paper. Oligosaccharides in samples soaked overnight and after 24, 48, 72 and 96 h of germination were analyzed and described above.

Proximate analysis

Proximate analysis was performed according to the Methods of Analytical Association of Official Chemists (AOAC, 1980).

## Results and Discussion

Proximate analysis

Carbohydrates (nitrogen-free extract) and proteins are the major constituents of the legume samples (Table 1). Protein content ranged from 20-30% with lima bean having the lowest level (20.35%) and sam-samping with the highest (30.12%). Conversely, sam-samping had the lowest carbohydrate content (44.37%) and lima, the highest (60.66%).

Oligosaccharide content and profile

HPTLC of the ethanol extracts showed the absence of simple sugars in the seed samples (Fig. 1). Sucrose represented 30-58% of the total sugar in the mature seeds, the highest amount being found in sam-samping (9.78%) which exceeded the range of 4.5-8.6% for legume flours reported by Sosulski et al [1982].

Sam-samping had the highest total oligosaccharide content (4.79%) followed by batao (3.66%), sabawel (3.46%), lima (3.08%), rice bean (2.40%, sword bean (2.35%) and jack bean (2.25%) (Table 2). Except for jack bean, the values obtained for the total oligosaccharides of the two accessions of batao, sabawel, lima

and rice bean were similar. However, a closer examination of the oligosaccharide profile shows pronounced varietal differences. The value of 4.79% obtained for sam-samping is lower than the 5.46% to 5.90% for lupins, chickpea and cowpea (Sosulski et al, 1982). Ologhobo and Fetuga (1982) reported a high 9.50% mean total oligosaccharide for 11 varieties of lima bean.

Stachyose was the principal oligosaccharide in sam-samping, batao, lima, jackbean and sword bean with values ranging from 1.4-2.4% of dry weight or 2.8-5.4% of total carbohydrates. These legumes also contained significant amounts of raffinose (0.3-2.1%) and minimal verbascose (trace-0.4%). Sosulski et al [1982] found a different oligosaccharide profile for lima bean using gas liquid chromatography although the total oligosaccharide was similar to that obtained in this study (3.75 vs 3.05). Stachyose was also found to be the major oligosaccharide in D. lablab var lignosus but other components differed (Salimath and Tharanathan, 1982).

On the other hand, raffinose was the major galactoside of rice bean with stachyose and verbascose being minor components. Verbascose was the predominant galactoside in sabawel which also contained a considerable amount of verbascose.

Localization

The vegetative parts and flowers of batao and sabawel were also examined for the presence of oligosaccharides at mature and immature stages of development (Table 3). Results show that oligosaccharides accumulated only to a limited degree in the different parts especially in the leaves and roots. Mature seeds

had the highest concentration of the oligosaccharides. In general batao had a greater concentration of the sugars in the different parts than sabawel.

Detoxification or removal of oligosaccharides

Several simple pre-cooking and cooking methods were evaluated to determine their effect on the oligosaccharide of whole seeds of Batao A-45. Batao was selected for this series of experiments because of its relatively high oligosaccharide content and its wider utilization among the legumes studied.

The separations of stachyose and verbascose was not complete in the detoxified samples. Verbascose accounted for only 10.8% of the total oligosaccharides in batao and this appeared as a shoulder in the stachyose peak. These two sugars were therefore estimated as a single constituent.

(a) Soaking. Soaking up to 24 h did not result in any significant reduction in oligosaccharides content regardless of the time or bean to water ratio used (Fig.2). Upadhyay and Garcia (1987) reported 0 to 36% lowering of the different oligosaccharides in seeds of 4 cowpea varieties soaked in water for up to 18 hr. They cited solubility of the individual oligosaccharides and the diffusion rate as two factors that could influence sugar losses during soaking. The diffusion rate in turn would depend on the thickness and permeability of the seed coat. Mature seeds of batao have a thick and hard seed coat which could inhibit significant diffusion of oligosaccharides.

(b) Boiling. Boiling Batao A-45 mature raw seeds resulted in a net decrease of sucrose in the 1:3 (16.4%) and 1:10 (46.0%) (w/v) bean to water ratios after 90 min. The level of raffinose and stachyose + verbascose increased at the 1:3 ratio (23.3% and 31.1%, respectively). However, in the 1:10 ratio, only the level of raffinose decreased by 62.5%. Stachyose + verbascose increased by 22.2% (Fig. 3).

Ku et al. (1976) and Silva and Braga (1982) reported the greater losses of oligosaccharides when beans were cooked in 1:10 bean and water ratio than in the lower ratios. This is probably due to the increased solubility or leaching of the sugars in the medium at the higher bean to water ratio. Higher losses (47-77%) in various oligosaccharides were observed in cowpea after boiling (Upadhyay and Garcia, 1987).

The observed increase in the oligosaccharides could be attributed to hydrolysis of oligosaccharides which are bound to proteins or other macromolecules, or present as constituent of high molecular weight polysaccharides. Cerning-Beroard and Filiatre (1976) have reported the presence of high molecular weight galactosides in some legumes. A similar trend of increased oligosaccharide levels after cooking was also observed by Rao and Belavady (1978).

(c) Dry roasting. Dry-roasting at high temperature resulted in a complete reduction in the oligosaccharide content of the beans (Fig. 4). The levels of sucrose and the oligosaccharides were higher at a roasting time of less than 0.5 min. However, prolonging the roasting time to 2 min almost completely removed

the oligosaccharide probably because of non-enzymatic browning reaction, oxidation of sugars or pyrolysis.

(d) Germination. Raffinose and stachyose + verbascose decreased by 85 and 88%, respectively during germination (Fig. 5), highest decrease being observed at 4 days. Sucrose increased 3-fold whereas no monossacharides were detected. This decrease is comparable to the 89.5% decrease reported for Phaseolus mungoreus, a cross between black gram and green gram (Gupta and Wagle, 1980) during a 4-day germination test, and 88.6% and 87.8% decrease, respectively, in Bengal gram and red gram during a 3-day germination period (Rao and Belavady, 1978). It is, however, lower when compared to the 100% decrease in black eye beans and pink beans (Silva and Luh, 1979). Reddy and Salunkhe (1980) have reported increased -galactosidase activity in germinated black gram bears which is responsible for the lower oligosaccharide content of germinated beans. Other investigators reported the same findings in different types of germinated beans (Pazur et al., 1962 and Upadhyay, 1987). The increased -galactosidase activity could be responsible for the increase in sucrose and reduction of the oligosaccharide content. The absence of monosaccharides indicates their rapid utilization. The difference in the degree of decrease in the oligosaccharides during germination could be partially due to origin and cultivar differences in the levels of indigenous -galactosidase activity in different beans (Sathe et al, 1982).

Of the several processing methods used for dry bean processing, germination is a relatively simple method, does not require intensive energy input and yields a natural product.

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**Table 1. Proximate composition of mature seeds of some indigenous food legumes.**

| Variety | % Moisture | % Fat | Protein (% N x 6.25) | % Ash | % Crude | % Nitrogen Free Extract |
|---|---|---|---|---|---|---|
| Sam-samping 7-2 (*Clitoria ternatea*) | 6.20 | 2.20 | 30.12 | 6.41 | 10.54 | 44.37 |
| Batao A-45 | 6.78 | 1.70 | 21.46 | 4.05 | 4.80 | 61.21 |
| A-57 (*Dolichos lablab*) | 7.10 | 2.06 | 23.24 | 4.10 | 5.50 | 57.90 |
| Sabawel A-2 | 7.76 | 2.67 | 29.36 | 4.00 | 4.40 | 51.81 |
| A-5 (*Mucuna pruriens*) | 7.40 | 2.48 | 29.42 | 4.70 | 3.15 | 53.45 |
| Lima A-535 | 9.47 | 1.32 | 20.35 | 4.73 | 3.47 | 60.66 |
| A-537 (*Phaseolus lunatus*) | 10.20 | 1.25 | 24.65 | 4.54 | 3.20 | 56.16 |
| Rice bean 28 (*Vigna umbellata*) | 8.97 | 3.46 | 16.82 | 4.31 | 7.43 | 64.73 |
| Swordbean A-12 (*Canavalia gladiata*) | 8.76 | 2.51 | 28.05 | 4.70 | 6.35 | 49.63 |
| Jackbean A-1 | 8.40 | 2.46 | 27.82 | 3.95 | 5.50 | 51.87 |
| B-8 (*Canavalia ensiformis*) | 8.56 | 2.66 | 29.41 | 4.59 | 5.30 | 49.48 |

**Table 2. Non-reducing sugar profile of seven Philippine indigenous food legumes.**

| Sample | % Non-reducing sugar | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | % Sucrose | | % Raffinose | | % Stachyose | | % Verbascose | | % Total oligo-saccharides |
| | A | B | A | B | A | B | A | B | A |
| Sam-sampling 7-2 (*Clitoria ternatea*) | 4.34 | 9.78 | 2.11 | 4.76 | 2.42 | 5.45 | 0.26 | 0.59 | 4.79 |
| Batao A-45 | 1.62 | 2.65 | 1.20 | 1.96 | 2.12 | 3.46 | 0.36 | 0.59 | 3.68 |
| A-57 (*Dolichos lablab*) | 1.79 | 3.09 | 1.13 | 1.95 | 2.07 | 3.58 | 0.44 | 0.76 | 3.64 |
| Sabawel A-2 | 2.60 | 5.02 | 1.40 | 2.70 | 0.97 | 1.87 | 1.20 | 2.32 | 3.57 |
| A-5 (*Mucuna pruriens*) | 2.37 | 4.43 | 1.12 | 2.10 | 1.02 | 1.91 | 1.22 | 2.28 | 3.36 |
| Lima A-535 | 1.68 | 2.77 | 0.93 | 1.53 | 1.87 | 3.08 | 0.09 | 0.15 | 2.89 |
| A-537 (*Phaseolus lunatus*) | 2.02 | 3.60 | 1.11 | 1.98 | 1.[illegible] | 3.54 | 0.12 | 0.21 | 3.22 |
| Rice bean 28 | 1.86 | 3.17 | 1.14 | 1.94 | 0.37 | 0.63 | 0.74 | 1.26 | 2.25 |
| 46 (*Vigna umbellata*) | 3.50 | 5.90 | 1.73 | 2.92 | 0.37 | 0.62 | 0.45 | 0.76 | 2.55 |
| Swordbean A-12 (*Canavalia gladiata*) | 2.57 | 5.18 | 0.91 | 1.83 | 1.44 | 2.90 | tr | tr | 2.35 |
| Jackbean A-1 | 1.49 | 2.87 | 0.34 | 0.66 | 1.44 | 2.78 | 0.01 | 0.02 | 1.79 |
| B-8 (*Canavalia ensiformis*) | 2.47 | 4.99 | 0.86 | 1.74 | 1.74 | 3.52 | 0.11 | 0.22 | 2.71 |

A- percentage of dry mature seeds

B- percentage of carbohydrates (Nitrogen-free extract)

**Table 3.** Oligossacharide contents of the different plant parts of Batao and Sabawel profile at the mature and immature stages.

| Plant parts | % Raffinose | % Stachyose + Verbascose |
|---|---|---|
| Batao A=45 | | |
| Mature seeds | 1.20 | 2.48 |
| Immature stem | 0.37 | 0.18 |
| Mature stem | 0.17 | tr |
| Immature leaves | 0.24 | 1.10 |
| Mature leaves | 0.59 | 0.39 |
| Immature roots | 0.05 | 0.02 |
| Mature roots | 0.55 | 0.19 |
| Flowers | 0.79 | 0.22 |
| Batao A=52 | | |
| Mature seeds | 1.13 | 2.51 |
| Immature stem | 0.32 | 0.13 |
| Mature stem | 0.20 | 0.01 |
| Immature leaves | 0.26 | 0.09 |
| Mature leaves | 0.46 | 0.39 |
| Immature roots | 0.03 | 0.01 |
| Mature roots | 0.49 | 0.21 |
| Flowers | 0.55 | 0.17 |
| Sabawel A=2 | | |
| Mature seeds | 1.40 | 2.17 |
| Immature stem | 0.01 | tr |
| Mature stem | 0.13 | tr |
| Immature leaves | 0.02 | tr |
| Mature leaves | 0.03 | tr |
| Immature roots | 0.03 | tr |
| Mature roots | tr | tr |
| Flowers | 0.01 | tr |
| Sabawel A=5 | | |
| Mature seeds | 1.12 | 2.24 |
| Immature stem | tr | tr |
| Mature stem | 0.03 | 0.01 |
| Immature leaves | 0.03 | 0.01 |
| Mature leaves | 0.05 | 0.05 |
| Immature roots | 0.02 | 0.01 |
| Mature roots | 0.02 | tr |
| Flowers | 0.01 | 0.10 |

Legends to Figures

1. HPTLC chromagtogram of sugars in the raw mature seeds of 1-Batao A-45, 2-Jackbean 8-8, 3-Sword bean, 4-Sabawel A-2, 5-Sam-samping, 6-Lima bean and standards, G-glucose, S-sucrose, R-raffinose and ST-stachyose.

2. Effect of soaking in water on the soluble sugars of Batao A-45 at 1:3 and 1:10 (w/v) bean to water ratios.
   ● or ○ sucrose; ■ or □ raffinose, ▲ or △ stachyose + verbascose; bean to water ratio, ____ , 1:3 and ----, 1:10.

3. Effect of boiling on the soluble sugars of Batao A-45 at 1:3 and 1:10 (w/v) bean to water ratios.
   ● or ○ sucrose; ■ or □ raffinose, ▲ or △ stachyose + verbascose; bean to water ratio, ____ , 1:3 and ----, 1:10.

4. Effect of dry roasting on the soluble sugars of Batao A-45.
   ● sucrose; ■ raffinose; ▲ stachyose + verbascose.

5. Effect of germination on the soluble sugars of Batao A-45.
   ● sucrose; ■ raffinose, ▲ stachyose + verbascose.

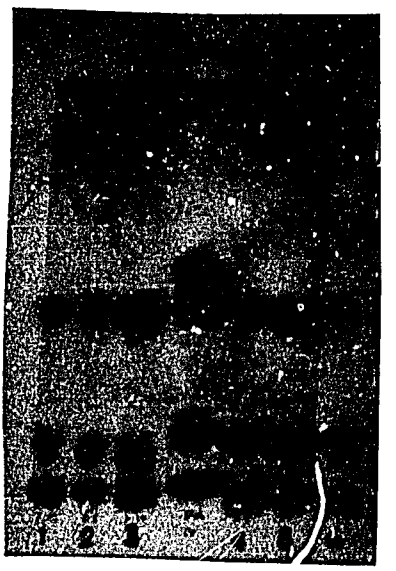

Fig. 1. HPTLC of sugars in the raw mature seeds of 1-Batao A-45, 2-Jackbean 8-8, 3-Swordbean A-12; 4-sabawel A-2; 5-Sam-samping, 6-Lima A-535 with standards: G-glucose; S-sucrose; R-raffinose and ST-stachyose.

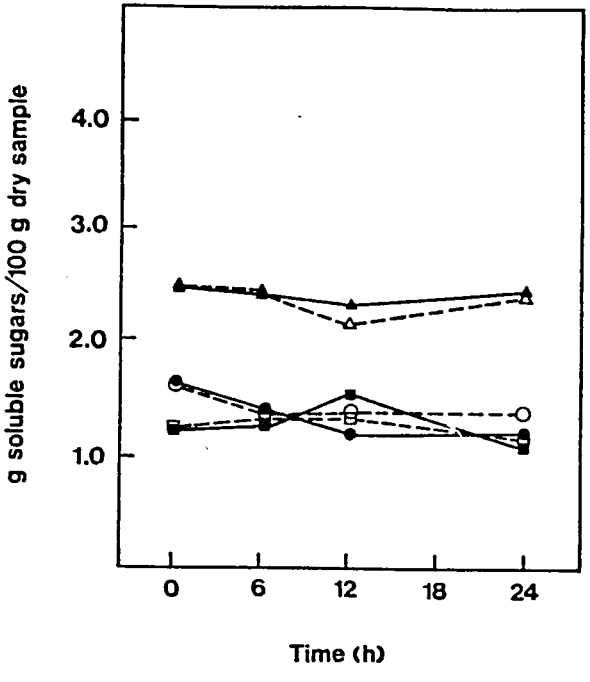


Fig. 2. Effect of soaking in water on the soluble sugars of Batao A-45 at 1:3 and 1:10 (w/v) bean to water ratios.

● or ○ sucrose; ■ or □ raffinose, ▲ or △ stachyose + verbascose; bean water ratio, ____, 1:3 and ----, 1:10.

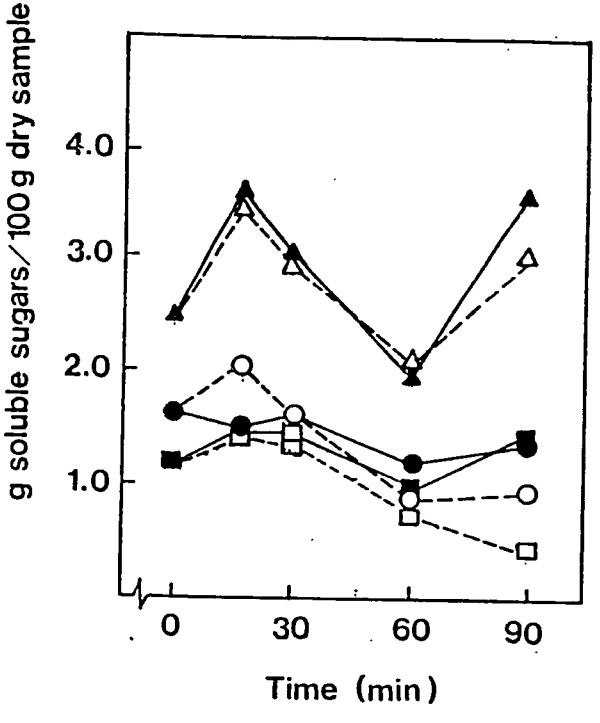


Fig. 3. Effect of boiling on the soluble sugars of Batao A-45 at 1:3 and 1:10 (w/v) bean to water ratios.

● or ○ sucrose; ■ or □ raffinose, ▲ or △ stachyose + verbascose; bean to water ratio, ____, 1:3 and ----, 1:10.

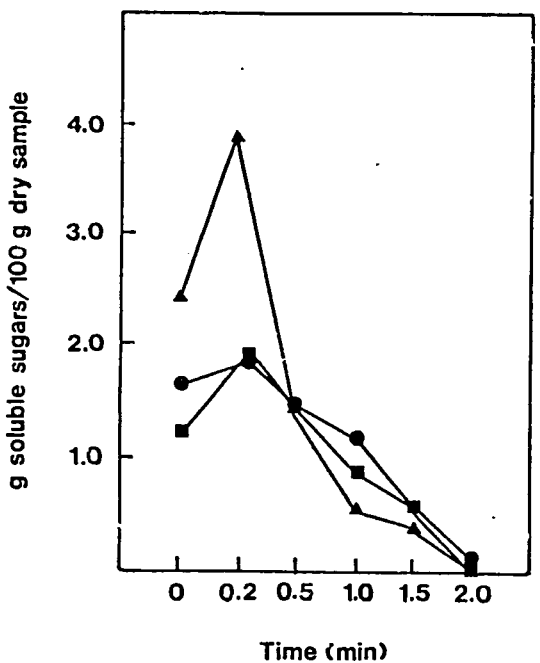


Fig. 4. Effect of dry roasting on the soluble sugars of Batao A-45.

● sucrose; ■ raffinose;
▲ stachyose + verbascose.

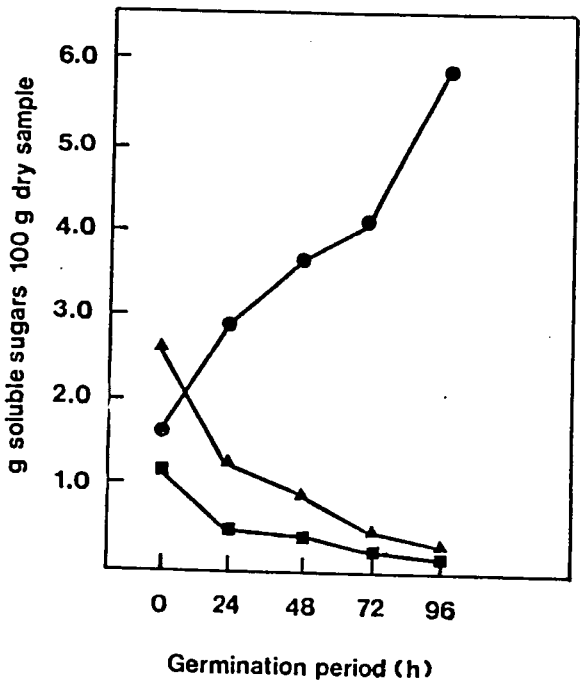


Fig. 5. Effect of germination on the soluble sugars of Batao A-45.

● sucrose; ■ raffinose;
▲ stachyose + verbascose

# Changes in Oligosaccharides, Soluble Sugars and Hydrolyzable Polysaccharides in Developing Seeds of Batao (*Dolichos lablab*) and Sabawel (*Mucuna pruriens* or *cochinchinensis*)[a]

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**Keywords:** oligosaccharides, raffinose, stachyose, verbascose, batao or hyacinth bean (*Dolichos lablab*), sabawel (*Mucuna pruriens* or *cochinchinensis*), ontogeny, hydrolyzable polysaccharides.

**Abstract:** During seed maturation of batao and sabawel, hydrolyzable polysaccharide increased in the cotyledon while it decreased in the pod wall. As disaccharides decreased in the cotyledon, the oligosaccharides raffinose and stachyose started to increase. Verbascose was detected one week later in sabawel and even later in batao. The oligosaccharides were very low or nondetectable in the podwall and seed coat of both batao and sabawel. Monosaccharides were detected in the podwall and seed coat but not in the cotyledon.

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This study aimed at determining the levels and patterns of the monosaccharides, disaccharides, oligosaccharides and the hydrolyzable polysaccharides in the developing seed of batao and sabawel. Results from this study are expected to give a better understanding of the metabolism of carbohydrates in the maturing seed with emphasis on oligosaccharide synthesis.

**Materials and Methods**

Samples

Seeds of two accessions each of Batao (A-45 and A-57) (*Dolichos lablab*) and sabawel (*Mucuna pruriens* or *conchichinensis*) (A-2 and A-5) were obtained from the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding.

Ten plants of each accession were planted 1 m apart. The first flowers that appeared in the lateral branches of the plants were tagged for subsequent sample collections.

Chemicals

Raffinose and stachyose were obtained from Nakarai Chemicals Ltd. Verbascose was prepared from mung bean by ethanol extraction and gel filtration chromatography using Bio-gel P.2, 100-200 mesh with distilled water. HPTLC plates precoated with silica gel 60 without indicator were from Merck (West Germany). Other chemicals used were of analytical grade.

Hydrolyzable polysaccharides

Total hydrolyzable polysaccharides were determined according to the method of Dawson et al (1969). Fifty mg dry sugar-free sample were hydrolyzed with 10 mL of 5% $H_2SO_4$ in a boiling water bath for 2.5 h. An aliquot containing 2 to 10 ug sugar was color developed with 3.0 mL of 0.2% anthrone in sulfuric acid in a boiling water bath for 10 min. The amount of hydrolyzable polysaccharides was calculated from a standard curve of glucose with a correction factor of 0.9 to account for the water that was lost during the condensation of monosaccharides to form the polysaccharides.

Determination of Oligosaccharide Profile and Content

The procedures described by Revilleza et al (1988) were followed.

**Results and Discussion**

Physical changes during development

Pod wall and seed formation commenced immediately after anthesis (Fig. 1). The start of seed maturation was indicated by a decrease in the fresh weight of the cotyledon and seed coat. For batao, maximum pod wall and seed coat weight was attained at 21 days after anthesis while cotyledon weight started to decrease at 24 days. Batao matured after 28 days.

For sabawel, pod wall weight was highest at 49 days after anthesis, seed coat and cotyledon weight were maximal starting at 42 days and started to decrease at 56 days. Sabawel matured after 63 days.

Moisture content of the different pod parts decreased gradually as pod development progressed but declined sharply when the pod was completely mature (data not shown).

The differences in rates of maturation for pod and seed were similarly observed in developing chickpea (Singh and Jambunathan, 1982) and french and soybeans (Onebedeany and Chollet, 1975).

Changes in monosaccharides, disaccharides, oligosaccharides and hydrolyzable polysaccharides

Raiap. As the cotyledon developed, disaccharides decreased until maturity while hydrolyzable polysaccharides which represent the storage polysaccharide in the seed, increased (Fig. 2a). Monosaccharides were not detected in the cotyledon. Raffinose and stachyose were at relatively high levels (1%) at 14 days and increased steadily. Verbascose appeared later at 24 days. In contrast, oligosaccharides were detected much later in the drying phase of the ripening lupin seed (Saini and Lymbery, 1983) and in the later period of seed development in chickpea (Singh and Jambunathan, 1982). The sequential formation of the oligosaccharides has been cited by several authors (Shallenberg and Moyer, 1961; Saini and Lymbery, 1983).

In the seed coat, monosaccharides and disaccharides increased up to 20 days after anthesis and decreased thereafter (Fig. 2b). Hydrolyzable polysaccharide level was steady until 24 days after which it increased concomittant with the decrease in the soluble sugars. Stachyose and raffinose were very low (0.02-0.03%) and verbascose was not detected in the seed c at.

In the pod wall, the increase in hydrolyzable polysaccharides was accompanied by a decrease in mono-and di-saccharides. Stachyose and raffinose were very low (0.01-0.25%) in the maturing pod wall.

Although an increase in the accumulation of hydrolyzable polysaccharides in the cotyledon was accompanied by a marked decline of the same in the pod wall, it is not possible to determine whether the hydrolyzable polysaccharide in the pod wall was mobilized and translocated in the cotyledon. Radioisotope experiments with bean cultivars showed the non-dependence of the seed on the pod for photosynthetic product [Oliker et al, 1978]. It can also be noted that the decrease in the hydrolyzable polysaccharide of the pod wall cannot account for its large increase in the cotyledon.

Sahawei. As the cotyledon developed and matured, disaccharides increased and started to decline at 42 days after anthesis (Fig. 3a) during the yellowing stage of maturation. Hydrolyzable polysaccharide increased steadily. At 35 days in the later stage of seed development, the oligosaccharides stachyose and raffinose started to increase from 0.1 to 1.3%. Verbascose was detected later at 49 days.

Monosaccharides were detected in the seed coat (0.2-0.5%) and podwall (0.2-1.2%) but not in the cotyledon. The oligosaccharides were very low (traces-0.05%) in both seed coat and podwall.

In the pod wall, hydrolyzable polysaccharides decreased steadily, while both mono-and dissacharides increased up to 35 and 42 days, respectively, and then declined.

Similar patterns of changes for the various carbohydrate components were obtained for the two batao and two sabawel accessions studied. Thus only the results for one accession for each of the two legumes were presented.

The results above show variation in carbohydrate changes in batao and sabawel. Differences were also evident in levels of the mono- and dissacharides and hydrolyzable polysaccharides for the two legumes although the trends especially during the latter part of maturation were similar.

The oligosaccharide synthesis in both batao and sabawel was accompanied by a decrease in dissacharide (sucrose). Part of the sucrose is probably used in the synthesis of the oligosaccharides. It has been shown that raffinose could be synthesized with a mixture of sucrose, -galactose-1-phosphate and UTP in the presence of crude enzyme preparation for sword bean [Pridham and Hassid, 1965].

In both batao and sabawel, raffinose and stachyose were synthesized earlier than verbascose which agree with the sequential synthesis pattern for the oligosaccharides.

**Acknowledgement**

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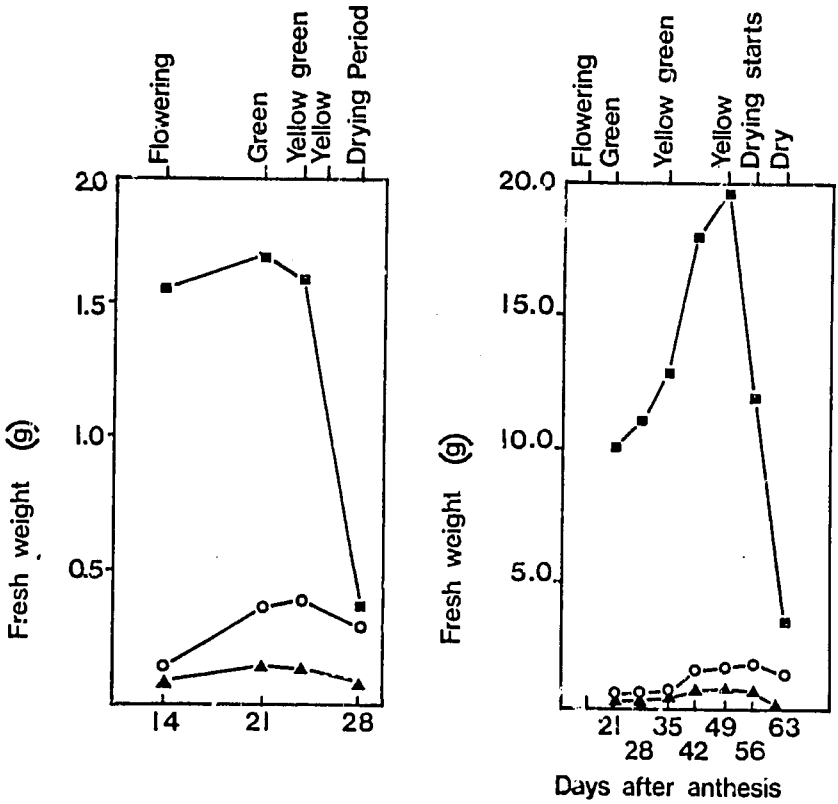


Fig. 1. Pod development of (a) Batac - A45 and (b) Sabawel A-2
■ pod wall; ○ cotyledon; ▲ seed coat.

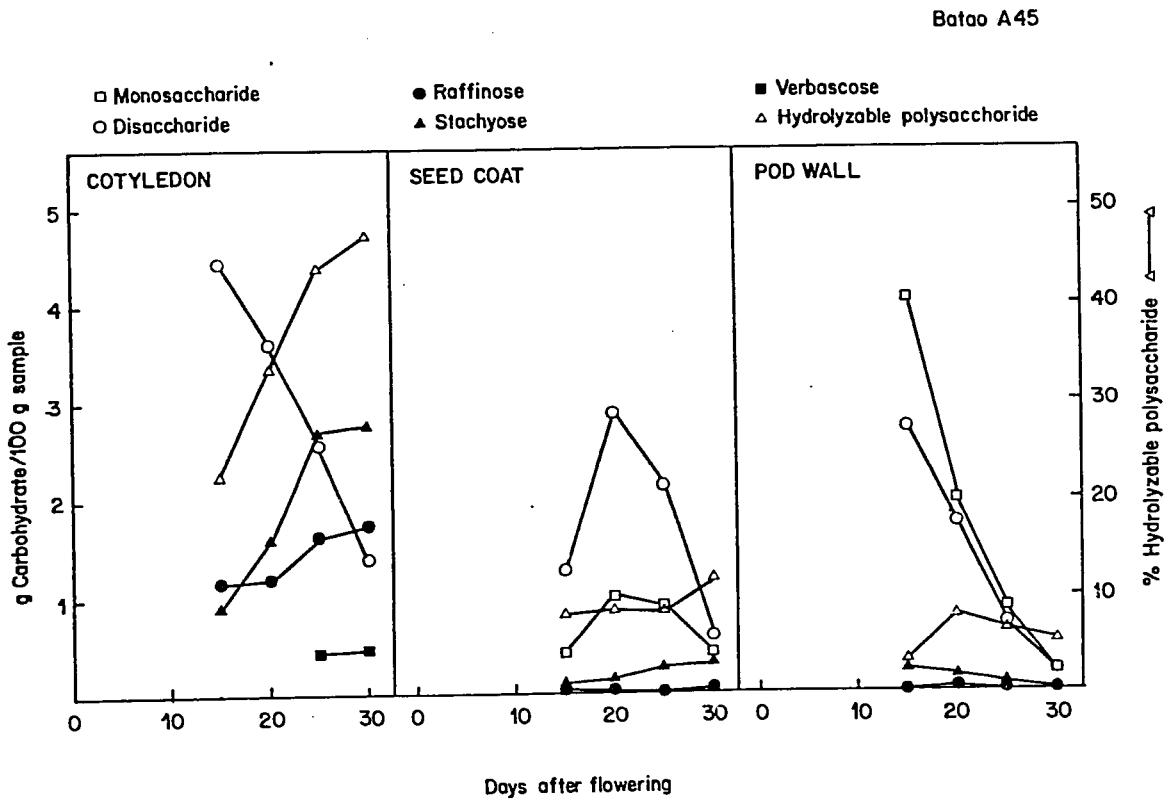


Fig. 2. Changes in monosaccharides, disaccharides, oligosaccharides and hydrolyzable polysaccharides in maturing seed of batao (Dolichos lablab).

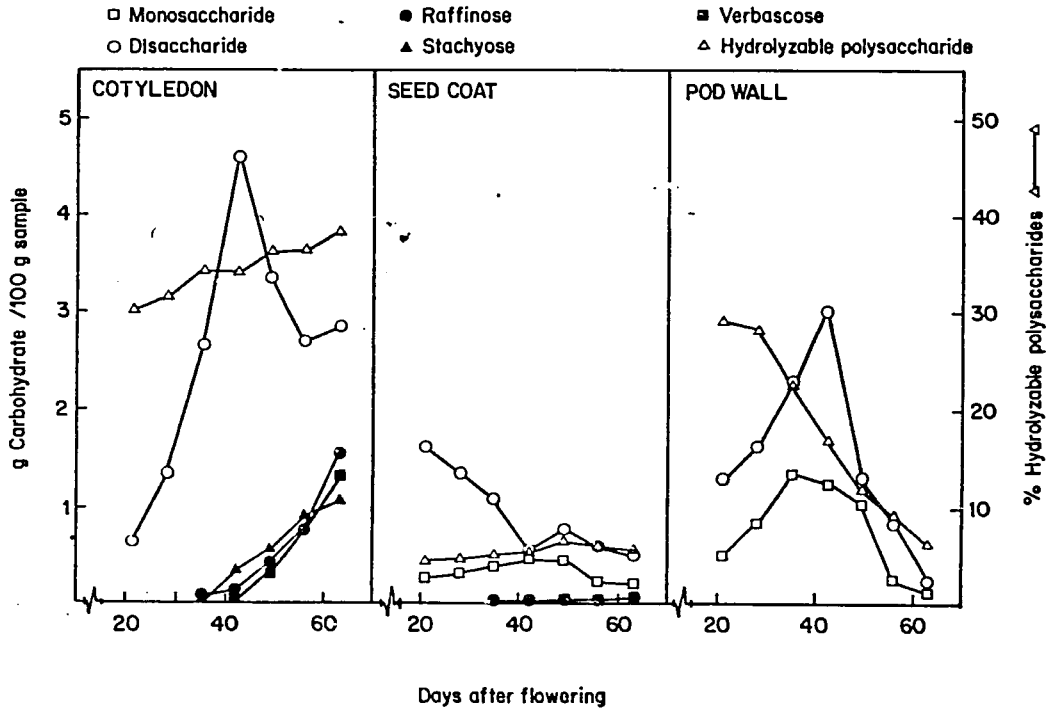


Fig. 3. Changes in monosaccharides, disaccharides, oligosaccharides and hydrolyzable polysaccharides in maturing seed of sabawel (Mucuna pruriens or conchinchinensis).

# DETERMINATION OF SAPONINS IN PHILIPPINE INDIGENOUS FOOD LEGUMES

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## ABSTRACT

Saponin content in mature seeds of seven legumes ranged from 0.09 to 0.44%. Sam-samping had the highest content of 0.44%. In mature pods, saponin ranged from 0.17 to 1.63%. Batao pods had high levels (1.00 to 1.63%) in four accessions. No correlation was found between results of the froth test and Liebermann-Burchard colorimetric test for saponins.

## INTRODUCTION

Saponins are glycosides of either triterpenoid or steroid aglycone structure described as having a spiroketal chain. They have the ability to froth when agitated as an aqueous solution and have a bitter taste. They exhibit strong hemolytic activity on red blood cells (RBC). These two properties--ability to froth and hemolyze RBC, are the basis for biological assays for saponins. Saponins are toxic when injected into the blood stream but are harmless when taken orally.

The exact mechanisms of the anti-physiological activity of saponins are not well established but could possibly be either or both of the following: (a) they lower food intake due to poor palatability or irritation of the gastrointestinal tract and (b) inhibition of enzymes and reduced availability of nutrients due to the formation of saponin-mineral complex (Cheeke, 1976).

This study aimed to estimate the level of saponins in food legumes which are indigenous to the Philippines by at least two methods.

## MATERIALS AND METHODS

### Materials

Raw mature seeds of legumes indigenous to the Philippines were obtained from the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding. These were planted following recommended practices and newly harvested seeds and raw mature pods were used for the analyses. The samples used

were: batao or hyacinth bean (Dolichos lablab), jackbean (Canavalia ensiformis) lima bean (Phaseolus lunatus), sabawel (Mucuna pruriens or cochinchinensis) sword bean (Canavalia gladiata), sam-samping (Clitoria ternatea) and rice bean (Vigna umbellata).

All chemicals used were analytical grade. Soybean as a source of saponin was used as a standard.

**Preparation of Samples**

Finally ground seeds (60 mesh) were defatted and extracted with 80% ethanol (0.5 g: 5 mL) under reflux condition at 50 C in an evapo-mix for 15 min and centrifuged at 2000 rpm in a Kubota centrifuge. The samples were re-extracted two more times using above procedure. The supernates were combined and made to 15 mL. About 3 mL of the extract was passed through a column of polyvinylpyrollidone (0.50 x 15.0 cm) previously hydrated overnight. Filtrates were set aside for the analyses.

**Tests for Saponin**

(1) Modified Liebermann-Burchard Test. The Liebermann-Burchard test was modified so that the color is clear and stable to determine saponin quantitatively. Plant extract (0.10 mL) was treated with 0.5 mL of concentrated acetic acid, followed by 3.0 mL of the Liebermann-Burchard reagent (30% acetic anhydride in 52% sulfuric acid) (Oakenfull, 1984). One tube for each sample was heated in a 90-100 C water bath for 10 min; another tube which is unheated is used as reference to account for further interfering compounds. After cooling to room temperature, absorbance was read at 450 nm. Saponin values were calculated

relative to soybean UPL SY2 (0.63% saponin).

2) Froth Test. To 1 mL of extract was added 3.0 mL of distilled water. The mixture was shaken by hand for 1 min, allowed to stand for another min and froth formation recorded as follows: - no froth; +, froth at side of tube only; ++, froth covers tube surface and +++, froth height greater than 0.50 cm.

## RESULTS AND DISCUSSION

Among the legumes analyzed, sam-samping had the highest level (0.44%) and two accessions of sword bean had the lowest level (0.06 and 0.09%) (Table 1). In general, the values obtained for the samples were much lower than the 0.63% saponin of soybean.

In general, no correlation was observed between the values of the froth test and the Liebermann-Burchard tests, similar to those previously reported (Birk, 1969). The Liebermann-Burchard test is a general test for triterpenoids and steroids. Strong hemolytic activity was also not always associated with frothing ability and vice-versa (Birk et al, 1963; Gestetner et al, 1963). Since more of the tests for saponins can be considered as unequivocal indication of the presence of saponins, it is necessary that at least two of the tests show highly positive responses.

Among the five samples of mature pods tested, four accessions of batao had very high saponin (0.98 to 1.63%) followed by a jack bean accession (0.74%) and sam-samping (0.67%)

(Table 2). The mature pods had higher saponin than the mature seeds. Notably, sam-samping was high in saponin in both mature seeds and pods.

Batao A-45 had high Liebermann-Burchard and froth test results. Again no correlation was evident between the colorimetric and froth tests.

Soybean saponins are the most widely studied perhaps because of the economic uses of soybean in food and feed industries. An exhaustive study showed no adverse effects on growth, food utilization, blood counts, blood glucose, blood nonprotein nitrogen and urine analysis or in gross or histological findings post mortem, on chicks, mice and rats by soybean saponins (reviewed by Birk, 1969). Saponins have also been reported to be present in peanut (Diechert and Morris, 1958), navy bean, cowpea, northern bean, lima bean and lentil (Elkowics and Sosulski, 1982) but their nutritional significance still has to established.

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**Table 1. Saponin content of mature seeds of several Philippine indigenous legumes.**

| Sample | | % Saponin[a] | Froth test |
|---|---|---|---|
| *Dolichos lablab* | | | |
| Batao | A-53 | 0.27 | + |
| *Canavalia ensiformis* | | | |
| Jackbean | A-1 | 0.11 | ++ |
| *Canavalia piadiata* | | | |
| Sword bean | A-9 | 0.06 | + |
| | A-4 | 0.09 | + |
| | B-4 | 0.19 | ++ |
| | 6-3 | 0.16 | +++ |
| | B-6 | 0.10 | +++ |
| | B-9 | 0.13 | +++ |
| *Mucuna pruriens* | | | |
| Sabawel | A-2 | 0.24 | + |
| | 6-1 | 0.12 | +++ |
| | 6-2 | 0.22 | +++ |
| *Phaseolus lunatus* | | | |
| Lima bean | A537 | 0.33 | +++ |
| | A535 | 0.32 | + |
| | A544 | 0.17 | +++ |
| *Clitoria ternatea* | | | |
| Sam-samping | 7-2 | 0.44 | nd |
| *Vigna umbellata* | | | |
| Rice bean | 46 | 0.33 | nd |

[a] Fat free basis: used soybean UPL Sy2 as standard with 0.63% saponin.

**Table 2. Saponin content of mature pods of several Philippine indigenous legumes.**

| Sample | | | %Saponin[a] | Froth test |
|---|---|---|---|---|
| *Dolichos lablab* | | | | |
| Batao | A-15 | | 1.63 | ++ |
| | A-35 | | 1.20 | ++ |
| | A-37 | | 1.20 | + |
| | A-45 | | 1.00 | +++ |
| | A-51 | | 0.98 | - |
| | A-52 | | 0.18 | - |
| *Canavalia ensiformis* | | | | |
| Jackbean | A-1 | | 0.10 | + |
| | A-3 | | 0.74 | ++ |
| | A-5 | | 0.17 | |
| *Canavalia gladiata* | | | | |
| Sword bean | A-9 | | 0.31 | ++ |
| *Mucuna pruriens* | | | | |
| Sabawel | A-8 | | 0.27 | + |
| *Clitoria ternatea* | | | | |
| Sam-samping | 7-2 | | 0.67 | ++ |

[a] Fat free basis; used soybean UPL SY2 as standard with 0.63% saponin.

# ALKALOIDS OF SEVERAL PHILIPPINE INDIGENOUS FOOD LEGUMES: DETERMINATION AND REMOVAL

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## ABSTRACT

Eight legumes indigenous to the Philippines were screened for the presence of alkaloids. These are: batao or hyacinth bean (Dolichos lablab), jack bean (Canavalia ensiformis), sword bean (C. gladiata), sam-samping (Mucuna pruriens or cochinchinensis), sabawel (Clitoria ternatea), pigeon pea (Cajanus cajan), lima bean (Phaseolus lunatus) and rice bean (Vigna umbellata). High to very high levels of alkaloids were detected only in mature and immature leaves of pigeon pea, jack bean and sword bean. Soaking the leaves in water at 60 C for 60 min reduced completely the alkaloids. Soaking at 30 C for 30 to 45 min and at 45 C for 30 min did not remove the alkaloids while soaking at 45 C for 60 min and at 60 C for 30 min partially removed the alkaloids.

## INTRODUCTION

Alkaloids are a chemically heterogeneous group. They all usually contain nitrogen usually in a heterocylic ring and many, if not all, are basic. Classification of alkaloids is based on the ring system present, e.g., pyridine, piperidine, tropane and isoquinoline (Robinson, 1975). Due perhaps to differences in their chemical structure, alkaloids have varying physiological effects; among these are emetric, anesthetic, antihemorragic, antispasmodic, tranquilizer, cardiac depressant, etc (Robinson, 1975).

The physiological role of alkaloids are not well established. Some of the roles ascribed to alkaloids are: (1) for protection against herbivorous animals, insect pests and pathogens, (2) for removal of toxic substances such as free ammonia, (3) for regulation as co-enzyme or precursors of co-enzymes or activators or inhibitors of enzymes. However, there seem to be no general observations that could be made for any of these. That they are not simply waste products of metabolism could be proved by the fact that 90 or more percent of plants do not form them and do manage well without them (James, ).

Alkaloids are considered anti-nutritional because in general, they have a bitter taste which could limit food intake and some have toxic effects. Some also have medicinal uses, thus the interest in screening for alkaloids in plants could be due to their potential medical and pharmacologic uses.

Aquinaldo et al (198 ) surveyed a total of 640 plant species located in selected areas of Luzon and found 43 species belonging

to 23 families representing 36 genera positive for alkaloids.

This study aimed at determining the presence of alkaloids in several Philippine indigenous food legumes and how they can be removed by simple methods.

## MATERIALS AND METHODS

### Materials

Seeds of indigenous food legumes were obtained from the National Plant Genetic Research Laboratory (NPGRL) of the Institute of Plant Breeding and planted to obtain fresh materials of leaves, pods and seeds for analysis. The following legumes were studied: batao or hyacinth bean (Dolichos lablab), pigeon pea (Cajanus cajan), jack bean (Canavalia ensiformis), sabawel (Mucuna pruriens or cochinchinensis), sam-samping (Clitoria ternatea), sword bean (Canavalia gladiata) and rice bean (Vigna umbellata).

All chemicals used were of analytical grade.

Samples of seeds, leaves and pods were dried at 45 C for 48 hr, ground and passed through 40 mesh.

### Analysis for Alkaloids

Screening for the presence of alkaloids was done according to Hultin and Torsell (1965). Each sample (4 g dried materials, 40 mesh) was incubated with 40 mL of methanol overnight and then warmed for 4 hr at 50 C. The mixture was filtered; the residue was washed with 20 mL of methanol and the combined extracts evaporated *in vacuo*. The residue was suspended in methanol (2 mL) and 12 mL of 1% HCl added. The mixture was shaken and filtered; another 8 mL of 1% HCl was used to wash the residue.

The filtrate was made basic with concentrated NH3 and extracted with three 20 mL of chloroform (Fraction A). The aqueous solution was next made half-saturated with sodium sulfate and extracted three times with 20 mL of chloroform: ethanol (3:2 v/v) (fraction B). The organic phases were washed with 5 mL of half-saturated sodium sulfate solution and dried with anhydrous sodium sulfate.

The two extracts were evaporated separately in vacuo and 1.0 mL of HCl and 1.0 mL of chloroform were added to each with vigorous shaking. The aqueous phase from each was pipetted off, filtered through cotton and divided into six portions. These samples were tested with the following alkaloid reagents: Mayer's, Wagner's, Dragendorff's, Sonnenschien's silicotungstic, and Hager's reagent. The amounts of precipitate found were compared to those resulting from caffeic acid solutions.

**Treatments for Removal of Alkaloids**

Two hundred g of dried ground sample was mixed with 250 mL of distilled water. The mixture was placed in a water bath at 30 C for 15 min and centrifuged, and the supernate removed. The residue was recentrifuged and the remaining liquid decanted. The residue was dried and subjected to alkaloid extraction and analysis, according to Hultin and Torsell (1965).

The process was repeated for the following treatments: 30 C, 60 min; 45 C, 30min; and 45 C, 60min.

## RESULTS AND DISCUSSION

### Screening

Among the 8 legumes analyzed, only pigeon pea, jack bean and sword bean were positive to highly positive for the presence of alkaloids (Table 1). In these legumes, only the mature and immature leaves had alkaloids. Jack bean A-8, sword bean A-4, A-9, and A-12 had high levels of alkaloids in both mature and immature leaves. Batao, sabawel, sam-samping, and rice bean were all negative to alkaloids in all plant parts analyzed, viz, mature seeds, immature and mature pods, and immature and mature leaves.

Leaves of pigeon pea have been reported to have many folk medicinal uses such as for sores, jaundice, to expel bladderstones, skin irritations etc (Morton, 1976). Such medicinal uses could be accounted for by the alkaloids. No previous report on such alkaloids in pigeon pea has been made. Jack bean has been reported to contain choline and trigonelline (Duke, 1981) both nitrogen containing heterocyclic structures. Choline is an essential nutrient for man while trigonelline is a product from the metabolism of niacin; it is devoid of the antiblacktongue activity and is quantitatively excreted on administration to man and dogs (White et al. 1973). Some folk medicinal uses have been ascribed to sword bean but not to jack bean (Duke, 1981). Aguinaldo et al (198 ) singularly found leaves of *D. lablab* and *C. cajan* to be positive for alkaloids.

**Removal of Alkaloids**

Simple treatments consisting of soaking in water at 30, 45 and 60 C for 30 to 60 min were tried on leaves of pigeon pea, jack bean and sword bean. Soaking mature leaves of these legumes at 60 C and for 60 min was the only treatment which was effective in removing the alkaloids completely (Table 2). Soaking at 45 C for 60 min or at 60 C for 30 min decreased the alkaloid level.

Similar results were obtained with immature leaves (Table 3). Only the 60 C - 60 min soaking treatment completely removed the alkaloids from the immature leaves.

Young et al (1983) showed that total alkaloid contents of wheat ergot sclerotia were reduced by 90% within 4 hr by treatment using chlorine and heat (150 and 200 C) but only slightly (< 20%) by sulfur dioxide and hydrogen chloride. Autoclaving ergot sclerotia at 121 C for 30 min reduced alkaloids by 26% only. The need for chemical treatment in reducing alkaloids in ergot sclerotia could be due to the difficulty in penetration of the sclerotial tissues.

**Remarks**

This study identified pigeon pea, jack bean and sword bean leaves as rich sources of alkaloids. The nature of these alkaloids can be further studied as well as their physiological effects and medicinal uses.

**ACKNOWLEDGEMENT**

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**Table 1. Alkaloid content of the raw mature seeds, mature and immature pods and mature and immature leaves of some indigenous food legumes.**

| Variety/ Accession No. | | Alkaloid content | | | | |
|---|---|---|---|---|---|---|
| | | mature seeds | immature pods | mature pods | immature leaves | mature leaves |
| *Dolichos lablab* | | | | | | |
| Batao | A-37 | - | - | - | - | - |
| | A-45 | - | - | - | - | - |
| | A-41 | - | - | - | - | - |
| | A-52 | - | - | - | - | - |
| | A-57 | - | - | - | - | - |
| Cajanus cajan BP-W | | | | | | |
| Pigeon pea | | - | nd | nd | + | +++ |
| *Phaseolus lunatus* | | | | | | |
| Lima bean | A-508 | - | nd | nd | nd | nd |
| | A-513 | - | - | - | - | - |
| | A-517 | - | - | - | - | - |
| | A-525 | - | - | - | - | - |
| | A-537 | - | - | - | - | - |
| | A-538 | - | nd | nd | nd | nd |
| | A-541 | - | - | - | - | - |
| | A-544 | - | - | - | - | - |
| | 546 | - | - | - | - | - |
| *Canavalia ensiformis* | | | | | | |
| Jackbean | A-1 | - | nd | nd | nd | nd |
| | A-3 | - | - | - | + | + |
| | A-5 | - | - | - | +++ | + |
| | A-6 | - | nd | nd | nd | nd |
| | A-8 | - | - | - | +++ | +++ |
| | 8-8 | - | nd | nd | nd | nd |
| *Mucuna curanii* | | | | | | |
| Sabawel | A-2 | - | - | - | - | - |
| | A-5 | - | - | - | - | - |
| | A-8 | - | - | - | - | - |
| *Clitoria ternates* | | | | | | |
| Sam-samping | 7-2 | - | nd | nd | nd | nd |

| Variety/ Accession No. | | Alkaloid content | | | | |
|---|---|---|---|---|---|---|
| | | mature seeds | immature pods | mature pods | immature leaves | mature leaves |
| *Canavalia gladiata* | | | | | | |
| Swordbean | A-3 | - | - | - | + | + |
| | A-4 | - | - | - | +++ | +++ |
| | A-5 | - | | | | |
| | A-9 | - | - | - | +++ | +++ |
| | A-12 | - | - | - | +++ | +++ |
| *Vigna umbellata* | | | | | | |
| Rice bean | 26 | - | nd | nd | nd | nd |
| | 28 | - | nd | nd | - | - |
| | 46 | - | nd | nd | - | - |

Higly positive +++; positive +; negative -
nd, not determined.

**Table 2. Removal of alkaloids from mature leaves of pigeon pea, jack bean and sword bean.**

| Accession | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | 30 C | | 45 C | | 60 C | |
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min |
| Pigeon pea | +++ | + | + | - | - | - |
| Jack bean A-3 | + | + | + | - | + | - |
| A-5 | + | + | +++ | + | + | - |
| A-8 | +++ | +++ | +++ | + | + | + |
| Sword bean A-3 | + | + | + | - | + | - |
| A-4 | +++ | +++ | +++ | + | + | - |
| A-9 | +++ | +++ | +++ | + | + | - |
| A-12 | +++ | +++ | +++ | + | + | - |

Highly positive, +++; positive, +; negative - .

**Table 3. Removal of alkaloids from immature leaves of pigeon pea, jack bean and sword bean.**

| Accession | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | 30 C | | 45 C | | 60 C | |
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min |
| Pigeon pea | + | + | + | + | - | - |
| Jack bean A-3 | + | + | + | - | - | - |
| A-5 | +++ | +++ | +++ | + | + | - |
| A-8 | +++ | +++ | +++ | + | + | - |
| Sword bean A-3 | + | + | + | + | + | - |
| A-4 | +++ | +++ | +++ | + | + | - |
| A-9 | +++ | +++ | +++ | + | + | - |
| A-12 | +++ | +++ | +++ | + | + | - |

Highly positive, +++; positive, +; negative, -.

# Lectins in Philippine Indigenous Food Legumes. I. Screening

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## ABSTRACT

Several accessions each of seven different legumes were screened for the presence of hemagglutinins or lectins. Jackbean, sword bean and batao had consistently high levels of hemagglutininins while lima bean had the least amount. Batao had a strong hemagglutinating activity against human type A RBC while others had strong reaction with either or both rabbit and human type O RBC. Varietal differences were also observed.

**Keywords:** lectins, phytohemagglutinins, legumes, lima bean (*Phaseolus lunatus* L.), sword bean (*Canavalia gladiata* (Jacq.) DC.), jack bean (*Canavalia ensiformis* (L.) DC.), sabawel (*Mucuna pruriens* or *conchinchinensis*), batao or hyacinth bean (*Dolichos lablab* L.) and rice bean (*Vigna umbellata* Ohashi.).

## INTRODUCTION

Legumes with protein content ranging from 20-40% are an important inexpensive sources of protein. However, they are limiting in the sulfur amino acids -- cysteine and methionine. Moreover, they contain substances which have adverse effects on men and animals, and in extreme cases, can cause fatal poisoning. These so-called anti-nutritional factors include cyanogenic glucosides, phytates, trypsin inhibitors, saponins, alkaloids, and phytohemagglutinins or lectins.

Lectins are of special interest because of their ability to bind saccharides and saccharide-containing proteins in a highly specific manner (Liener, 1976). The toxic effect of lectins when ingested orally could probably be due to their ability to bind to specific receptor sites on the intestinal epithelial cells which thus causes a non-specific interference with the absorption of nutrients across the intestinal wall (Jaffe, 1989).

Although hemagglutinins are proteinous in nature and thus could be inactivated by heat, inadequate cooking of some legume seeds has resulted in reported cases of food poisoning characterized by nausea and diarrhea. Slow cooking or cooking in large cooking vessels where temperature could only reach 70-80 C will not destroy hemagglutinins but actually increase their toxicity (Bender, 1983). Thompson et al (1983) showed that heating presoaked and kidney beans at 100 C for 15 min or at 80 C for 2 hr or pressure cooking (15 psi) for 45 min without presoaking lowered the hemagglutinin activity to nondetectable levels.

Among the seven kinds of legumes tested by Bender (1983) kidney beans (Phaseolus vulgaris) had 50 times as much hemagglutinin as those with low levels such as split peas (Lens culinaris) and black eye beans or cowpea (Vigna unguiculata). Varietal differences in type and amount of lectins in thirteen cultivars (Puztai et al, 1979) and variable stability to heat (Bender, 1983) have been shown.

This study aims to investigate the presence of lectins in underutilized Philippine indigenous food legumes.

## MATERIALS AND METHODS

### Materials

Mature whole seeds of Philippine indigenous food legumes were obtained from the National Plant Genetic Resources Laboratory of the IPB.

Red bloods cells of rabbit, bovine, human A and human O types were obtained from Sigma Chemical Co. All chemicals used were of the highest purity available.

### Preparation of Crude Extract

Seeds were ground and sieved through 60 mesh. Ground samples (250 mg) were extracted with 5 mL of 0.90% NaCl for one hr in a shaker and centrifuged for 15 min at 3000 rpm in a Kubota KA-1000 centrifuge. The supernate was analyzed for hemagglutination immediately.

**Hemagglutination Test**

To each well of the microtiter plates, the following were added: 50 uL of phosphate buffered saline (PBS) pH 7.2, 50 uL of crude extract (diluted 1:1 with PBS) and 50 uL of 0.5% trypsin-sensitized RBC (prepared according to the procedure of Sigma Chemical Co.). The contents were mixed by gentle shaking and hemagglutination reaction was allowed to take place overnight at 4 C. Agglutination was determined and rated +, ++, and +++ for slight, moderate and strong hemagglutinating activities, respectively.

## RESULTS AND DISCUSSION

Among the food legumes tested, jack bean, sword bean and batao had consistently high titers of hemagglutinins (Table 1) against rabbit RBC. Three out of five sabawel varieties had hemagglutinating activity (HA). Lima had slight or no hemagglutinating activity while sam-samping and rice bean had more.

The jack bean varieties had little or no HA with bovine and Human A RBC; however they reacted strongly with human O RBC. Sword bean showed similar reactions although a certain accession, sword bean A-9 was negative to rabbit and bovine RBC but slightly and strongly positive to human A and O RBC.

The reaction of Sabawel A-2 was very different from the 4 other accessions. A-2 reacted strongly to all 4 types of RBC while the others had no, little or moderate HA to any 1 or 2 types of RBC.

Batao reacted differently from jack bean and sword bean in that it had strong HA against human A RBC.

Jackbean is known to contain the lectin concanavalin A. Diets containing raw jack beans and concanavalin A were lethal to conventional quail but non-lethal to germ-free birds (Jayne-Williams, 1973). Similar results with diets containing raw navy beans (Phaseolus vulgaris) (Jayne-Williams and Hewitt, 1972). Jayne-Williams and Hewitt (1972) have suggested that one are more of the biological activities of phytohemagglutinins could be to interfere with normal body defense mechanisms that the host is not able to prevent normally harmless intestinal bacteria which pass from the lumen of the gut to lymph, blood and liver.

Thus the high activities of 7 out of 9 accessions of jack bean studied could be attributed to concanavalin A. Lectins in swordbean are not well studied. Altamirano (1985) reported the immunological similarity of lectin from sword bean with that of jack bean.

Lima bean lectin had been shown to have a glycopeptide of molecular weight of 1380 and can be adsorbed by human blood groups of A and O (Misaki and Goldstein, 1977). The present results showed slight hemagglutinating activity of lima bean lectin to these blood groups.

Two hemagglutinins A and B had been isolated from D. lablab (Salgarkar and Sohonie, 1965a and b). Hemagglutinin a when fed to rat at 2.5% to level produced zonal necrosis of the liver.

Mucuna flagellipes was shown to contain large amounts (about 14% of the protein) glutinin specific to human blood B type (Mbadine and Agogbua, 1978).

The results show varietal differences as well as possible differences in the active site for batao which has stronger affinity to human blood type A than the other lectins.

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**Table 1. Hemagglutinating Activity in Several Philippine Indigenous Food Legumes.**

| Food legumes | RBC Rabbit | Bovine | Human A | Human O |
|---|---|---|---|---|
| Jack bean A-1 | +++ | + | - | +++ |
| Jack bean A-3 | +++ | + | - | +++ |
| Jack bean A-5 | +++ | + | - | +++ |
| Jack bean A-6 | ++ | + | - | ++ |
| Jack bean A-8 | ++ | - | - | ++ |
| Jack bean B-6 | +++ | ++ | - | ++ |
| Jack bean B-8 | +++ | - | - | ++ |
| Jack bean 44 | + | - | - | ++ |
| Jack bean 45 | + | - | - | ++ |
| Sword bean B-4 | +++ | - | - | ++ |
| Sword bean B-9 | ++ | - | - | +++ |
| Sword bean A-4 | + | - | + | +++ |
| Sword bean A-9 | - | - | + | +++ |
| Sword bean A-12 | +++ | +++ | ++ | +++ |
| Sabawel A-2 | +++ | +++ | ++ | +++ |
| Sabawel A-5 | + | - | + | - |
| Sabawel A-8 | + | - | + | + |
| Sabawel 6-1 | - | - | - | ++ |
| Sabawel 6-2 | - | - | - | + |
| Batao A-45 | +++ | - | +++ | + |
| Batao A-51 | +++ | + | +++ | +++ |
| Batao A-52 | ++ | + | ++ | +++ |
| Batao A-57 | ++ | + | +++ | +++ |
| Lima Bean A-544 | - | - | - | - |
| Lima bean A-537 | + | - | + | ++ |
| Sam-samping | - | - | - | - |
| Rice bean (PPOC) | - | ++ | - | - |
| Rice bean (Tapilan 28) | - | ++ | - | - |

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# Lectins in Philippine Indigenous Food Legumes. II. Sword bean Lectins: Removal and/or Inactivation

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## ABSTRACT

Soaking up to 36 hr did not reduce the lectins of mature green and mature dry seeds of sword bean as measured by hemagglutination and double immunodiffusion (Ouchterlony) technique. Fifteen min boiling of mature green seeds already removed lectins although 45 min were needed to completely cook the seeds. Mature dry seeds of swordbean A-4 took 45 min to remove the lectins although another accession (A-9) took only 15 min. Cooking by boiling of whole seeds (without seed coat) cut into 4 and minced or granulated resulted in removal of lectin as determined by immunological method. However, hemagglutinating activity was still detected in the whole and quartered seeds.

Cooking for 45 min in water , 0.9% NaCl, coconut milk with water and coconut milk without water resulted in removal of lectin immunologically but hemagglutinating activity was maintained/increased in the second and third media. Autoclaving and roasting removed lectins as measured immunologically but not the hemagglutinating activity.

**Keywords:** sword bean (*Canavalia gladiata*), lectins, phytohemagglutinin, hemagglutinin, detoxification, immunodiffusion, Ouchterlony technique.

## INTRODUCTION

Among the food legumes screened for lectins, swordbean is one of those with high protein contents (22.58 - 28.05%) but which exhibits strong hemagglutinating activity (HA) (Barroga et al, 1988). Since studies on this crop and its lectins are limited, swordbean was chosen as material for the detoxification (removal or inactivation) of lectins.

## MATERIALS AND METHODS

### Materials

Two accessions of swordbean A4 and A9 were grown in clay pots (30 cm in diameter) arranged 0.50 m apart from each other. Flowers upon dehiscence were tagged for subsequent sample collections.

Samples were classified as immature seeds, mature green seeds and mature dry seeds. Mature green seeds were detoxified fresh while the mature dry seeds were taken as composite sample before detoxification.

Lectin standard (concanavalin A), con A antiserum and red blood cells were obtained from Sigma Chemical Co. Bacto agar. Other chemicals were of analytical grade.

### Preparation of Crude Extract

Ground samples (250 mg) were extracted with 5 mL of 0.90% NaCl for one hr in a shaker. The residue was removed by centrifugation for 15 min at 3000 rpm in a Kubota KA-1000 centrifuge. The supernate was saved for analyses.

**Double Immunodiffusion (Ouchterly technique)**

Bacto agar (1.5% (w/v) was dissolved in pre-heated 0.05 M tris-barbital buffer. The solution was heated in a boiling water bath until clear (about 45 min depending on total volume of agar). After cooling, 15 mL of the gel was poured into petri plates (9.0 cm in diameter) and allowed to solidify. A 6-mm hole/well was then punched into the gel using a gel punch set at 2.50 cm apart from the center well, center to center. About 20 uL of agar was applied into each well to seal the bottom of wells to prevent leakage of antigen/antisera into the plate. Forty uL of undiluted antisera was placed into the center well; standard concanavalin A / crude extracts were placed in the outer wells. The plates were covered and kept in plastic boxes at 4 C. Immunodiffusion was allowed to take place for 14 days, after which the gels were flooded with 0.90% NaCl for 2-3 days to remove unreacted antigen and or antibody. The gels were then washed and soaked in distilled water for another day and later stained with coomassie brilliant blue. The radii of precipitins formed were measured. The amount of lectin was estimated by logarithmic regression using a standard curve.

**Hemagglutination Test**

To each well of the microtiter plates, the following were added: 50 uL of phosphate buffered saline (PBS), pH 7.2 was added; 50 uL of crude extracts (dilluted 1:1 with PBS) and 50 uL of 0.5 % trypsin-sensitized red blood cells (prepared according to (Sigma Chemical Co.'s procedure). The contents were mixed by gentle shaking and hemagglutination reaction was allowed to take

place overnight at 4 C. Agglutination titer was determined under an illumination lamp and rated as +, ++ and +++ for slight, moderate and strong hemagglutinating activities, respectively.

**Methods of Detoxification**

Different treatments were employed to detoxify the lectins in two swordbean accessions; soaking, boiling in water and in different cooking media, autoclaving and dry heat treatment or roasting.

1. Soaking

Twenty-five g of whole mature green and mature dry seeds of swordbean A-4 and swordbean A-9 were soaked in water at 1:2 (w/v) bean to water ratio for 1 to 36 hr. Soaked mature green seeds were collected every hour for 4 hr and at 4 hr interval thereafter, while mature dry seeds were collected every after 6 hr.

2. Boiling

Twenty-five g of whole seeds were cooked in boiling water for 15, 30, 45, and 60 min (up to 75 min for the mature dry seeds) at 1:2 bean to water ratio, the volume of which was maintained until the desired cooking time was reached. Coconut milk (with and without NaCl), 0.9% NaCl and 1.0% acetic acid were also used for cooking.

3. Autoclaving

Twenty-five g of whole mature dry seeds were autoclaved at 1:3 bean to water ratio for 15 and 30 min at 10 psi.

4. Roasting

Twenty-five g of whole mature dry seeds were roasted for 15 min and until coffee-like, which lasted for 55 min.

## RESULTS AND DISCUSSION

The erythroagglutinin activity of detoxified swordbean seeds was determined by its ability to specifically agglutinate bovine RBC. The use of trypsinated bovine cells appeared to be the most useful tool for detecting potentially toxic beans, as shown by Jaffe and Brucher (1972).

Lectin was also measured by double immunodiffusion (Ouchterlony technique) using concanavalin A as standard. Although the values obtained are only rough estimates (the use of con A as standard is based on the premise that jackbean and swordbean lectins are immunologically similar; Altamirano, 1985), they provide basis for determining percent lectin lost during detoxification. Hemagglutinating activity was also determined since the antigenic property of lectin may disappear during heat treatment but not its other biological properties.

Precipitins for standard con A were already evident after 5 days and for the samples-- 9-10 days, however, the precipitins formed were not sharp. Sharpness of precipitins was optimum at 14 days.

1. Soaking

Results (Table 1) showed that soaking of the mature green seeds for 36 hr had little effect on lectin concentration as determined immunologically and hemagglutinating activity, but

considerably affect the organoleptic properties of the seeds. The color changed from pink to brown and the seeds developed a slight fermented odor. The former took place 3 hr after soaking with an accompanying increase in seed size and breakage of seed coat, while the latter became distinct 12 hr after.

Soaking of mature dry seeds for 36 hr removed only 16% of the lectin, but when soaked for another 6 hr the lectin concentration dropped by approximately 29.6% (Table 2). This coincided with physical changes such as swelling and partial breakage of the seed coat, and the development of the distinct fermented odor. In this connection the loss of lectin may be attributed to leaching, as the change in seed coat permeability and its rupture provide ease for diffusion to take place. On the contrary, soaking of beans did not affect the hemagglutinating activity of the lectin, instead a slight increase in hemagglutination was observed 4 hr after soaking. This was also observed in soaked kidney beans, though the apparent increase was considered insignificant (Thompson et al., 1983). Bender (1983) reported 18-66% decrease in hemagglutin activity after 16-18 hr soaking of kidney beans.

**Table 1. Effect of soaking at room temperature on lectin contents of mature green seeds of swordbean.**

| Soaking time(hr) | Sword bean A-4 | | Sword bean A-9 | |
|---|---|---|---|---|
| | Lectin (mg/g sample)[a] | Hemagglutinating activity[b] | Lectin (mg/g sample[a] | Hemagglutinating activity |
| 0 | 22.45 | + | 12.75 | - |
| 1 | 16.69 | - | 12.75 | + |
| 2 | 18.12 | - | 13.05 | + |
| 3 | 14.30 | - | 13.05 | + |
| 4 | 18.30 | ++ | 12.50 | ++ |
| 8 | 9.21 | ++ | 16.15 | ++ |
| 12 | 16.34 | ++ | 8.15 | + |
| 16 | 20.52 | ++ | 18.87 | ++ |
| 20 | 20.07 | ++ | 5.94 | + |
| 24 | 18.45 | + | 11.15 | ++ |
| 28 | 18.45 | + | 8.39 | ++ |
| 32 | 18.45 | + | 11.55 | + |
| 36 | 18.45 | + | 11.55 | + |

[a] Dry basis (mg/g sample); determined by immunological method.

[b] No agglutination (-); slight (+), moderate (++) and strong (+++) agglutination.

**Table 2. Effect of soaking at room temperature on lectin of mature dry swordbean seeds (Swordbean A-4).**

| Soaking time (hr | Lectin (mg/g)[a] | Hemagglutinating activity[b] |
|---|---|---|
| 0 | 25.58 | ++ |
| 6 | 20.00 | - |
| 12 | 20.00 | - |
| 18 | 14.46 | - |
| 24 | 28.95 | ++ |
| 30 | 19.34 | ++ |
| 36 | 21.49 | ++ |
| 42 | 15.13 | ++ |

[a] Determined by immunological method.

[b] No agglutination (-); slight (+), moderate (++) and strong (+++) agglutination.

2. Cooking

Cooking of mature green seeds in boiling water for 15 min removed the hemagglutinating and antigenic properties of swordbean lectins (Table 3). However, 45-min cooking is required for the bean to be considered thoroughly cooked. Boiling the mature dry seeds in water for 30 min removed only 86.20% of the lectins and trace amount was observed in seeds cooked for 45 min, as shown by a broad precipitin band formed in the immunological assay (Table 4).

Cooking of beans of different particle sizes (Table 5) for 1- 1/2 hr was insufficient to destroy the hemagglutinating activity of lectins, although the immunological activity was already destroyed. This can be attributed to impaired heat penetration as the amount of sample used was ten times greater than what was normally used for other detoxification experiments (250 g per treatment), and the slurry was very viscous. This in

**Table 3. Effect of cooking in water on concentration and hemagglutinating activity of lectins in mature green seeds of swordbean.**

| Cooking time (min) | Swordbean A-4 | | Swordbean A-9 | |
|---|---|---|---|---|
| | Lectin (mg/g sample)[a] | Hemagglutinating activity[b] | Lectin (mg/g sample)[a] | Hemagglutinating activity |
| 0 | 22.45 | + | 12.75 | - |
| 15 | - | - | - | - |
| 30 | - | - | - | - |
| 45 | - | - | - | - |
| 60 | - | - | - | - |
| overnight soaking + 45 min cooking | - | - | - | - |

a
Determined by immunological method.

b
No agglutination (-); slight (+), moderate (++) and strong (+++) allglutination.

**Table 4. Effect of cooking in water on concentration and hemagglutinating activity of lectins in mature dry sword - bean seeds.**

| Cooking time (min) | Swordbean A-4 | | Swordbean A-9 | |
|---|---|---|---|---|
| | Lectin (mg/g sample)[a] | Hemagglutinating activity[b] | Lectin (mg/g sample)[a] | Hemagglutinating activity |
| 0 | 25.58 | + | 14.88 | - |
| 15 | 23.00 | ++ | trace | + |
| 30 | 3.53 | ++ | - | - |
| 45 | trace | - | - | - |
| 60 | - | - | - | - |
| 75 | - | - | - | - |
| overnight soaking + cooking | - | - | - | - |

a Determined by immunological method.

b No agglutination (-); slight (+), moderate (++) and strong (+++) agglutination.

**Table 5. Effect of cooking swordbean seeds of different particle sizes on hemagglutinating and immunological properties of lectins.**

| Particle size | Lectin (mg/g sample)[a] | Hemagglutinating activity[b] |
|---|---|---|
| cooked whole | - | ++ |
| seeds cut into 4 (w/o seed coat) | - | + |
| granules (with out seed coat) | - | - |
| control | 14.88 | + |

a Determined by immunological method.

b No allutinationg (-); slight (+), moderate (++) and strong (+++) agglutination.

agreement with the observation of Jaffe (1973), that heat transfer is imperfect with a tough viscous mass like cooked beans, and in the absence of vigorous and constant stirring , the temperature reached in parts of the preparation is inadequate for the destruction of the lectin. Jaffe (1980) also suggested that a reduction in boiling point of water in mountainous regions may result in incomplete elimination of the lectin.

On the other hand, boiling of whole bean seeds separately in 0.9% NaCl and cocomilk (Table 5) did not destroy the hemagglutinating activity of the lectin. When the two were combined, HA was destroyed and a negative result was obtained. Cooking in 1% acetic acid similarly resulted in negative HA.

3. Autoclaving

Autoclaving of mature dry swordbean seeds for 15 min destroyed only the antigenic property of the lectin but not its hemagglutinating activity (Table 7) Further autoclaving for another 15 min completely destroyed the lectin.

4. Roasting

Roasting removed the immunological activity of lectins but was ineffective in destroying its hemagglutinating activity (Table 8). The same result was obtained by De Muelenaere (1964) in some varieties of *P. vulgaris*.

**Table 6. Detoxification of lectins in mature green seeds of swordbean by cooking in different media for 45 min.**

| Cooking Media | Swordbean A-4 | | Swordbean A-9 | |
|---|---|---|---|---|
| | Lectin (mg/g sample) [a] | Hemagglutinating activity [b] | Lectin (mg/g sample) [a] | Hemagglutinating activity |
| Water | - | - | - | - |
| 0.9 % NaCl | - | + | - | + |
| Coconut milk (2:1, coconut meat:water) | - | ++ | - | ++ |
| Coconut milk with 0.9% NaCl | - | - | - | - |
| 1 % HAC | - | - | - | - |
| Control | 22.45 | + | 12.75 | - |

[a] Determined by immunological method.

[b] No agglutination (-); slight (+), moderate (++) and strong (+++) agglutination.

**Table 7. Effect of autoclaving on lectin activity of mature dry swordbean seeds.**

| Autoclaving Time (min) | Swordbean A-4 | | Swordbean A-9 | |
|---|---|---|---|---|
| | Lectin (mg/g sample) [a] | Hemagglutinating activity [b] | Lectin (mg/g sample) [a] | Hemagglutinating activity |
| 15 min | - | + | - | + |
| 30 min | - | - | - | - |
| Control | 25.58 | +++ | 14.88 | + |

[a] Determined by immunological method.

[b] No agglutination (-); slight (+), moderate (++) and strong (+++) agglutination.

**Table 8. Effect of roasting on swordbean lectins.**

| Treatment | Swordbean A-4 | | Swordbean A-9 | |
|---|---|---|---|---|
| | Lectin (mg/g sample)[a] | Hemagglutinating activity[b] | Lectin (mg/g sample)[a] | Hemagglutinating activity |
| Control | 25.58 | ++ | 14.89 | + |
| Roasting for 15 min | - | ++ | - | + |
| Roasting until coffee-like | - | + | - | + |

a
Determined by immunological method.

b
No agglutination (-); slight (+), moderate (++) and strong (+++) agglutination.

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# Isolation and Characterization of Soluble Proteins from Rice Bean [*Vigna Umbellata* (Thumb.) Ohwi Ohashi][1]

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## ABSTRACT

Globulins comprise the largest fraction in rice bean seed proteins and accounted for 56-60% of total proteins, with albumin (17-26%), prolamin (1.5-1.8%) and glutelin (3.2 to 4.2%).

When fractionated on Sepharose 48, globulins separated into three peaks with molecular weights of approximately >700,000, for the first peak, 175,000 and 18,000 for Tapilan 28; 139,000 and 7,000 for Tapilan 46 and 60,000 and 3,000 for Tapilan PROC 1. Albumins exhibited 3 major peaks and a minor one. The major peak had a molecular weight >210,000. The other peaks had: 75,000, 3,900 and 1,700 for Tapilan 28; 144,000, 14,800 and 2,000 for Tapilan 26 and 56,000, 7,800 and 3,400 for Tapilan PROC 1. Both albumins and globulins exhibited heterogeneity showing 7-10 bands on polyacrylamide gel electrophoresis.

The most limiting amino acids were cysteine and methionine with chemical scores of 38 to 59% only.

In vitro protein digestibility (IVPD) ranged for 82-86% for the seed meal, 86-88.5% for the albumins and 75.9 to 83.3% for the globulins.

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## Introduction

Food legumes have become a supplementary and even primary source of dietary proteins in developing countries. As of 1975, roughly 20% of the protein consumed by man is derived from legumes (Pimentel et al, 1975). The protein content of legumes ranges from 20-50%, and of the essential amino acids, the sulfur-containing units have been found to be limiting.

The nutritional contribution of legumes may be increased by improving not only the quantity but also the quality of seed proteins available. In this regard, studies are being done on assessing the potentials of indigenous legumes that are not widely used as food, as a dietary source of protein, as well as a genetic resource for the improvement of the traditional legume crops.

Among the indigenous legumes to which the Institute of Plant Breeding of the University of the Philippines at Los Baños has given attention is rice bean (*Vigna umbellata*) or "tapilan". Rice bean grows in various tropical countries and some varieties are being used as a dietary source of proteins in countries like India, China and the Philippines. Although rice bean is reported to possess a relatively low protein content of approximately 17% (Mendoza et al, 1981), its characteristic resistance to pests and diseases has raised considerable interest in breeding researches.

In view of the possibility that rice bean may serve as a protein source in human and animal diet, or as a genetic resource, it is important that a biochemical evaluation of the

protein quality of rice bean be done. This study was undertaken to characterize and partially assess the nutritional quality of rice bean proteins.

It is hoped that this study would provide information that would be useful in the current thrusts for the improvement of legume protein production, particularly in studies on rediscovering the indigenous legumes as a source of protein.

## Materials and Methods

### Source and Preparation of Seed Samples

Dried seed samples of three accessions of rice bean were obtained from the Institute of Plant Breeding, College of Agriculture, University of the Philippines at Los Baños (UPLB). These accessions were 28 (yellow brown), 46 (black) and PROC 1 (maroon).

Seeds were ground to pass 60-mesh in a Wiley Mill and were kept in airtight containers at 4 C until used.

### Proximate Analysis

Kjeldahl nitrogen, crude fat, crude fiber, ash and carbohydrate content of the seed meals were determined according to standard AOAC procedures (1975). Crude protein content was calculated by multiplying Kjeldahl nitrogen by a factor of 6.25.

### Amino Acid Analysis

Enough samples to contain 6 mg protein each were hydrolyzed in 3 mL of 6N HCl under nitrogen at 110 C for 24 hr. The hydrolysate was filtered and evaporated to dryness under reduced pressure at 40 C. The residue was dissolved in 10.0 mL of 0.2 M

pH 2.2 sodium citrate loading buffer. The resulting solution was analyzed in an LKB 4150 Alpha Amino Acid Analyser using ULTROPAC II cation exchange resin. Amino acid analysis was done at the Institute of Chemistry, University of the Philippines at Los Baños.

The chemical score was calculated as:

$$\text{chemical score} = \frac{\text{mg of amino acid in 1 g testfood} \times 100}{\text{mg of amino acid in 1 g reference protein}}$$

Isolation, Fractionation and Characterization of the Soluble Proteins

Fractionation of proteins from the seed meals was done based on solubility differences (Osborne, 1998). The whole seed meals were defatted thrice with n-hexane (1:10 w:v meal to solvent ratio) at 4 C with continuous stirring for 4 hr each. The defatted meal, which did not differ appreciably in appearance from the non-defatted meals, was kept in air-tight containers in a freezer until used.

Determination of % Protein Fractions

One hundred mg of the defatted meals were placed in separate test tubes. Two-and-a-half mL of 5% NaCl solution were added to each of the samples. The resulting mixtures were mixed in a Vortex genie mixer, allowed to stand for 30 min with occasional mixing, and centrifuged at 7,000 rpm for 15 min. The clear supernates, which contained the albumins and globulins, were decanted off and diluted to 5.0 mL with distilled water and their protein content analyzed.

The supernatants were dialyzed against water at 4 C to precipitate the globulins. The dialysates, which contained the albumin fraction of the soluble proteins, were analyzed for protein.

The residues from the NaCl extraction were reextracted twice more with 5% NaCl using the same procedure. Protein content was determined on the second and third extracts of soluble proteins.

The residues from the third extraction of soluble proteins were extracted with 70% ethyl alcohol. The ethanolic extracts containing the prolamins exhibited colors similar to the color of the seed coats of the whole samples.

The glutelins were extracted with 0.5M NaOH solution from the residue. The alkaline extracts were brownish in color, regardless of the color of the seed coats of the samples.

Preparative Fractionation of Soluble Proteins

Fifty g each of the defatted rice bean meals were stirred in 0.5M NaCl (20 mL: 1 g of meal) at 4 C for 1 hr. The slurry was centrifuged at 12,000 x g and 4 C for 20 min in a Sorvall RC 5 centrifuge.

The clear extracts were dialyzed at 4 C against distilled water until free from NaCl and precipitation of the globulins was completed. The mixtures were centrifuged at 12,000 x g and 4 C for 20 min. The precipitated globulins were washed with distilled water, collected and lyophilized to give a gray fluffy powder for Acc 28 and brown powders for Acc 46 and Acc PROC 1.

The supernatants, which contained the albumin fraction, were combined with the washings and were lyophilized. The albumin

fraction of Acc 28 proteins was collected as a light yellow powder while those of Acc 46 and Acc PROC 1 were both brown powders.

The lyophilized albumin and globulin fractions were kept in air-tight containers in a freezer until used for analysis.

Gel Filtration of the Albumin Fraction

Fifty mg samples of each of the albumin fractions were dissolved in 3.0 mL of 0.05M sodium phosphate buffer pH 7.0. The mixtures were passed through a Whatman No. 1 filter paper. The filtrates were chromatographed on Sephadex G-200 column (1.5 x 70 cm) using the same buffer. Three mL fractions were collected at the rate of 12 mL/hr using a Buchler fractomette Alpha 200 automatic fraction collector. The elution of the protein components was monitored by measuring the absorbance at 280 nm. The molecular weights of the eluted proteins were estimated using the calibration curve prepared with catalase (Mw 232,000), bovine serum albumin (Mw 69,000) and cytochrome C (Mw 12,400).

Gel Filtration of the Globulin Fractions

Thirty mg samples of each of the globulin fractions were dissolved in 2.0 mL of 0.05M sodium phosphate buffer pH 7.0 in 0.5M NaCl and chromatographed individually on the Sepharose 4B column (1.5 x 70 cm). Three mL fractions were collected and a total of 150 fractions were obtained for each sample. Protein was monitored by measuring the absorbance at 280 nm. A calibration curve was prepared with standards (thyroglobulin Mw 669,000, catalase Mw 210,000, bovine serum albumin Mw 69,000, and cytochromec (Mw 12,000).

Disc Gel Electrophoresis

The protein fractions were further characterized by disc gel electrophoresis following the modified procedure of Davis (1964). The molecular weights of polypeptide chains that comprise the protein fractions were determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (Weber and Osborne, 1969).

Determination of In Vitro Protein Digestibility

The digestibility of the protein fractions and the seed meals were determined *in vitro* following the method of Hsu et al (1977) using trypsin, chymotrypsin and peptidase as enzymes.

## Results and Discussion

Proximate Compsition of Rice Bean Seeds

The chemical composition of the three varieties of rice bean seeds is given in Table 1. Protein content is relatively low at 17-21% compared to other legumes. The reported average value of the protein content of rice bean seeds is 20.9% (Duke, 1981) which is not very different from that obtained for Tapilan 46.

**Table 1. Proximate composition of seeds of three rice bean varieties.**

| Accessions | Moisture (%) | Protein (%) | Fat (%) | Ash (%) | Fiber (%) | NFE |
|---|---|---|---|---|---|---|
| 28 | 8.97 | 16.82 | 3.46 | 4.31 | 7.43 | 64.73 |
| 46 | 8.64 | 21.42 | 3.77 | 4.58 | 6.55 | 61.09 |
| PROC 1 | 9.16 | 17.26 | 4.03 | 3.99 | 5.22 | 63.74 |

[a] Except for moisture, values are expressed on dry basis.
NFE, nitrogen-free extract.

The fat content of rice bean is 3-4% is well in agreement with previous reports of 3.9% (Duke, 1981). The seeds are good sources of carbohydrates, more than half of their weights being due to carbohydrates (61-65%). Crude fiber and ash are within range of the reported values of 5-7% and 3-4% respectively (Mendoza et al, 1981).

Fractionation and Characterization of the Seed Proteins

The distribution of protein in rice bean as determined from Osborne fractionation is given in Table 2. Like most legumes, the globulins comprise the largest fraction and accounted for 56-60% of the total proteins. Albumins contributed 14-17%, glutelins 3-4% and the prolamins composed the smallest fraction at 1.5 - 1.8%. The total nitrogen extracted was in the range of 76-83%.

**Table 2. Distribution of total protein in rice bean seeds.**

| Fraction | Amount of protein (g/100 g seed meal) | | |
|---|---|---|---|
| | Acc 28 | Acc 46 | Acc PROC 1 |
| Albumin | 2.90 + 0.28 (17) | 3.80 + 0.42 (18) | 4.45 + 0.42 (26) |
| Globulin | 9.51 + 0.87 (56) | 12.89 + 0.93 (60) | 9.96 + 0.56 (58) |
| Prolamin | 0.26 + 0.08 (1.5) | 0.38 + 0.07 (1.8) | 0.28 + 0.05 (1.6) |
| Glutelin | 0.71 + 0.12 (4.2) | 0.82 + 0.09 (3.8) | 0.56 + 0.09 (3.2) |
| Residue | 3.44 (20) | 3.53 (16) | 2.01 (12) |

[a] Values are average of three replicates.

[b] Difference between total protein and sum of solubilized protein.

Percent of total protein in parentheses.

Gopinathan et al (1987) reported globulins to constitute 38-54% of total seed proteins in several populations of Vigna minima and V. umbillata. Studies done on other legumes reported similar values for the globulin fractions. In Phaseolus seeds, 56-61% of the proteins were globulins (Pant and Tulsani, 1969; Powrie, 1961). Bhatty (1982) reported that globulins comprised 66.5% of the total proteins in mungbean and 62.7% in peas.

The contribution of the albumins obtained for rice bean were slightly higher that the reported value of 7% in cowpea (Hernandez et al 1982) and 8.1% in mung bean (Bhatty, 1982). Chickpeas, Phaseolus seeds and peas exhibited similar values as rice bean at 12.2%, 13% and 14.1% of albumins in the total protein, respectively (Bhatty, 1982).

The amount of nitrogen extracted is similar to the 74-82% solubilization reported by Evans and Kerr (1963) and Powrie (1961). On the other hand, Jambunathan and Mertz (1973) reported a low 70% nitrogen recovery using fractionation procedure of Osborne.

Differences in solubilization of nitrogen may be due to factors other than those inherent in the seed samples. These factors may be extraction temperature, meal-solvent ratio and particle size of the sample (Smith et al, 1959; Cagampang et al, 1966). It is also reported that the alcohol and glutelin fractions tend to gel and thus cause losses in extractable nitrogen (Jambunathan and Mertz, 1973).

The prolamins and glutelins comprise a very small fraction of the total proteins of rice bean. Although that they are a

part of the nutritional contribution of the rice bean protein, most of the succeeding analyses were done on the two major protein fractions, the albumins and the globulins.

The albumin and globulin fractions obtained were all colored. The albumins of Acc 28 was light yellow while the globulins were slightly gray. The protein fractions from Acc 46 and Acc PROC 1 were reddish brown and dense. These two latter accessions gave albumin and globulin fractions of higher nitrogen content than Acc 28, as shown in Table 3.

The globulin fractions were of higher nitrogen content than the albumins of the same variety. It is believed that more non-nitrogenous materials that are also soluble in water contaminate the albumins than the globulins. Moreover, the carbohydrate moities that are attached to the proteins tend to vary. A determination of the starch content of the albumin fractions of some legumes revealed only traces of starch in faba beans and peas but 1.8% in mung bean albumin (Bhatty, 1982). The protein fractions of cowpea were also found positive for glycoproteins Hernandez et al, 1983).

**Table 3. Protein content of albumin and globulin fractions isolated from rice bean meal (g/100 g protein extract).**

| Variety | Amount of Protein (%) | |
|---|---|---|
| | Albumin | Globulin |
| Acc 28 | 39.32 | 68.82 |
| Acc 46 | 53.46 | 76.53 |
| Acc PROC 1 | 52.64 | 71.62 |

Gel Filtration

The albumins exhibited 3 major peaks and a minor one (Fig. 1). All of the first fractions were eluted near the exclusion limit of Sephadex G-200 corresponding to molecular weights greater than 210,000. The estimated molecular weights of the other fractions are 75,800, 3,900 and 1,700 for Acc 28; 144,000, 14,800 and 2,000 for Acc 46 and 56,000, 7,800 and 3,400 for Acc PROC 1. With the exception of the third fraction of Acc 46, the molecular weights of the 3rd and 4th fractions were extrapolated from the calibration curve. The molecular weights of the corresponding fractions of the albumin samples differ markedly but the elution pattern are quite similar (Fig. 1). Cowpea albumins had only 3 peaks (Hernandez et al, 1983), with the first fraction also coming out near the void volume.

The globulins separated into three fractions each, the first one in all of the samples eluting at the void volume of Sepharose 4B (Fig. 2) with molecular weight greater than 700,000. The approximate molecular weights of the other fractions are 175,000 and 18,600 for Acc 28; 138,000 and 7,000 for Acc 46 and 60,000 and 3,000 for Acc PROC 1. The molecular weights of the third fraction of Acc 46 and Acc PROC 1 were extrapolated from the calibration curve for Sepharose 4B. As in the albumins, the molecular weights of the fractions differ but the elution patterns of the globulins were very similar. A similar run on cowpea gave 3 protein peaks with molecular weights from greater than 700,000 to 27,000 daltons (Hernandez et al, 1988).

Polyacrylamide Gel Electrophoresis

The albumins exhibited 9 to 10 bands of varying intensities (Fig. 3). The major subunits for the three varieties had similar mobilities from Rf 0.17 - 0.27. The samples gave two to three major subunits which may be of similar charge densities. The fast moving subunits of the albumins were lightly stained or diffused. Similarities can also be observed among the fast moving subunits such as the wide diffused band near midgel and the three lightly stained narrow bands from Rf 0.8 - 1.0.

The globulins gave 7 to 8 bands, with the major sub-units appearing as a wide, intense band around midgel and a narrower zone at Rf 0.2 - 0.4 (Fig. 4). Again, the major subunits for each of the varieties appeared to be quite similar. The disc gel pattern of Vicia faba also showed major bands at Rf 0.1 - 0.3 (Mcleester et al, 1973).

Electrophoretic patterns of albumins and globulins of other legumes had varying number of protein zones for each legume. Hu and Esen (1982) isolated soybean albumins and globulins which gave rise to 50-60 major subunits. Albumins of Phaseolus vulgaris gave at least 68 bands while the globulins exhibited an extremely diffused banding pattern. (McLeester et al, 1973).

The major polypeptides of the albumin and globulin fractions were determined by SDS-polyacrylamide gel electrophoresis (Fig. 5-6). The albumins gave three to five fine well-stained bands corresponding to polypeptides with molecular weights over 66,000. The samples gave two major polypeptides each but the apparent molecular weights of these major units differed from one sample to another. For Acc 28, the major units had molecular weights of

22,000 and 50,000 daltons. The polypeptides of Acc 46 were in the range of 55,000 and 37,000 and those of AccPROC 1 had molecular weights of 29,000 and 36,000. There were several lightly stained bands and each electrophoretogram was characterized by a diffused band at Rf 0.4 to 0.8.

The globulins exhibited four to five very closely spaced bands representing polypeptides with molecular weights over 66,000. Major subunits occurred in a narrower range of molecular weights than the albumins, from 70,000 (extrapolated) to 45,000.

The heterogeneity observed from the protein fractions has also been seen in other legume species (Bhatty, 1982; McLeester, 1973, Hu and Esen, 1982). Similar observations were also reported in rice by Iwasaki et al (1982).

One of the main causes of heterogeneity and multiplicity of a few kinds of subunits is the occurrence of genetic variants arising in several minor bands (Bhatty, 1982). Other causes are post-translational, among which is the occurrence of several degrees of oligomerization resulting to a series of sedimentation coefficients. There is also a possible interchange of some of the subunits, the contigent variation in size and the nature of the bound carbohydrate groups (Mosse and Pernollet, 1982).

Amino Acid Composition

The amino acid composition of the seed meals and the protein fractions are given in the Table 4. Tryptophan was not determined. Only acid hydrolysates were analyzed, thus cysteine and methionine are underestimated since these are destroyed during acid hydrolysis.

Except for the sulfur-containing amino acids, all the other essential amino acids determined had chemical scores above 60% and some exceeding 100% (Table 5). The most limiting amino acids were sulfur-containing units, cysteine and methionine. Chemical score for lysine, as expected of legumes, were high, reaching 174% in the albumin fraction of Tapilan PROC 1 proteins.

The chemical scores for the meals and the protein fractions of Acc 28 and Acc 46 were from 38% to 59% and were based on the limiting cysteine and methionine levels of 0.36 - 1.07 g and 0.85 - 1.43 g per 100 g protein respectively.

**Table 4. Amino acid content of dried defatted seed samples of Acc 28 46 and PROC 1 and their soluble protein fractions (g aa/100g protein).**

| Amino Acid | Acc 28 | | | Acc 46 | | | Acc PROC | | |
|---|---|---|---|---|---|---|---|---|---|
| | Defatted Meal | Albumin | Globulin | Defatted Meal | Albumin | Globulin | Defatted Meal | Albumin | Globulin |
| Lysine | 7.17 | 3.93 | 5.83 | 7.46 | 6.16 | 5.91 | 7.48 | 9.59 | 7.57 |
| Histidine | 3.19 | 1.62 | 2.48 | 3.18 | 1.81 | 2.12 | 3.22 | 2.46 | 2.18 |
| Arginine | 6.35 | 3.53 | 5.08 | 6.02 | 3.08 | 4.50 | 6.45 | 3.93 | 4.22 |
| Aspartic acid | 11.96 | 8.36 | 10.30 | 11.30 | 8.21 | 9.83 | 12.11 | 12.36 | 10.20 |
| Threonine | 3.84 | 3.63 | 3.05 | 3.48 | 2.48 | 2.96 | 3.82 | 5.79 | 3.70 |
| Serine | 5.96 | 4.18 | 3.88 | 4.79 | 2.98 | 4.31 | 5.09 | 4.29 | 3.83 |
| Glutamic acid | 19.25 | 9.14 | 14.66 | 17.15 | 11.53 | 15.50 | 17.16 | 18.03 | 15.07 |
| Proline | 4.16 | 3.67 | 3.66 | 3.75 | 2.87 | 3.73 | 3.79 | 3.65 | 3.89 |
| Glycine | 4.18 | 3.34 | 3.67 | 3.93 | 3.51 | 3.52 | 4.30 | 5.27 | 3.90 |
| Alanine | 4.79 | 3.57 | 3.79 | 4.40 | 3.85 | 4.03 | 4.68 | 5.53 | 3.64 |
| Cystine | 1.21 | 1.07 | 0.91 | 0.36 | 0.62 | 0.57 | 0.44 | trace | trace |
| Valine | 4.54 | 2.83 | 4.39 | 4.75 | 4.63 | 4.81 | 5.08 | 8.03 | 6.89 |
| Methionine | 1.19 | 0.95 | 0.86 | 0.96 | 1.43 | 0.85 | 0.79 | 0.52 | 0.69 |
| Isoleucine | 3.85 | 2.86 | 4.04 | 4.19 | 4.18 | 4.02 | 4.14 | 6.36 | 5.68 |
| Leucine | 8.28 | 4.08 | 6.68 | 7.88 | 7.25 | 8.36 | 8.29 | 9.48 | 10.51 |
| Tyrosine | 3.32 | 2.53 | 3.14 | 3.11 | 3.56 | 3.64 | 3.11 | 4.97 | 4.23 |
| Phenylalanine | 5.97 | 3.16 | 5.36 | 5.43 | 5.04 | 5.94 | 5.94 | 7.14 | 7.61 |
| Chemical Score [a], % | 43 | 55 | 51 | 38 | 59 | 41 | 35 | 15 | 20 |

[a] Calculated based on the amino acid scoring pattern prepared by FAO/WHO (1973).

**Table 5. Chemical score of seed meals, albumin and globulin fractions of three rice bean accessions.**

| SAMPLE | ILE | LEU | LYS | MET CYS | PHE TYR | THR | VAL | HIS [c] |
|---|---|---|---|---|---|---|---|---|
| Acc 28 | | | | | | | | |
| Seed meal | 96 | 118 | 130 | 43 | 155 | 96 | 91 | 133 |
| Albumin | 72 | 58 | 70 | 55 | 95 | 91 | 57 | 67 |
| Globulin | 101 | 95 | 106 | 51 | 142 | 76 | 88 | 103 |
| Acc 46 | | | | | | | | |
| Seed meal | 104 | 113 | 136 | 38 | 142 | 87 | 95 | 132 |
| Albumin | 104 | 104 | 112 | 59 | 143 | 62 | 93 | 75 |
| Globulin | 100 | 119 | 107 | 41 | 160 | 74 | 96 | 88 |
| Acc PROC 1 | | | | | | | | |
| Seed meal | 104 | 118 | 136 | 35 | 151 | 96 | 102 | 134 |
| Albumin | 159 | 150 | 174 | 15 | 202 | 144 | 161 | 102 |
| Globulin | 142 | 135 | 138 | 20 | 197 | 92 | 138 | 91 |

[a] Calculated based on the provisional amino acid scoring pattern prepared by FAO/WHO (1973).

[b] Underestimated values, will be re-analyzed; rice bean meal Cys + Met chemical score according to Duke (1981) is 89%.

[c] Food and Nutrition board requirement of albino rat or infant (1963).

Data on amino acid composition of rice bean collected by Duke (1991) showed that the meal contained 2.5 g methionine and 0.7 g cysteine in 100 g protein. At these levels, the sulfur-containing amino acids are no longer limiting. Combined cysteine and methionine levels would give a chemical score of 113%.

The actual chemical scores of the rice bean samples used should probably be higher than those obtained in this study since acid hydrolysis tend to oxidize and render undetectable some cysteine and methionine (Schram et al, 1954).

The albumin fractions of Acc 28 and Acc 46 were found to contain higher methionine and cysteine levels than their counterparts. This is similar to the findings of Bhatty (1982) in his study of the albumin fractions of some food legumes.

All samples exhibited high levels of aspartic and glutamic acids. In Acc 28 and Acc 46, the two amino acids were highest in the meal and lowest in the albumin fraction. A different trend is observed for Acc PROC 1 where the aspartic acid and glutamic acid were highest in tne albumin fraction.

A closer inspection of the aminograms (not shown) of the samples showed that the seed meals exhibited an additional peak in the red region which eluted before aspartic acid. Literature showing similarly positioned peaks that strongly absorb at 440 nm identify these as hydroxyproline (Andrews, in Protein Chemistry Notes, a publication of LKB Biochrom Ltd.). The peaks were not quantified due to the absence of the amino acid in the standard. The aminogram of the globulins and the albumins did not show this peak suspected to be hydroxyproline since this amino acid is expected to be present, along with greater amounts of proline, in the prolamine fraction of the proteins. The albumin and globulin fractions had nitrogen recoveries between 67-98% with the exception of Acc PROC 1 which had a nitrogen recovery of 98%. The recovery from the whole seed meals were consistently high at 92-93%. Reported recoveries of nitrogen in amino acid analysis of protein fractions usually lie in the range of 76-92% (Bhatty, 1982; Reichert and McKenzie, 1982).

*In Vitro Protein Digestibility*

The albumin fractions were found to be most digestible with 86.10 - 88.47% digestibility, almost parallel in value to the casein standard used which had as *in vitro* digestibility value of 88.50% (Table 6).

The raw seed meals exhibited digestibilities of 82-85%, slightly higher than the 80% digestibility of mungbean and 74% for cowpea (Mendoza et al, 1982, Hernandez et al, 1983). A similar determination on other Philippine indigenous legumes reported digestibility values of 69 - 77% (Mendoza et al, 1982).

The globulins were least digestible at 75 - 83% contrary to the greater digestibility obtained for globulins in cowpea compared to albumins (Hernandez et al, 1983). The authors attributed the difference in the digestibilities of the fractions to the higher trypsin inhibitor activity (TIA) of cowpea albumins. Although the trypsin inhibitor activities of the rice bean meals and the protein fractions were not determined, Mendoza et al (1982) reported a low TIA of 5-7 units/mg sample. Duke (1981) reported the absence of both trypsin and chymotrypsin inhibitors in rice bean. The low level or absence of trypsin and chymotrypsin inhibitors may account for the high digestibility values of rice bean.

Table 6. In *vitro* protein digestibility of seed meals and protein fractions of rice bean.

| Sample | % Digestibility |
|---|---|
| Acc 28 | |
| Seed meal | 85.70 |
| Albumin | 88.43 |
| Globulin | 83.34 |
| Acc 46 | |
| Seed meal | 83.13 |
| Albumin | 88.47 |
| Globulin | 79.11 |
| Acc PROC 1 | |
| Seed meal | 82.19 |
| Albumin | 86.10 |
| Globulin | 75.92 |
| Casein (standard) | 98.50 |

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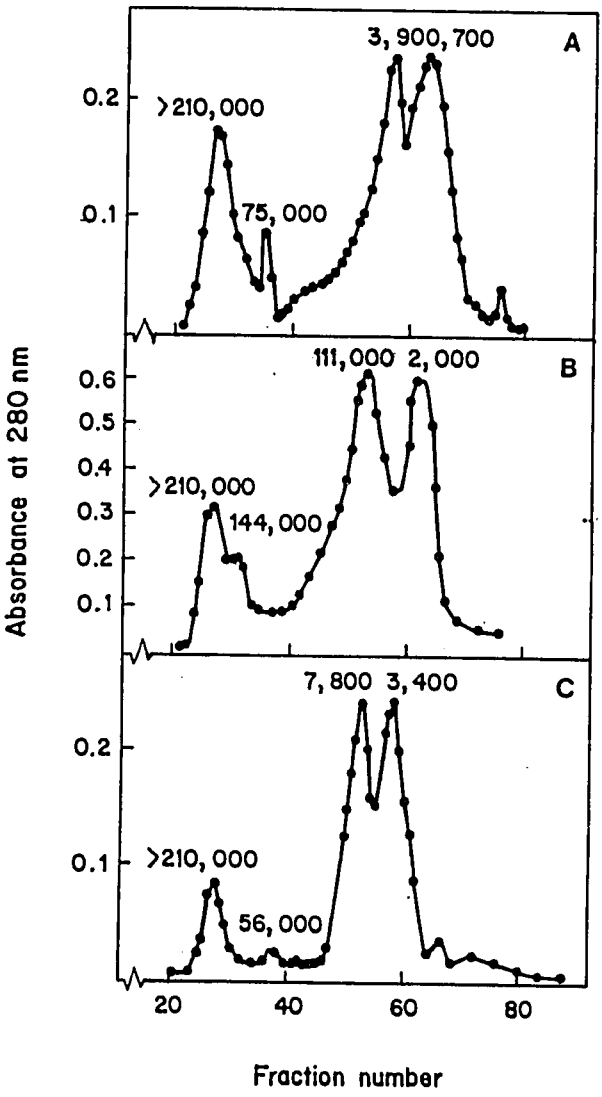


Fig. 1. Elution patterns of the albumin fractions of Tapilan 28 (A), Tapilan 46 (B) and Tapilan PROC 1 (C) in Sephadex G-200, 0.05M phosphate buffer pH 7.0.

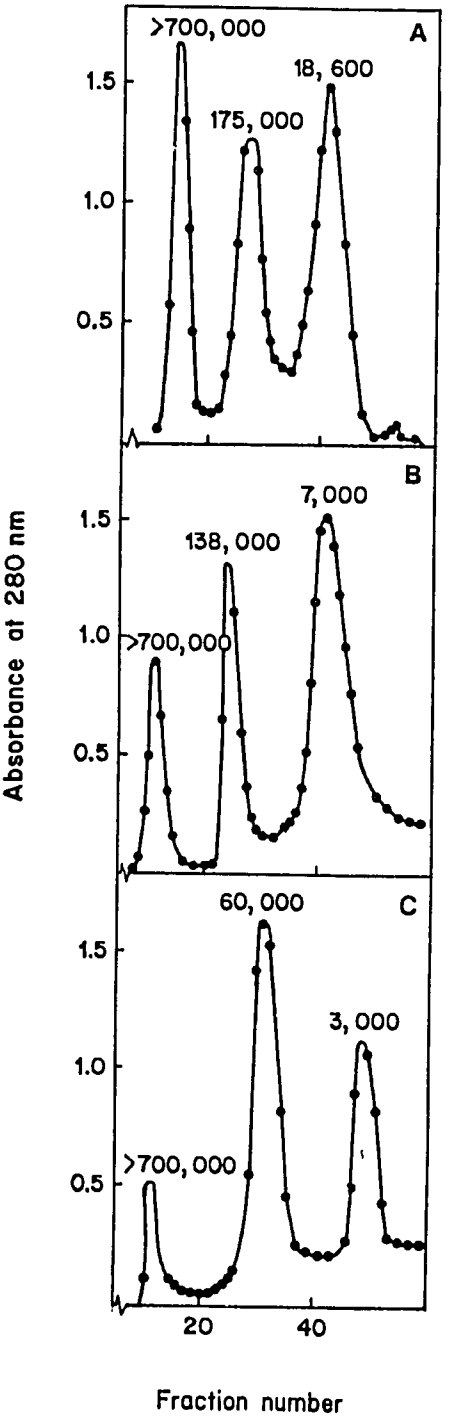


Fig. 2. Elution patterns of the globulin fractions of Acc 28 (A), Acc 46 (B) and Acc PROC 1 (C) in Sepharose 4B, 0.05M phosphate buffer pH 7.0 in 0.5M NaCl.

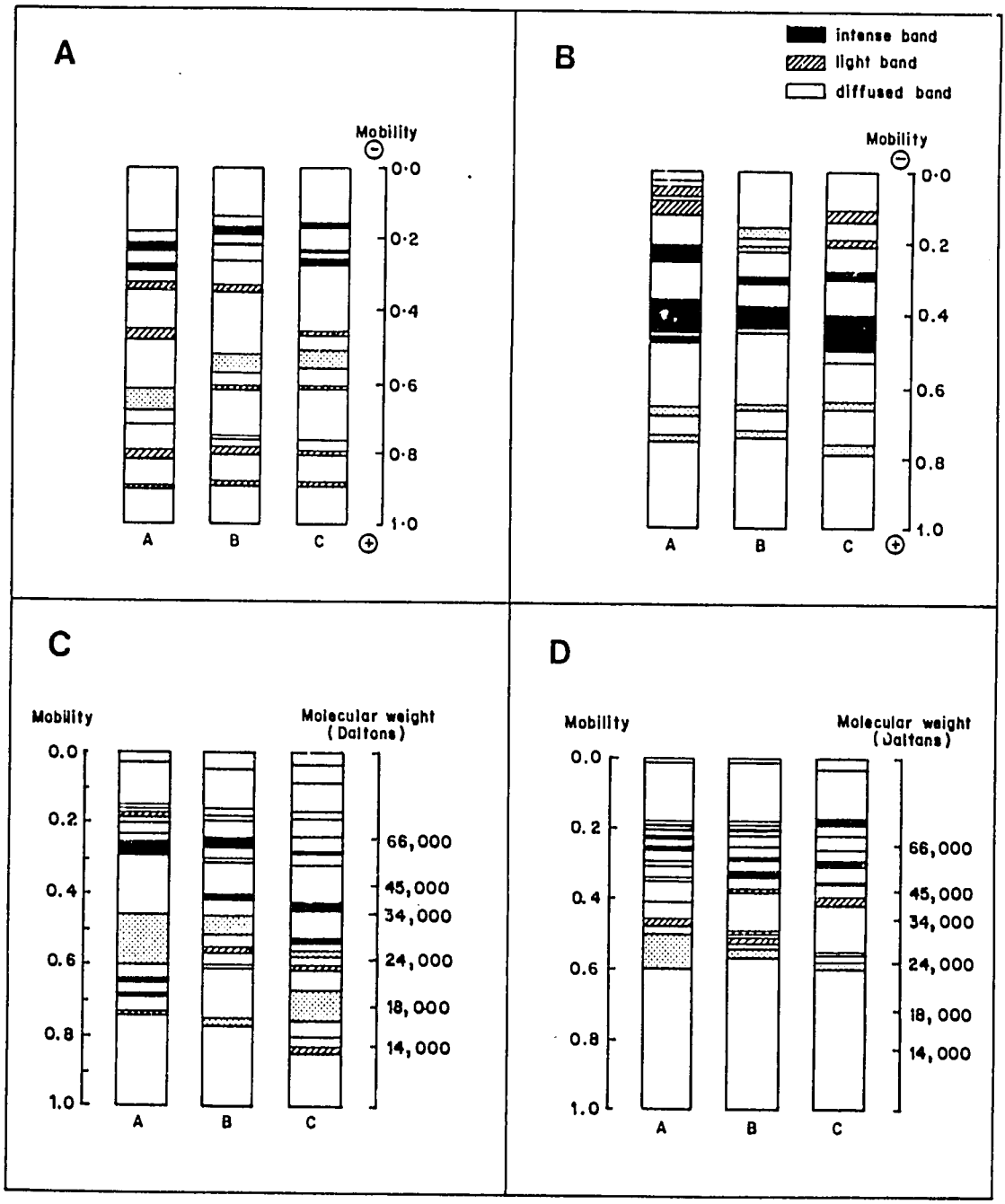


Fig. 3. Polyacrylamide gel electrophoregrams of albumins [A] and globulins [B], and SDS-polyacrylamide gel electrophoregrams of albumins [C] and globulins [D]; samples: Acc 28 (A), Acc 46 (B) and Acc PROC 1 (C).

# CHARACTERIZATION OF SEED PROTEINS FROM SABAWEL (*MUCUNA PRURIENS*) AND SAM-SAMPING (*CLITORIA TERNATEA*)

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## ABSTRACT

Seed proteins of sabawel (*Mucuna pruriens* or *cochinchinensis*) and sam-samping (*Clitoria ternatea*) were fractionated according to solubility. Globulins were the major fraction (73-76%), with albumin and glutelin having similar proportion (8-12%) and prolamin at 3.5% only.

Albumins of sabawel consist of proteins of molecular weight (mw) from 21,100 to 152,300 and a polypeptide of low mw (6,600). Albumins of sam-samping had larger mw constituents: 7,700, 24,500, 73,100 and 430,000. Globulins had larger proteins, 37,600 to >700,000 for the two legumes.

Electrophoresis of the fractions revealed that the fractions and sub-fractions were mostly heterogeneous. Methionine contents of the factions were determined.

## INTRODUCTION

The seed proteins of sabawel (*Mucuna pruriens* or *cochinchinensis* and sam-samping (*Clitoria ternatea*) were fractionated according to solubility and size and characterized. This study is in line with our efforts to obtain a better understanding of legume seed proteins towards improvement of their nutritional quality.

## MATERIALS AND METHODS

### Preparation

Dried seeds of Sabawel A-8 and Sam-samping 7-2 were obtained from the Institute of Plant Breeding. The seeds were ground to pass 60-mesh in a Wiley mill and were stored in air tight containers at 4 C before fractionation.

### Determination of % Protein Fractions

Based on the Osborne fractionation scheme (Osborne, 1898), proteins of the seed samples were classified according to their solubilities in different solvents: albumins, water-soluble; globulins, salt-soluble; prolamins, alcohol soluble and glutelins, alkali-soluble. The amount of the seed proteins of the 2 samples studied were determined on a test tube scale. All operations were carried out at 4 C.

Two hundred and fifty (250) mg of ground and defatted sample were placed in screw-capped test tubes. Five mL of 0.5 M NaCl solution at pH 7.5 were added to each sample and the resulting mixtures were shaken for one hr in the Roto-torque at setting 6.

The mixtures were allowed to stand for 15 min before decanting and centrifuged at 12,000 x g for 20 min. The supernates, which contained the globulins and albumins, were decanted off and diluted to 6 mL with 0.5 M NaCl solution. From these, 0.3 and 0.6 mL were taken and separately spotted on filter paper discs (1.5 cm diameter, Whatman no. 7) and dried. The discs were digested and analyzed for nitrogen by the Kjeldahl method (AOAC, 1980). Blanks were run using the discs only.

The rest of the supernates were dialyzed against distilled water to precipitate the globulins. The dialysates, which contained the albumins, were lyophilized.

The residues were reextracted four times more with 0.5 M NaCl using the same procedures. After the fourth extraction, the residues were then extracted three times with 70% ethanol (v/v) to determine the amount of prolamins. The supernates from each extraction were diluted to 5 mL with distilled water and analyzed for protein using 0.5 mL aliquots on filter paper discs.

Glutelin was extracted from the resulting residue with 0.05 M NaOH solution. Three extractions were also performed. The supernates obtained were also diluted to 5 mL and a 0.5 mL aliquot was analyzed for protein. The alkaline extract of sabawel was brownish in color while sam-samping had a dark brown alkaline extract.

Preparative Fractionation of Sam-samping and Sabawel Meals

Thirty g each of the defatted meals were stirred in 600 mL of 0.5 M NaCl solution for 4 hr at 4 C. The slurry was then filtered and dalyzed against distilled water to precipitate the globulins. Both the precipitate (globulins) and dialysates

(albumins) were lyophilized and stored dessicated in air tight containers in a freezer.

Extraction was carried out on both hulled and dehulled seeds. Dehulled seeds gave higher protein values and hence their proteins were used for gel filtration.

Gel Filtration of the Albumin Fraction

Fifty mg of the albumin fractions of sam-samping and sabawel were dissolved in 3 mL of 0.05 M sodium phosphate buffer pH 7.0. The mixtures were centrifuged in an Beckman Mini Centrifuge for 5 min. The clear supernates were applied with a pasteur pipette on the top of the gel bed of the Sephadex G-200 column (2.5 x 62 cm). Five mL fractions (110 drops) were collected at the rate of 10 mL/hour using an LKB 2112 Redi-Rac fraction collector. A total of 500 mL were collected. The elution pattern of the protein components was monitored by measuring the absorbance at 280 nm. The fraction numbers corresponding to the peaks were pooled and dialyzed against distilled water for twenty four hours. The resulting dialyzates were lyophilized and stored in a freezer.

Molecular weights of the eluted proteins were estimated by using a standard curve obtained using the following standards: ferritin (MW) (MW440,000) bovine serum albumins (MW 69,000) and cytochrome C (MW 12,400).

Preparation of the Sepharose 4B Column

The suspension of the Sepharose 4B was washed and equilibrated with 0.05 M sodium phosphate buffer, pH 7.0, in

0.05M NaCl. The slurry was deaerated and poured into a chromatography column (1.5 cm x 83 cm). Three bed volumes of the eluent buffer were run through the gel column at a water pressure of 42 cm and a flow rate of 16 mL/hr. Blue dextran was used to determine the void volume. The following standards were used to calibrate the column: thyroglobulin (MW 669,000), ferritin (MW 440,000), and bovine serum albumin (MW 69,000).

Gel Filtration of the Globulin Fraction

Fifty mg samples of the globulin fractions obtained from sam-samping and sabawel were dissolved in 2 mL of 0.05 M sodium phosphate buffer, pH 7.0, in 0.5 M NaCl. The mixtures were applied and chromatographed on the Sepharose 4B column. Three and one-half mL fractions were collected and a total of 100 fractions were obtained for each run. The elution patterns were monitored using a Beckman Spectrophotometer at 280 nm. The molecular weights of the peaks were estimated using the prepared calibration curve. As with the fractions obtained from the albumins, the fractions corresponding to each peak were pooled and dialyzed against distilled water. The precipitates were lyophilized and stored in a freezer.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The albumins and globulins together with their respective fractions as obtained from gel filtration were subjected to SDS-PAGE, according to Weber and Osborne (1869).

The proteins were stacked at 4 mA/tube for 20 minutes. Electrophoresis proper was carried out at 8 mA/tube until the

tracking dye was within 1 cm of the bottom of the gel tube.

After the run, the gels were rimmed out and stained with Coomassie brilliant blue R (1.25 g Coomassie blue in 454 mL 50% methanol and 46 mL glacial acetic acid) for 5 hr. The gels were destained in 7.5% acetic acid containing 5% methanol for 5 hr a Biorad diffusion destainer and stored in tubes containing the destaining solutions. A calibration curve was prepared by plotting the mobilities of protein standards against the natural logarithms of their known molecular weights. The molecular weights of the polypeptide sub-units were obtained from the standard curve.

Determination of Cysteine and Methionine

Cysteine and methionine contents of the fractions were determined on acid hydrolysates of samples previously subjected to performic acid oxidation, on a Waters-Millipore HPLC system.

## RESULTS AND DISCUSSION

### Seed Protein Distribution

The distribution of proteins in sabawel and sam-samping determined using the Osborne fractionation is shown in Tables 1 and 2. The globulins comprised the largest fraction and contributed 73-75% of the total proteins; albumins contributed 8-10%, glutelins 10-12.5% and prolamins 0.6-0.8%.

Studies done on other legume seeds show that globulins comprise 56-61% of Phaseolus seeds (Pant and Tulsani, 1969), 66.5% in mungbean and 62.7% in peas (Bhatty, 1982). This study gave comparatively higher values for the globulin fraction.

In sam-samping 7-2 seeds, the albumins contributed 8% to the total seed proteins. This value is similar to mungbean which has 8.1% albumins (Bhatty 1982). On the other hand, sabawel albumins contributed a higher value of 9.78%. This is higher than that reported for cowpea (Hernandez et al, 1983) but lower than the values for chickpeas (12.2%), Phaseolus seeds (13%) and peas (14.1%) as reported by Bhatty (1982).

The glutelin fractions for both sabawel and sam-samping were both larger than their respective albumin fractions. This is not in accordance with the reported data on legume seeds where glutelins are considered to be minor proteins, with globulins and albumins consisting the major portion of the seed proteins.

The total amount of protein extracted totaled 94.09% for sabawel and 95.58% for sam-samping. Both values are higher than those reported by Jambunathan (1973) with 70% recovery and by Evans and Kerr (1963) with 74-82% extraction.

**Preparative Fractionation**

The protein contents of albumins and globulins obtained preparatively from sabawel and sam-samping seeds (Table 3) ranged from 71 to 99%. These are much higher than those obtained for fractions of jack bean and sword bean (Samonte et al, 1988).

**Gel Filtration**

Sabawel albumins separated into three major peaks (Fig. 1a). on Sephadex G-200 with molecular weights of 152,000, 21,100 and 6,600 daltons. Sam-samping exhibited 3 major peaks of molecular

weights 430,000, 73,100 and 26,500 (Fig. 2a). Table 4 presents the MWs of sabawel and sam-samping albumin fractions.

Sabawel and sam-samping globulins both separated into 3 major peaks but with different elution profiles (Fig. 1b and 2b). Sabawel globulin separated into fractions of molecular weights >700,000, 251,000 and 42,600 daltons. Sam-samping globulin fractionated into peaks of >700,000, 251,000 and 42,600 daltons.

**Gel Electrophoresis**

SDS gel electrophoresis patterns of the albumins and globulins and their fractions of sam-samping and sabawel are given in Fig. 3a and 3b. These show the heterogeneity of the proteins, even those already further fractionated on gel chromatography. Notably sam-samping albumin has a large amount of low molecular weight (14.3 KD) components. For sabawel, major proteins are in the 18 to 24 KD range.

**Cystine and Methionine Contents**

The concentration of methionine and cystine in albumin and globulin fractions is shown in Table 6. For sabawel methionine is very high (>15%) in globulin and albumin and is relatively high in glutelin (12.04%). In sam-samping, the albumin and globulin had high met levels. However, when further fractionated, the met level decreased to 5%. As can be seen in Fig. 2b, globulin II had several bands thus, none of the proteins in this sub-fraction may have high met (>2.5%). In a preliminary experiment on Dolichos lablab in our laboratory with Dr. B.O. de Lumen of UC Berkeley a met rich protein with about 15% met was identified.

The method of de Lumen and Kho (1987) using radioactive iodoacetate in identifying met rich proteins is certainly faster than the method used in this study.

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**Table 1. Distribution of total protein in Sabawel A-8 seeds.**

| Fraction | Amount of protein (g/100 g seed meal) | % Contribution |
|---|---|---|
| Albumin | 2.76 ± 0.14 | 9.78 |
| Globulin | 20.60 ± 1.14 | 73.0 |
| Prolamine | 0.18 ± 0.04 | 0.64 |
| Glutelin | 3.01 ± 0.03 | 10.67 |
| Residue | 1.68 | 5.95 |

**Table 2. Distribution of total protein in Sam-samping 7-2 seeds.**

| Fraction | Amount of protein (g/100 g seed meal) | % Contribution |
|---|---|---|
| Albumin | 2.41 ± 0.07 | 8.0 |
| Globulin | 22.79 ± 0.71 | 75.66 |
| Glutelin | 3.76 ± 0.08 | 12.48 |
| Prolamin | 0.18 ± 0.04 | 0.60 |
| Residue | 1.33 | 4.41 |

a
Average of 2 replicates
b
Difference between total protein and sum of solubilized protein.

**Table 3. Protein content of albumin and globulin fractions of Sabawel A-8 and Sam-samping 7-2 seeds.**

| | Protein Content (%) | |
|---|---|---|
| | Kjeldahl Method | Lowry Method |
| Sabawel A-8 | | |
| Albumin | 76.92 ± 0.50 | 79.17 |
| Globulins | 98.00 ± 0.10 | 78.54 |
| Sam-samping 7-2 | | |
| Albumins | 71.26 ± 2.20 | 34.42 |
| Globulins | 99.45 ± 0.40 | 95.98 |

[a] Average of 2 replicates.

**Table 4. Estimated molecular weights of the protein fractions of sabawel and sam-samping albumins separated on Sephadex G-200.**

| Peak number | Molecular weight | |
|---|---|---|
| | Sabawel | Sam-samping |
| I | 152,000 | 430,000 |
| II | 21,100 | 73,100 |
| III | 6,600 | 26,500 |

**Table 5. Estimated molecular weights of the protein fractions of sabawel and sam-samping globulins separated on Sephadex 4B.**

| Peak number | Molecular weight | |
|---|---|---|
| | Sabawel | Sam-samping |
| I | >700,000 | >700,000 |
| II | 150,400 | 251,000 |
| III | 37,600 | 42,600 |

**Table 6. Cystine and methionine content (mg/100 mg protein) of protein fractions of sabawel and sam-samping.**

| Sample | % Cystine | % Methionine |
|---|---|---|
| Sabawel | | |
| Globulin | 1.11 | 20.80 |
| Albumin T2P3 I | 2.93 | 33.31 |
| Albumin | 3.13 | 15.36 |
| Albumin T3P2 | 5.34 | 5.42 |
| Albumin PU | 0.24 | 5.14 |
| Glutelin 1 | 1.31 | 12.04 |
| Sam-samping | | |
| Albumin | 2.91 | 21.24 |
| Albumin T2P1 | 0.39 | 4.22 |
| Albumin T2P3 | 0.38 | 7.11 |
| Globulin | 16.68 | 22.54 |
| Globulin T4P1 | 0.20 | 5.07 |
| Globulin T3P2 | 0.29 | 4.57 |
| Globulin T3P1 | 0.40 | 5.24 |
| Globulin T3P2 II | 1.31 | 0.13 |
| Globulin T4P1 II | 3.44 | 0.14 |

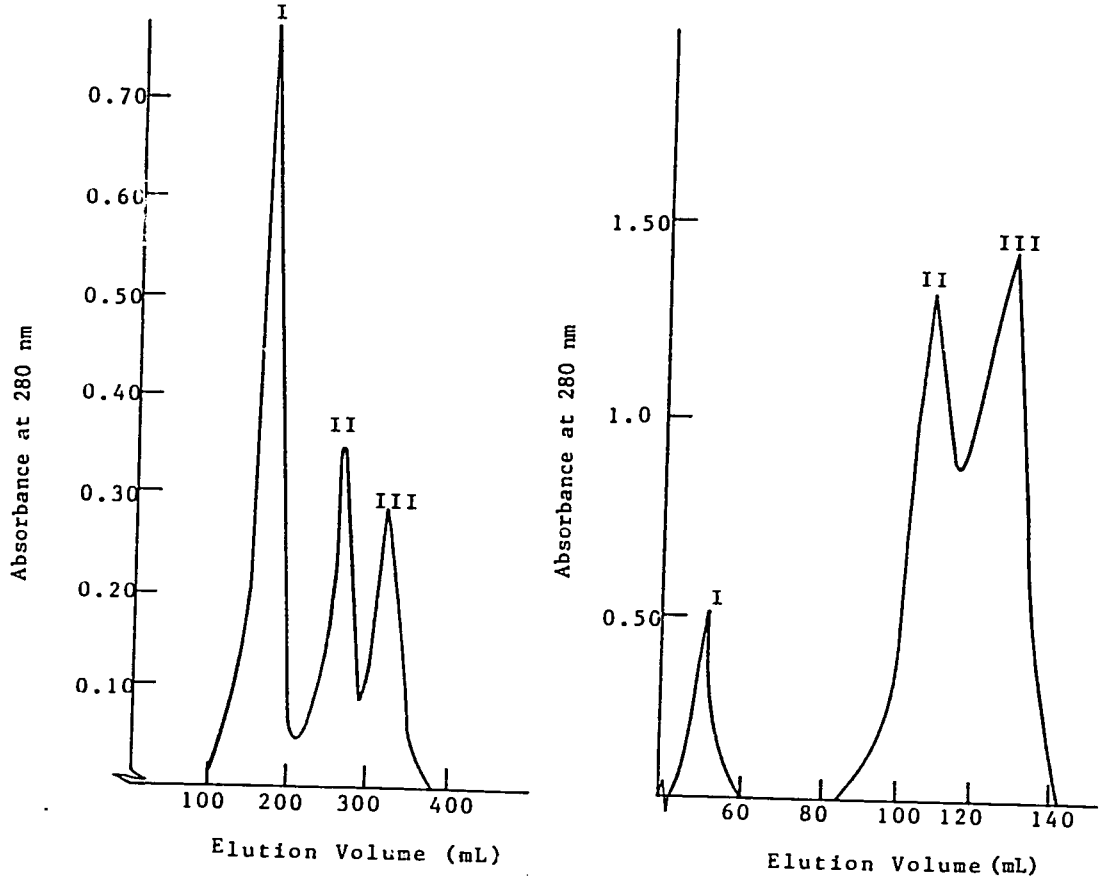



Fig. 1. Elution patterns of sabawel albumins (a) and globulins (b) on Sephadex G-200.

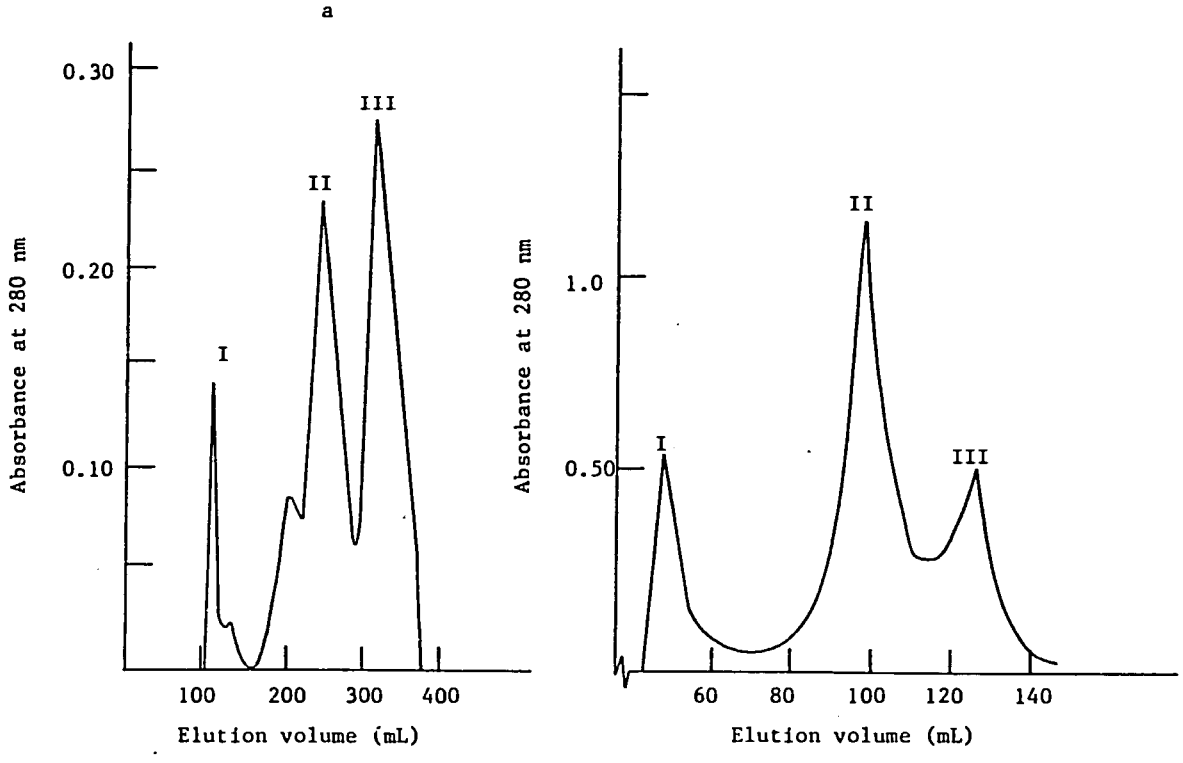
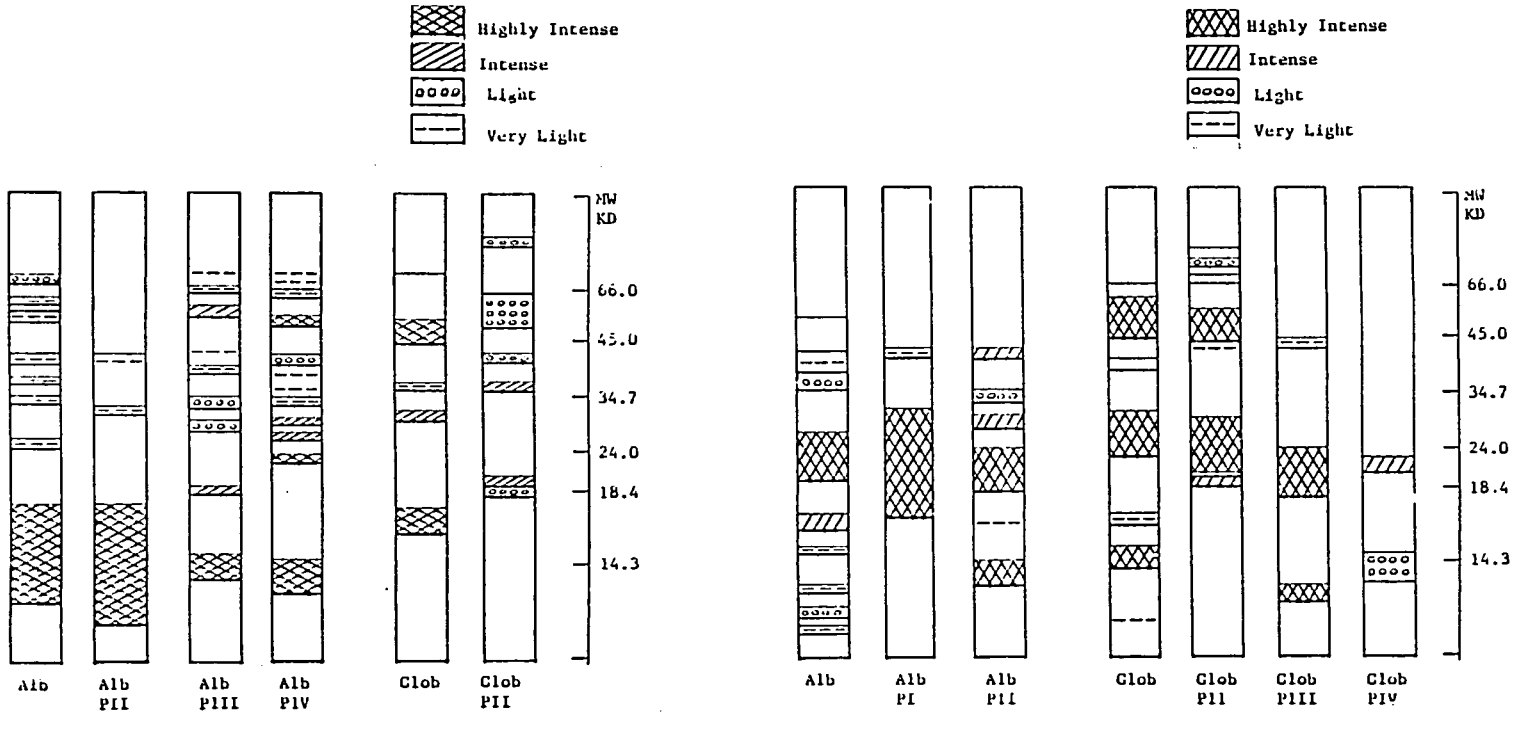



Fig. 2. Elution patterns of sam-samping albumins (a) and globulins (b) on Sephadex G-200.




b

Fig. 3. SDS gel electrophoretic patterns of sam-samping (a) and sabawel (b)

XIII

# Isolation, Fractionation and Characterization of Seed Proteins from Sword bean (*Canavalia gladiata*) and Jack bean (*Canavalia ensiformis*)

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## ABSTRACT

Based on solubility, sword bean has 79% globulin, 12% albumin, 6% prolamin and 0.3% glutelin. Jackbean has 66% glutelin, 14% jackbean, 14% prolamin and 0.8% glutelin.

A major portion of jackbean albumin fractionated into 40-60% saturated $(NH_4)_2SO_4$ while its globulin fractionated into 40-60 and 60-80%. Sword bean albumin and globulin fractionated into 60-80% and 40-60 and 60-80%, respectively.

The albumin and globulin of both sword bean globulin were eluted on Biogel 450m and SP-Sephadex to produce two to three peaks. These peaks were shown to be heterogenous by electrophoresis.

## INTRODUCTION

The seed proteins of jackbean (Canavalia ensiformis) and sword bean (Canavalia gladiata) were fractionated according to solubility and size and characterized. The cysteine and methionine contents of some of the fractions were also analyzed. This is in line with our efforts to obtain a better understanding of legume seed proteins towards the improvement of their nutritional quality.

## MATERIALS AND METHODS

### Materials

Mature seeds of sword bean and jack bean were obtained from plants grown at the Institute of Plant Breeding. The seeds were sun dried, dehulled and ground to pass through a 40 mesh sieve. The samples were then freeze dried and defatted by stirring with hexane (1 g meal: 10 mL) in the refrigerator (8 C) for 4 hr. The residue was re-extracted with hexane twice more. The defatted material was dried overnight in the hood and stored in airtight containers in the freezer until use.

All chemicals used were of analytical grade.

### Extraction of Proteins

Fifty g of seed meal was stirred with 0.5 M NaCl (1g: 20 mL). The slurry was stirred gently at 4 C for 1 hr. The mixture was centrifuged at 10,000 rpm at 4 C for 15 min and the supernate was collected. The residue was further extracted (3x).

The combined supernate were dialyzed against distilled water in the cold. The mixture that resulted was centrifuged at 10,000

rpm for 15 min at 4 C. The supernates were collected and the residue washed 2x with 100 mL distilled water. Both the supernate and residue were lyophilized.

**Solubility in $(NH_4)_2SO_4$ Solutions**

Five hundred mg of sword bean/jack bean albumins were dissolved in 20 mL distilled water. This solution was saturated with $(NH_4)_2SO_4$ up to 20%, stood for 1 hr at 4 C and then centrifuged to collect the precipitates. The supernate was further saturated to 40%, 60%, 80% and 100%. All the precipitates were collected after each saturation step and dialyzed against distilled water at 4 C.

For the sword bean and jack bean globulins, the same procedure was followed except these proteins were dissolved in 0.5 M NaCl before ammonium sulfate fractionation was done on it.

**Bio gel A-50 Gel Filtration**

Two ml of the sample containing 200 mg protein was applied on a Bio gel A-50 column (2.5 x 70 cm) and eluted with 0.5 M NaCl. The hydrostatic head was maintained at 62 cm and the flowrate at 25 mL/hr. The eluant used was 0.5 M NaCl solution. The fractions were collected at 3.0 mL per tube. The protein content of the fractions was monitored at 280 nm. The peaks obtained were pooled together, dialyzed against distilled water at 4 C, and lyophilized.

**SP-Sephadex Ion Exchange Chromatography**

Ion exchange chromatography of seed proteins was done using a 50 cm column with an internal diameter of 2.5 cm. The

hydrostatic pressure was maintained at 17.0 cm and flow rate at 2.50 mL/min. The eluant used was 0.05 M sodium acetate buffer pH 4.0 with an increasing NaCl gradient. The fractions were collected at 3.0 mL per tube.

Two mL of the sample solution containing 200 mg protein was applied on the column. Protein content was monitored at 280 nm. The peaks obtained were pooled together, dialyzed against distilled water at 4 C and lyophilized afterwards. The salt concentration of the eluates was determined using a conductivity meter.

**SDS Electrophoresis**

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was done on the samples according to the procedure of Weber and Osborn (1969). The gels used contained 5% acrylamide and 0.1% SDS in 0.1 M sodium phosphate buffer pH 7.0. The electrode buffer consisted of 0.1% SDS in 0.1 M Na phosphate buffer pH 7.0. About 50 ug of the proteins were applied per tube and a constant current of 5 mA per tube was used. The gels were stained with 0.25% Coomasie Brilliant Blue for 12 hr and destained in aqueous 7.5% acetic acid-5% methanol solution. The respective bands were recorded on a graphing paper. Ammonium sulfate fractions of globulins were run on slab PAGE under similar conditions. Standards of known molecular weight were run with the samples.

**Amino Acid Analysis**

Ground samples were heated in 6 N HCl under vacuum for 24 hr at 110 C. The amino acid composition of the acid hydrolyzate was

determined with a Waters-Millipore HPLC apparatus. Cysteine and methionine were determined separately as cysteic acid and methionine sulfur after performic acid oxidation. Each sample was analysed once.

## RESULTS AND DISCUSSION

### Isolation of Albumins and Globulins

Globulins were the major fractions in swordbean (78.7%) and jackbean (66.4%), followed by albumin in swordbean (12.1%) and albumin and prolamin in jackbean (13.5 and 14.0%) (Table 1). Glutelin was only 0.3% and 0.8% in swordbean and jackbean, respectively. These are similar to the protein composition of other legumes such cowpea (Hernandez et al, 1983), sam-samping and sabawel (Ocampo et al, 1988).

Large batch fractionation of albumin and globulin from jack bean and sword bean yielded fractions with 46-78% protein and 4 to 13% carbohydrate (Table 2).

### Ammonium Sulfate Fractionation

A. Fractions. Ammonium sulfate fractionation of sword bean and jack bean proteins showed that the highest concentration of proteins were found mostly in the 40-60% and 60-80% saturation fractions (Table 3). Jack bean albumins were higher at 40-60% while its globulins concentrated in the 60-80% fraction. In contrast, more sword bean albumins were found in 60-80% fraction and its globulins in 40-60% fraction.

If all of the proteins from jack bean and sword bean were almost of the same net charges during $(NH_4)_2SO_4$ fractionation, then those proteins precipitating at a lower salt saturation

level will be albumins (low MW) and those at the higher levels will be the globulins (high MW) (Khan et al, 1980). Results of ammonium sulfate fractionation showed that jack bean contained more "albumins" in the albuminous fraction and more "globulins" in the globulin fraction. However, sword bean albumins were concentrated more in the 60-80% fraction while its globulins were higher in the 40-60% fraction. This may be due to the differences in polarity or net charges of the amino acids in sword bean thereby causing precipitation of high molecular weight proteins at a lower saturation level.

The % recovery of the fractions was lower than 100%. However, this % recovery almost coincided with % proteins as estimated by the Kjeldahl N method. Probably, only the proteinaceous fraction of the albumin and globulin precipitate were isolated or reprecipitated by ammonium sulfate.

B. Electrophoresis. SDS gel electrophoresis of the (NH4)2 SO4 fraction of jack bean albumin showed multiple bands (Fig. 1). Protein of the bands were highly concentrated in the 20-40%, 40-60% and 80-100% (NH4)2 SO4 fractions. One major band with an Rf value of 0.5 was present in all these three fractions.

The electrophoretic pattern of jack bean globulins showed that multiple bands were found in 0-20%, 60-80%, and 80-100% fraction (Fig. 2). A major band of 0.30 Rf was present in almost all of the saturation levels. Comparing the band patterns of jack bean albumins and globulins, a large number of the globulins were observed to have lower Rf values than those for albumins. Both the albumins and globulins had fewer bands in the

60-80% fractions.

In sword bean albumins, the proteins were concentrated in the 40-60% and 60-80% fractions (Fig. 3). Major bands had Rf values of 0.35 and 0.50. Similar results were obtained for sword bean globulins (Fig. 4). Protein bands were highly concentrated in the 40-60% and 60-80% fractions. Very few proteins were found in the 20-40% and 80-100% fraction. The major bands were also found to have an Rf value of 0.30 and 0.50. The 80-100% fraction contained a few protein bands.

**Biogel A-50 m Gel Filtration**

Two major peaks (A and B) of jack bean albumins eluted at volumes corresponding to molecular weights of 407,600 and 185,300 daltons, respectively (Fig. 5). Jack bean globulins also had two peaks with molecular weights of 677,800 and 207,400 daltons, respectively (Fig. 5). On the other hand sword bean albumins had two peaks of different molecular weights of 571,000 and 196,000 daltons, respectively.

SDS electrophoresis of the various peaks revealed their heterogeneity (Fig. 7 and 9). Peak A of jack bean albumins had 3 bands less than peak B. In the globulin function, peaks A and B had similar patterns (Fig. 7).

Peak A of sword bean albumins had two strong bands which were not found in peak B. In the globulin fraction, peak B was more intense than peak A. Also, peak B had two extra bands that were found in peak A.

**Ion-Exchange Chromatography**

Jack bean albumins separated into three peaks upon elution on ion exchange column, with least absorbed peak A having the largest amount of protein (Fig. 9).

Sword bean albumins separated into 5 distinct peaks with peak B having the largest amount of proteins (Fig. 10-I). On the other hand, sword bean globulins had 4 peaks (Fig. 10-II). The position of the peaks shows the relative degree of positive or negative charge of the proteins. Thus, in general, the globulins of sword bean were more positively charged than the major albumins.

SDS gel electrophoresis patterns of the samples showed two major bands in peak A of jack bean albumins (Fig. 11). Two low molecular weight proteins were observed in peak C. Both peak B and C had more bands than A but of lesser intensity.

The electrophoretic patterns of sword bean albumins fractions from ion exchange chomatography were quite different from each other (Fig. 12). Peak A had bands of higher molecular weight than the other peaks. Peaks C, D and E had bands concentrated in Rf higher than 0.5.

Interestingly, sword bean globulins had a large number of intense bands in peaks B and D, with Rf's from 0.1 to 0.9 (Fig. 13). Peak A had little protein which failed to show up on the gel. Peak C had a few bands within the region of Rf 0.3 to 0.63.

**Methionine and Cysteine Contents**

In jack bean, glob 60-80 fraction had a relatively high met content of 11.56% (Table 4). In sword bean, albumins had much higher levels of met (14 to 38%) than in globulins (13-14%). However, as shown in fig. 1 to 4, these fractions are heterogeneous and, thus, the met content will be further spread out into the various protein components of the fractions.

**ACKNOWLEDGEMENT**

This study is part of the project "Biochemical and Nutritional Studies of Philippine Indigenous Food and Forage Legumes" supported by a grant to EMTM from the US Agency for International Development under its Program for Science and Technology Cooperation (AID-SCI-2N-04; Grant No. 936-5542-G-00-1347-00).

**Table 1. Protein fractions of swordbean and jackbean.**

| | Swordbean A-12 | Jackbean A-6 |
|---|---|---|
| % albumin | 3.95 ± 0.56 (12.1) | 3.94 ± 0.27 (13.5) |
| % globulin | 25.11 ± 1.31 (78.7) | 19.48 ± 0.26 (66.4) |
| % glutelin | 0.083 ± 0.003 (0.3) | 0.22 ± 0.01 (0.8) |
| % prolamine | 1.86 ± 0.16 (5.8) | 4.11 ± 0.19 (14.0) |
| % protein in residue | 1.00 ± 0.02 (3.1) | 1.77 ± 0.09 (6.1) |
| % Total protein | 31.90 | 29.27 |
| % protein in seed meal | 30.94 ± 0.72 | 28.12 ± 0.62 |

[a] Percent of total protein in parenthesis.

**Table 2. Albumin and globulin of mature seed of jack bean and sword bean.**

| Sample | Yield g/100 g meal | Carbohydrate (%) | Protein (%) |
|---|---|---|---|
| Jack bean | | | |
| albumin | 10.6190 | 22.84 | 33.66 |
| globulin | 14.9330 | 4.38 | 59.69 |
| residue | 55.80 | not det'd | 6.83 |
| Sword bean | | | |
| albumin | 13.6552 | 10.62 | 44.31 |
| globulin | 8.5354 | 4.11 | 77.46 |
| residue | 58.60 | not det'd | 2.19 |

**Table 3. Ammoniun sulfate fractionation of albumins and globulins of jack bean and sword bean.**

| $(NH_4)_2SO_4$ Saturation (%) | Protein (mg) in $(NH_4)_2SO_4$ fraction albumin | globulin |
|---|---|---|
| Jack bean | | |
| 0 - 20 | 6.0 | 7.2 |
| 20 - 40 | 9.6 | 34.9 |
| 40 - 60 | 104.6 | 133.0 |
| 60 - 80 | 30.6 | 176.9 |
| 80 - 100 | 22.0 | 13.4 |
| Total mg | 172.8 | 365.3 |
| % recovery | 34.5 | 73.06 |
| Sword bean | | |
| 0 - 20 | 6.5 | 6.9 |
| 20 - 40 | 17.9 | 53.0 |
| 40 - 60 | 53.0 | 188.2 |
| 60 - 80 | 129.0 | 130.9 |
| 80 - 100 | 18.0 | 15.9 |
| Total mg | 226.4 | 397.8 |
| % recovery | 45.28 | 78.96 |

**Table 4. Methionine and cystine content of albumin and globulin fractions of some indigenous food legumes.**

| Protein fraction | Cys | Met |
|---|---|---|
| Jackbean | | |
| Glob 0-20 II | 1.07 | 3.32 |
| Glob 40-60 II | 3.42 | 9.02 |
| Glob 60-80 II | 2.57 | 11.56 |
| Glob Residue II | 2.83 | 5.55 |
| Swordbean | | |
| Alb 2 | 2.79 | 14.23 |
| Alb 0-20 II | 1.69 | 5.59 |
| Alb 20-40 II | 6.92 | - |
| Alb 60-80 II | 3.25 | 18.10 |
| Alb 80-100 II | 2.38 | 0.18 |
| Swordbean | | |
| Alb | 5.45 | 23.00 |
| Alb 20-40 AS | 5.22 | 19.69 |
| Alb 40-60 As | 10.25 | 38.09 |
| Alb 80-100 II | 9.26 | 2.83 |
| Swordbean | | |
| Glob Residue I | 2.92 | 13.72 |
| Glob A-12 | 5.97 | 14.46 |
| Glob 0-20 I | 0.51 | 0.07 |
| Glob 20-40 I | 2.99 | 0.81 |
| Glob 40-60 I | 1.59 | 0.31 |
| Glob 1B IE | 3.32 | 13.51. |

Met and Cys determined by performic acid oxidation of the different fractions followed by acid hydrolysis. Glob refers to globulin fraction while alb refers to the albumin fraction. AS denotes ammonium sulfate fraction.

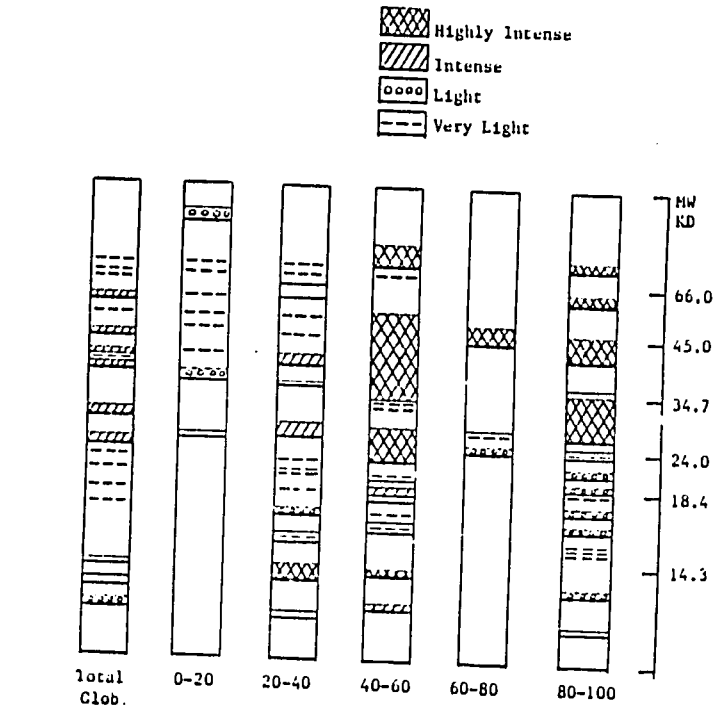


Fig. 1. SDS-electrophoretic patterns of jack bean albumins fractionated in various ammonium sulfate saturated solutions.

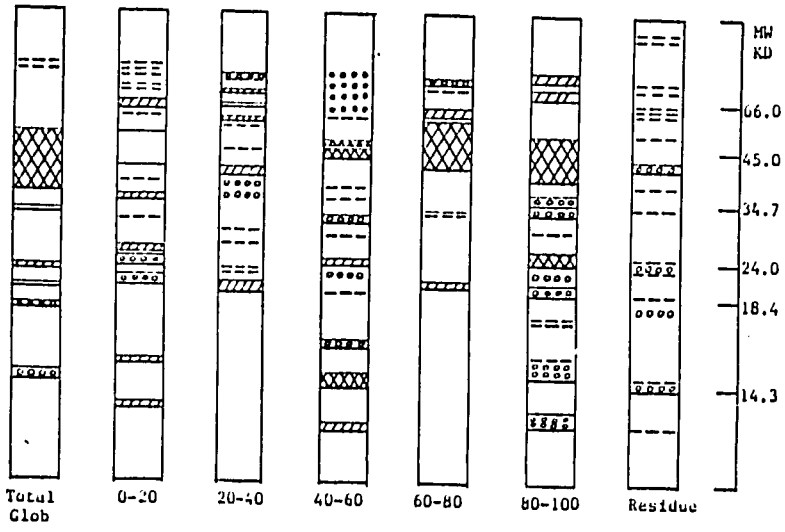


Fig. 2. SDS-electrophoretic patterns of jack bean globulins fractionated in various ammonium sulfate saturated solutions.

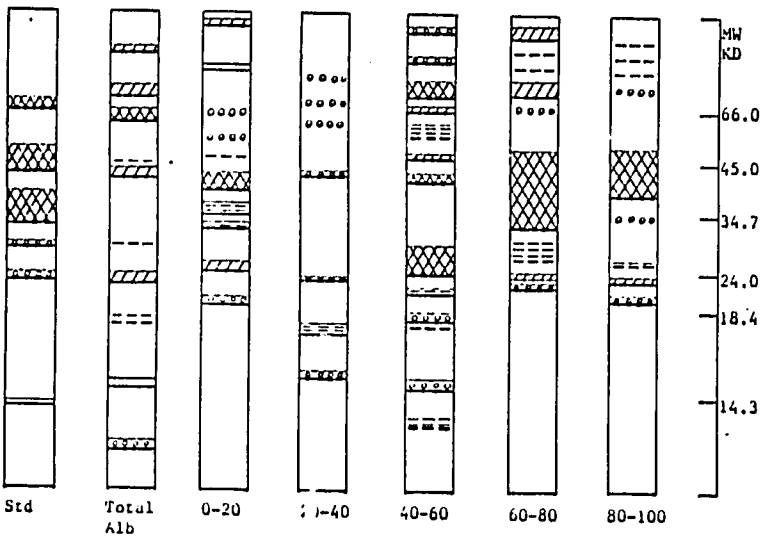


Fig. 3. SDS-electrophoretic pa ns of sword bean albumins fractionated into various ammonium fare saturated solutions.

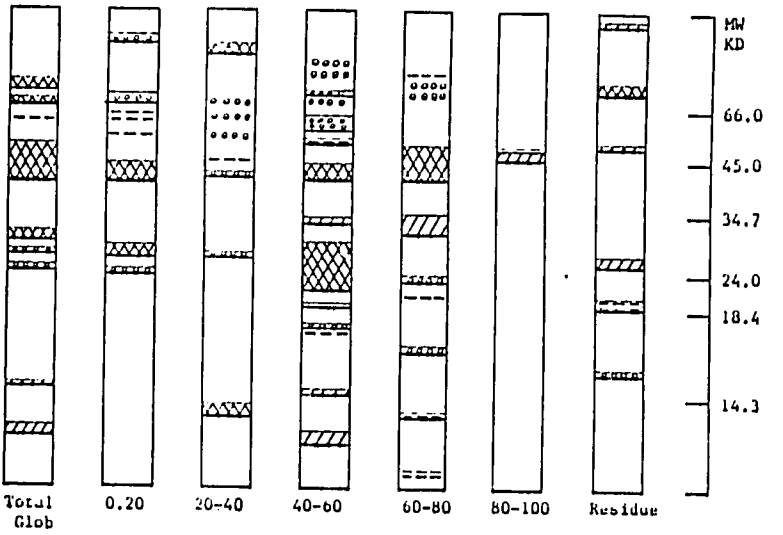


Fig. 4. SDS-electrophoretic patterns of sword bean globulins fractionated into various ammonium sulfate saturated solutions.

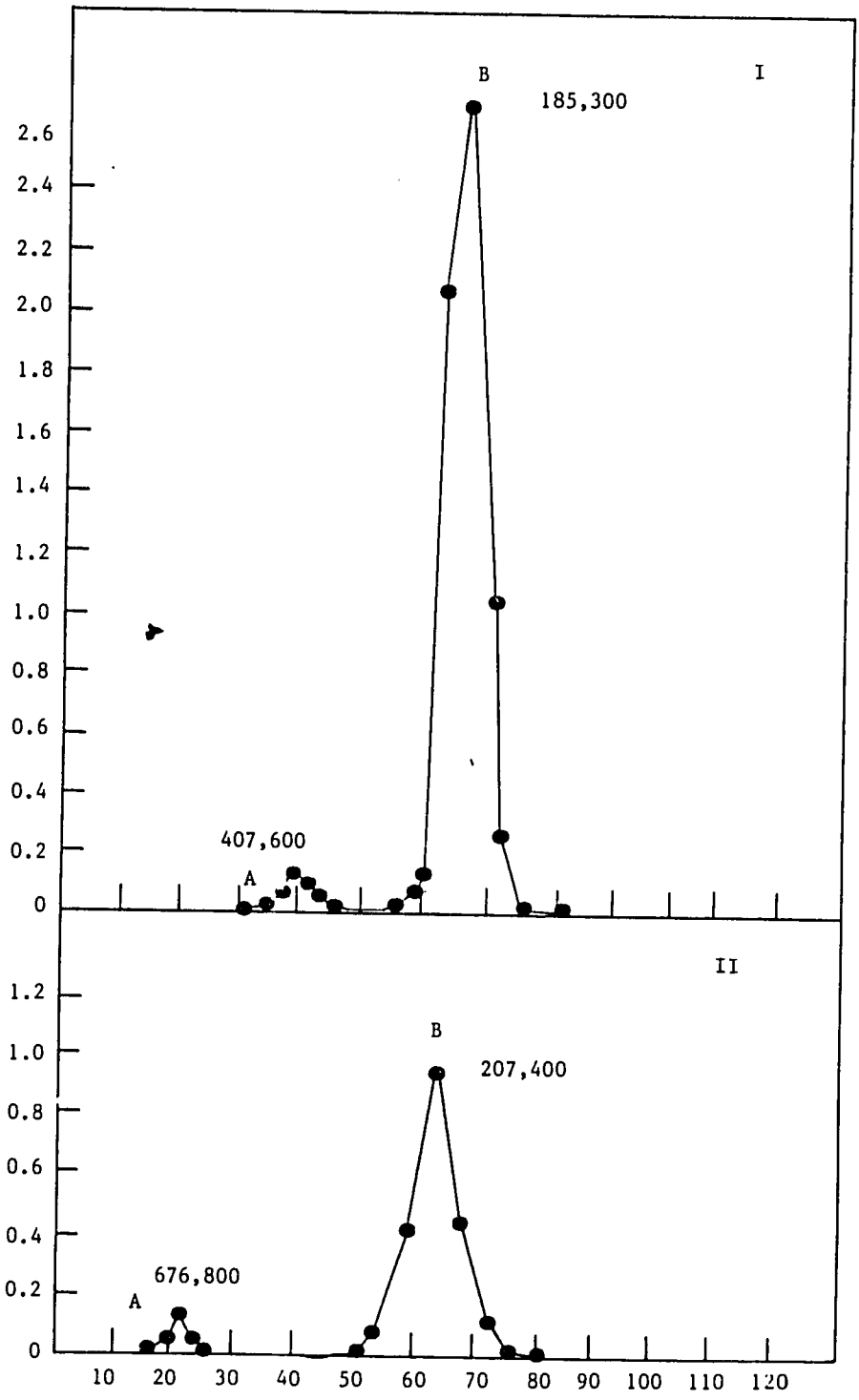


Fig. 5. Gel filtration of jack bean albumins (I) and globulins (II) on Biogel $A_{50}$ m.

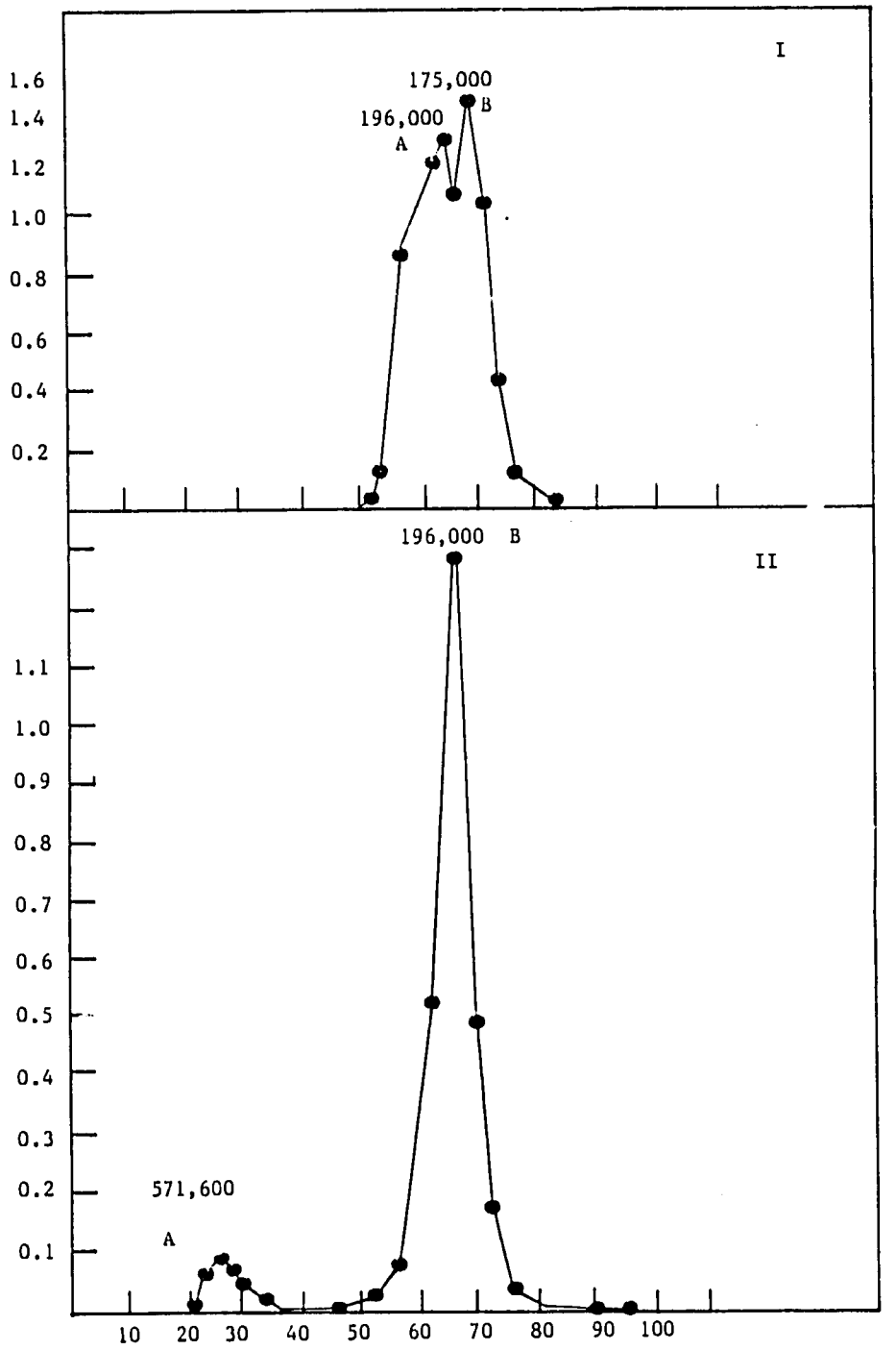


Fig. 6. Gel filtration of sword bean albumins (I) and globulins (II) on Biogel $A_{50}$ m.

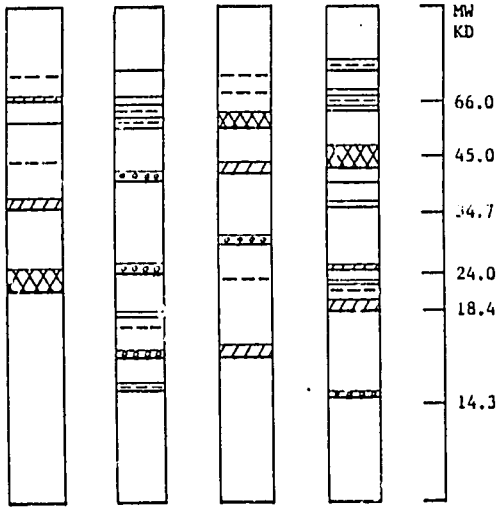


Fig. 7. SDS-electrophoretic patterns of jack bean albumins and globulins separated on Biogel $A_{50}$ m.

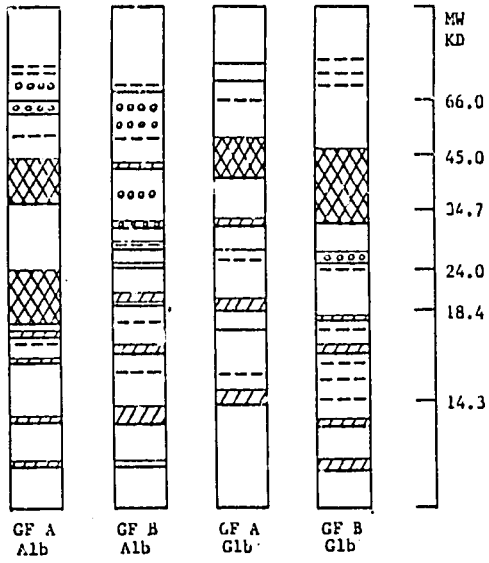


Fig. 8. SDS-electrophoretic patterns of sword bean albumins and globulins separated on Biogel $A_{50}$ m.

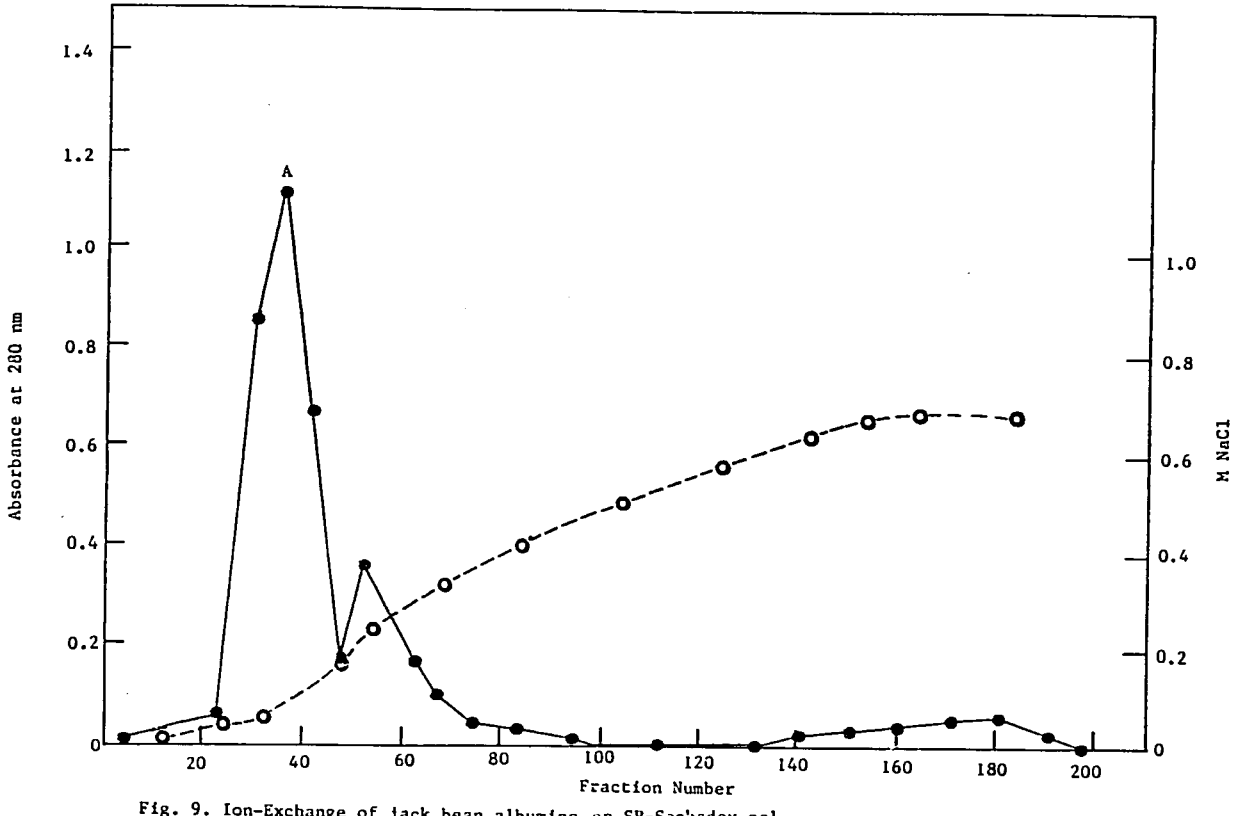


Fig. 9. Ion-Exchange of jack bean albumins on SP-Sephadex gel.

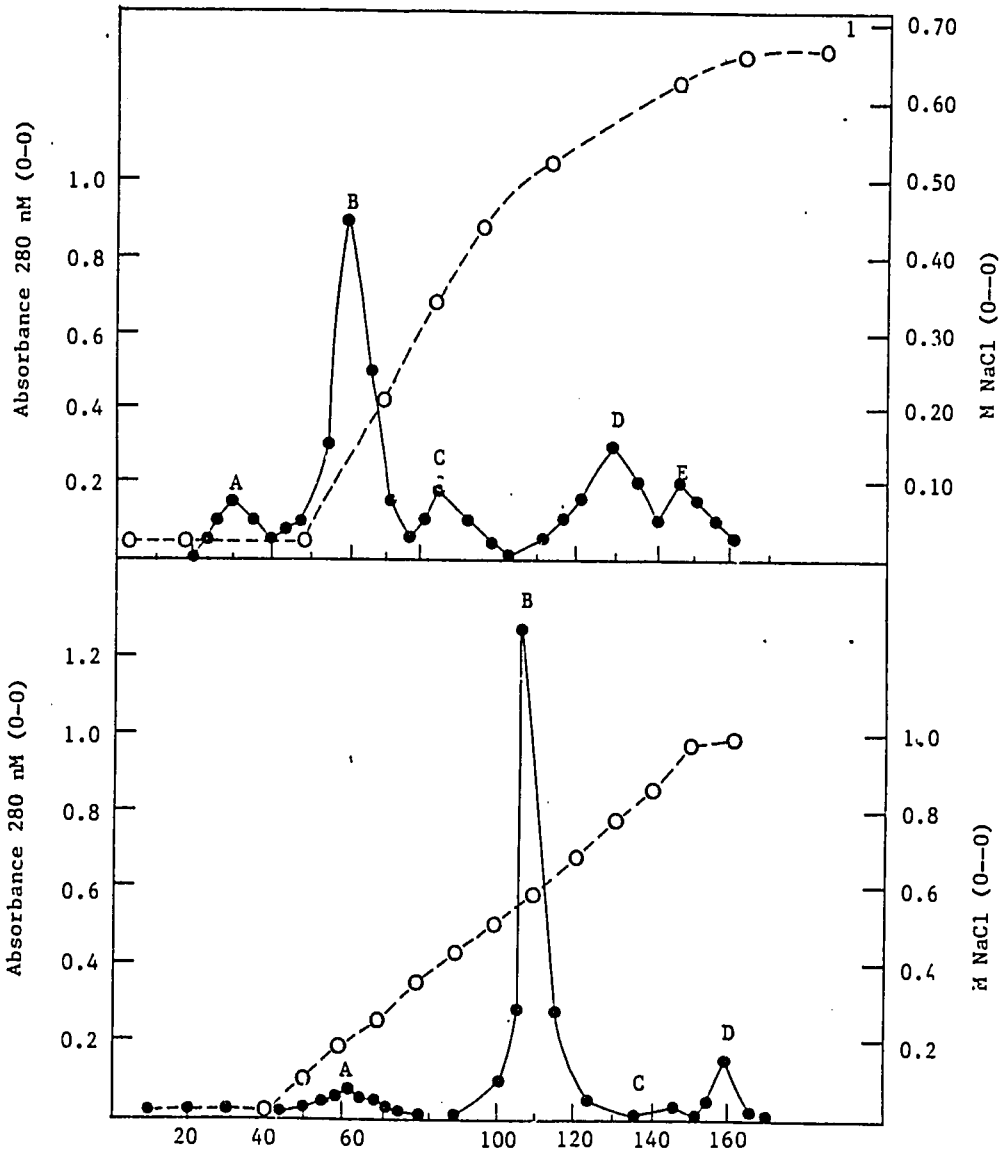


Fig. 10. Ion-exchange chromatography of sword bean albumins and globulins on SP-Sephadex $C_{20}$-150.

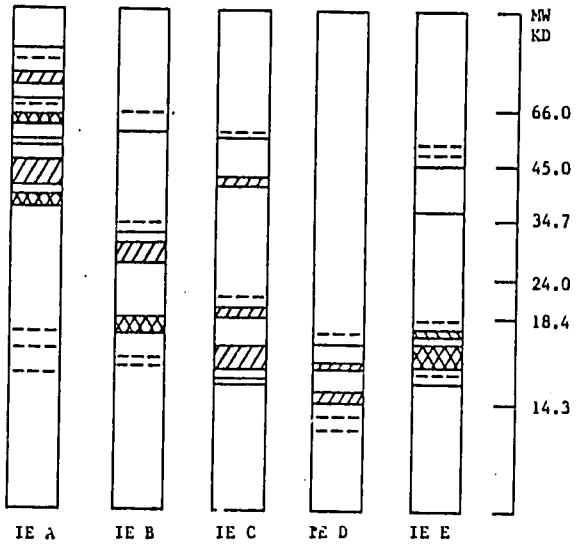


Fig. 12. SDS-electrophoretic patterns of sword bean albumins separated by ion exchange chromatography.

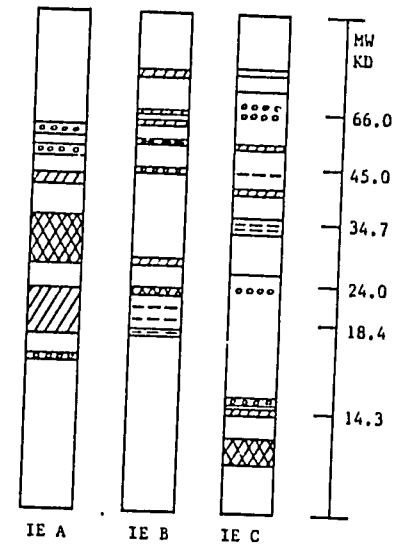


Fig. 11. SDS-electrophoretic patterns of jack bean albumins separated by ion-exchange chromatography.

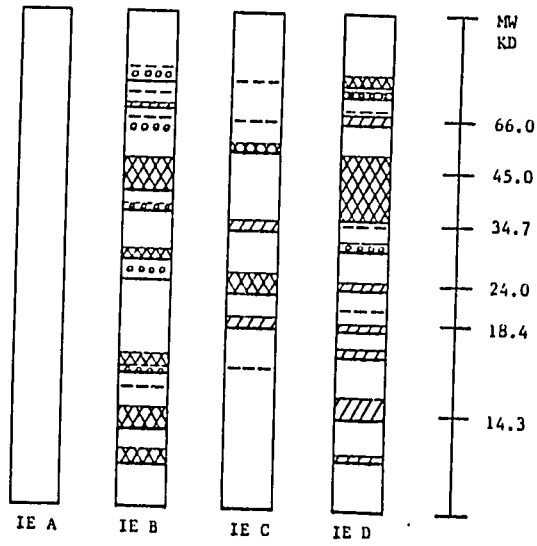


Fig. 13. SDS-electrophoretic patterns of sword bean globulins separated by ion exchange chromatography.

# PROXIMATE ANALYSES AND IN VITRO DRY MATTER DIGESTIBILITY OF SEVERAL PHILIPPINE INDIGENOUS FORAGE LEGUMES

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## ABSTRACT

The proximate composition of the mature and immature leaves, mature and immature pods and mature seeds of nine forage legumes was determined. These legumes include: Aeschynomene, Cassia occidentalis, Centrosema, Calopogonium, Crotolaria, Desmodium, Pueraria, Phaseolus lahyacinos and Porpogon.

In vitro dry matter digestibility (IVDMD) ranged from 16 to 38% for leaves, and 13 to 38% for pods. Seeds of two samples had IVDMD of 8 and 21%.

Metabolizable energy (ME) predicted from crude fiber contents ranged from 3.60 to 4.30 kcal/g for leaves, 3.50 to 4.25 kcal/g for pods and 3.42 to 4.29 kcal/g for seeds.

## INTRODUCTION

The nutritive value of feeds can be estimated through their digestibility and chemical composition. From the latter, especially, crude fiber, feed energy values can be predicted. However, although crude fiber is continued to be used, its inadequacy because of the great variability in lignocellulose composition between species and other factors, has been recognized. Thus, other methods have been proposed to replace crude fiber analysis; these are: acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin (Pigden et al, 1980).

Digestibility predicts more accurately the nutritive value of feeds. In vitro methods have been developed using either selected enzymes (mainly celluloses) or using rumen liquor. The two-stage *in vitro* rumen fermentation technique has become widely accepted as the method to predict digestibility of all types of forages (Martin and Barnes, 1980). High correlations between in vitro rumen and in vivo dry matter digestibility have been reported (Barnes, 1973). Lopez et al (1976) obtained correlation of 0.85 to 0.90 for 38 indigenous and introduced species of forages.

Feeding systems are based on metabolizable energy (ME) of the feed components (Minson, 1980). Tables of ME values of roughages have been prepared and equations established to predict ME values satisfactorily from chemical constituent contents of the feed.

Also essential for forage evaluation is the estimation of crude protein content although this does not accurately show the extent of its breakdown in the rumen (Leng, 1985).

Different parts of nine species indigenous to the Philippines were subjected to proximate analyses and in vitro dry matter acceptability to estimate their feeding values. These species are: Aeschynomene, Cassia occidentalis, Calopogonium, Centrosema, Crotolaria, Desmodium, Phaseolus lathyroides, Porpogon and Pueraria. ME values were also estimated from crude fiber contents.

## MATERIALS AND METHODS

### Materials

Seeds of the aforementioned forage legumes were obtained from the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding. These were planted to obtain fresh samples of immature and mature leaves, immature and mature pods and mature seeds.

All chemicals used were of analytical grade.

### Preparation of Samples

Leaves, pods and mature seeds were separately dried at 45 C C in a forced draft oven for 48 hr. Afterwards, they were ground in a Wiley mill and passed through 60 mesh sieve.

### Analyses

Moisture, fat, protein (% N x 6.25), ash and fiber were determined according to official AOAC methods (AOAC, 1960).

### Metabolizable Energy (ME)

ME was calculated from the regression equation (Y=4.65-0.062 CF) developed by Armstrong (1966) for temperate grasses.

**In Vitro Dry Matter Digestibility (IVDMD)**

IVDMD was determined using the procedure of Tilley and Terry (1963) as modified by Barnes (1967) and was done at the Animal Nutrition Laboratory of the Institute of Animal Science, CA, UPLB.

## RESULTS AND DISCUSSIONS

**Proximate Analyses**

The proximate composition of different parts of nine Philippine indigenous forage legumes is summarized in Table 1.

Moisture content of immature and mature leaves and pods ranged from 65 to 82% while that of mature seeds ranged from 4.5 to 9%. Fat content ranged from 1.3 to 4.4% in leaves and pods and 2 to 17% in mature seeds. Notably, the following had relatively high levels of fat content in mature seeds: Aeschynomene (12%), Calopogonium (10.7%), Desmodium (14%) and Porpogon (17%). Similar ranges of fat contents were reported by Gerpacio and Castillo (1979) for Centrosema, Aeschynomene and Kudzu. Protein content was from 6 to 7% in leaves 2 to 3.5% in pods and 18 to 33% in mature seeds. Relatively high protein contents were observed in seeds of: Aeschynomene (20%), Calopogonium (33%), Crotolaria (27%), Desmodium (30%), Kudzu (30%) and Porpogon (33%). Lower protein content was found in pods. Varietal differences were also evident; e.g., for Centrosema, protein content of seeds ranged from 18.45% to 22.73%.

The protein contents observed in the leaves of these legumes are comparatively lower than in Leucaena leaves (24%) (Gerpacio and Castillo, 1979; Endrinal and Mendoza, 1979); and acacia

leaves (21%) (Gerpacio and Castillo, 1979).

Ash ranged from 1 to 3.6% in leaves, 1 to 1.5% in pods and 2.4 to 4.7% in seeds. Fiber ranged from 1.3 to 5.8% in leaves, 2.2 to 3.7% in pods and 5.2 to 17% in seeds. Aeschynomene seeds were highest in fiber content.

Nitrogen free-extracts or carbohydrates were the major constituent of the legumes. Leaves had 10 to 20% of NFE, pods had 9.4 to 28% in pods, and 32 to 58% in seeds.

This is the first comprehensive study of the chemical composition of different parts of several accessions of forage legumes indigenous to the Philippines.

**In Vitro Dry Matter Digestibility** (IVDMD)

IVDMD of immature and mature leaves ranged from 16 to 38% and for immature and mature pods--13 to 38% (Table 2). Crotolaria had the highest IVDMD values for leaves and pods. Seeds of two samples Crotolaria and D. lathyroides had IVDMD of 8 and 21%. These values are much lower than the control sample of L. leucocephala leaves with 49.72-51.26% IVDMD and 33 to 66% IVDMD for 14 grasses and 28 to 68% for 7 legumes, obtained by Lopez et al (1976). The values for Desmodium, Pueraria and D. lathyroides obtained in this study were about 50% less than those reported by Lopez et al (1976). The differences could be due to varietal differences. These low IVDMD results could suggest the low feeding values of the legumes analyzed and need to be re-investigated.

**Prediction of Metabolizable Energy (ME)**

ME for the samples calculated from crude fiber content (organic matter basis) ranged from 3.60 to 4.30 kcal/g for leaves, 3.50 to 4.25 Kcal/g for pods and 3.42 to 4.29 kcal/g for seeds (Table 3). High ME values (4.0 to 4.30) were obtained for leaves of Aeschynomene, Cassia occidentalis, Crotolaria and C. lathyroides. Medium levels of 3.75 to 3.99 Kcal/g were obtained for leaves of Centrosema, Calopogonium, Pueraria and Perogon. Low levels of lower than 3.75 Kcal/g were obtained for leaves of Desmodium (2 accessions). ME values for immature and mature pods were similar to those obtained for leaves. ME values for seeds were generally lowest in 6 of the 15 samples and comparable with the other parts in the other samples.

The regression equation (Y = 4.65 - 0.062 CF) used in this study was obtained from data for temperature grasses (Armstrong, 1964). Minson and Milford (1966) had reported mean energy contents of 4.37, 4.19 and 4.53 kcal/g digestible organic matter for samples of Sorghum almum, Digitaria decumbens and Macroptilium atropurpureum. A range of 3.86 - 4.77 Kcal/g for 23 legumes was later reported (Minson, 1980). The estimated ME values for the legumes in this study are within the range of the reported values for tropical legumes.

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**Table 1. Proximate composition and in vitro dry matter digestibility (IVDMD) of different parts of several Philippine indigenous forage legumes.**

| Legume/Acc/ Part | Moisture (%) | Fat (%) | Protein (% Nx6.25) | Ash (%) | Fiber (%) | NFE (%) |
|---|---|---|---|---|---|---|
| Aeschynomene | | | | | | |
| Acc IPB-1 | | | | | | |
| Immature leaves | 74.21 | 2.08 | 6.14 | 1.87 | 1.36 | 14.34 |
| Mature leaves | 69.69 | 4.17 | 8.36 | 2.70 | 1.68 | 14.20 |
| Immature pods | 74.72 | 2.08 | 5.56 | 1.11 | 2.15 | 14.38 |
| Mature pods | 66.37 | 2.08 | 5.56 | 1.11 | 2.15 | 14.38 |
| Mature seeds | 9.13 | 11.93 | 24.77 | 3.59 | 17.25 | 33.33 |
| Acc IPB-2 | | | | | | |
| Immature leaves | 72.54 | 2.24 | 6.33 | 1.91 | 1.41 | 15.57 |
| Mature leaves | 62.79 | 4.45 | 8.42 | 2.51 | 1.93 | 19.90 |
| Immature pods | 75.16 | 2.20 | 5.31 | 1.28 | 2.36 | 13.69 |
| Mature pods | 64.49 | 2.20 | 5.31 | 1.28 | 2.36 | 13.69 |
| Mature seeds | 9.44 | 11.47 | 24.56 | 3.80 | 17.14 | 33.59 |
| Balatong Aso (*Cassia occidentalis*) | | | | | | |
| Acc BP | | | | | | |
| Immature leaves | 74.32 | 2.43 | 6.33 | 1.91 | 1.41 | 15.57 |
| Mature leaves | 70.17 | 2.34 | 8.84 | 2.64 | 2.20 | 13.81 |
| Immature pods | 91.17 | 1.37 | 2.24 | 1.20 | 2.91 | 10.90 |
| Mature pods | 70.86 | 1.37 | 2.24 | 1.20 | 2.91 | 10.90 |
| Mature seeds | 5.94 | 2.07 | 21.08 | 4.69 | 8.23 | 57.99 |
| Acc 24 | | | | | | |
| Immature leaves | 76.33 | 1.79 | 8.91 | 1.97 | 1.42 | 9.58 |
| Mature leaves | 69.75 | 2.19 | 8.46 | 2.76 | 3.04 | 13.80 |
| Immature pods | 81.61 | 1.18 | 2.06 | 1.23 | 3.14 | 10.79 |
| Mature pods | 72.54 | 1.34 | 2.14 | 1.21 | 3.67 | 9.41 |
| Mature seeds | 6.48 | 2.68 | 20.63 | 3.96 | 11.67 | 54.38 |
| Centrosema | | | | | | |
| Acc BM-1 | | | | | | |
| Immature leaves | 70.83 | 3.06 | 7.80 | 2.09 | 2.26 | 13.96 |
| Mature leaves | 62.15 | 2.46 | 9.84 | 2.27 | 4.66 | 18.62 |
| Immature pods | 75.46 | 1.44 | 3.56 | 1.06 | 2.78 | 15.70 |
| Mature pods | 65.58 | 1.24 | 1.78 | 0.97 | 2.18 | 28.35 |
| Mature seeds | 6.28 | 5.86 | 22.73 | 2.48 | 8.90 | 57.85 |
| Acc BPI-T1 | | | | | | |
| Immature leaves | 68.75 | 3.18 | 9.41 | 2.85 | 3.14 | 12.67 |
| Mature leaves | 63.38 | 3.36 | 9.67 | 2.71 | 5.80 | 15.08 |
| Immature pods | 72.44 | 1.75 | 3.27 | 1.17 | 2.44 | 18.93 |
| Mature pods | 64.78 | 1.45 | 2.37 | 1.04 | 2.75 | 27.61 |
| Mature seeds | 4.75 | 7.17 | 18.45 | 2.98 | 8.90 | 57.96 |

| Legume/Acc/ Part | Moisture (%) | Fat (%) | Protein (%) | Ash (%) | Fiber (%) | NFE (%) |
|---|---|---|---|---|---|---|
| Acc JS-1 | | | | | | |
| Immature leaves | 67.62 | 2.70 | 8.38 | 2.23 | 3.45 | 15.62 |
| Mature leaves | 64.51 | 2.89 | 9.25 | 2.85 | 3.30 | 17.20 |
| Immature pods | 78.64 | 1.67 | 3.14 | 1.14 | 2.38 | 13.03 |
| Mature pods | 67.46 | 1.31 | 2.45 | 0.95 | 2.42 | 25.41 |
| Mature seeds | 5.98 | 5.36 | 19.72 | 2.44 | 8.30 | 57.96 |
| Acc BP-2 | | | | | | |
| Immature leaves | 68.14 | 2.94 | 8.46 | 2.44 | 2.68 | 15.34 |
| Mature leaves | 64.72 | 3.79 | 9.27 | 2.43 | 4.57 | 16.23 |
| Immature pods | | | | | | |
| Mature pods | | | | | | |
| Mature seeds | | | | | | |
| Calopogonium | | | | | | |
| Acc JS-1 | | | | | | |
| Immature leaves | 75.00 | 2.55 | 5.69 | 1.79 | 2.56 | 12.47 |
| Mature leaves | 66.89 | 3.31 | 6.51 | 2.95 | 2.97 | 17.37 |
| Immature pods | 79.65 | 1.32 | 3.19 | 1.18 | 3.16 | 12.50 |
| Mature pods | 67.94 | 1.67 | 4.41 | 1.47 | 3.12 | 21.39 |
| Mature seeds | 4.49 | 10.69 | 33.23 | 4.26 | 13.60 | 33.69 |
| Crotolaria | | | | | | |
| Acc PC-1 (NB) | | | | | | |
| Immature leaves | 72.84 | 2.69 | 7.31 | 1.47 | 2.43 | 13.27 |
| Mature leaves | 66.76 | 2.90 | 6.45 | 1.94 | 3.37 | 18.59 |
| Immature pods | 75.89 | 1.59 | 2.74 | 1.15 | 2.94 | 15.69 |
| Mature pods | 65.15 | 1.56 | 4.12 | 1.56 | 3.42 | 24.19 |
| Mature seeds | 6.39 | 6.98 | 26.79 | 3.75 | 13.25 | 42.85 |
| Acc PC-1 (WB) | | | | | | |
| Immature leaves | 74.12 | 2.24 | 6.59 | 1.59 | 2.33 | 13.13 |
| Mature leaves | 68.95 | 2.94 | 7.19 | 1.86 | 3.07 | 15.99 |
| Immature pods | 76.45 | 1.75 | 3.68 | 0.87 | 2.65 | 15.60 |
| Mature pods | 64.36 | 1.59 | 3.99 | 1.32 | 3.51 | 25.24 |
| Mature seeds | 4.57 | 7.81 | 28.09 | 3.70 | 10.45 | 45.19 |
| Acc JS-1 | | | | | | |
| Immature leaves | 73.67 | 2.45 | 7.23 | 1.78 | 2.61 | 13.24 |
| Mature leaves | 68.44 | 4.43 | 6.67 | 1.75 | 3.16 | 15.55 |
| Immature pods | 78.24 | 1.28 | 2.98 | 1.02 | 3.12 | 12.76 |
| Mature pods | 63.49 | 1.74 | 3.76 | 1.53 | 3.31 | 26.17 |
| Mature seeds | | | | | | |
| Desmodium | | | | | | |
| Acc DO-1 | | | | | | |
| Immature leaves | 69.81 | 2.54 | 6.50 | 1.08 | 4.88 | 15.19 |
| Mature leaves | 66.05 | 3.45 | 7.71 | 3.69 | 4.35 | 13.77 |
| Immature pods | | | | | | |
| Mature pods | | | | | | |
| Mature seeds | 6.48 | 14.10 | 30.73 | 3.29 | 6.15 | 39.23 |

| Legume/Acc/ Part | Moisture (%) | Fat (%) | Protein (%) | Ash (%) | Fiber (%) | NFE (%) |
|---|---|---|---|---|---|---|
| Acc DO-2 | | | | | | |
| Immature leaves | 71.81 | 2.28 | 5.29 | 1.00 | 4.59 | 15.03 |
| Mature leaves | 65.60 | 3.26 | 7.43 | 2.90 | 5.26 | 14.36 |
| Immature pods | | | | | | |
| Mature pods | | | | | | |
| Mature seeds | 6.14 | 13.84 | 30.33 | 4.10 | 6.46 | 39.13 |
| Acc CT | | | | | | |
| Immature leaves | 72.79 | 1.82 | 5.46 | 1.17 | 4.36 | 14.90 |
| Mature leaves | 63.50 | 3.26 | 6.35 | 3.56 | 4.11 | 19.22 |
| Immature pods | | | | | | |
| Mature pods | | | | | | |
| Mature seeds | 6.49 | 13.44 | 30.17 | 4.08 | 6.31 | 39.51 |
| Acc 93 | | | | | | |
| Mature seeds | 5.44 | 14.17 | 29.69 | 4.22 | 8.15 | 38.06 |
| Acc 195 | | | | | | |
| Immature leaves | | | | | | |
| Mature leaves | | | | | | |
| Immature pods | | | | | | |
| Mature pods | | | | | | |
| Mature seeds | 6.36 | 13.95 | 30.07 | 4.26 | 6.95 | 38.51 |
| Pueraria | | | | | | |
| Kudzu | | | | | | |
| Acc DO-1 | | | | | | |
| Immature leaves | 79.04 | 1.82 | 4.52 | 1.77 | 2.06 | 10.79 |
| Mature leaves | 71.48 | 3.09 | 5.58 | 2.63 | 2.58 | 14.64 |
| Immature pods | | | | | | |
| Mature pods | | | | | | |
| Mature seeds | 5.40 | 6.13 | 27.43 | 5.17 | 5.20 | 50.67 |
| Phaseolus | | | | | | |
| Lathyroides | | | | | | |
| Acc IPB | | | | | | |
| Immature leaves | 81.36 | 1.01 | 5.37 | 1.34 | 1.79 | 9.13 |
| Mature leaves | 76.56 | 2.41 | 6.84 | 2.23 | 2.06 | 9.90 |
| Immature pods | 74.56 | 1.51 | 4.37 | 1.38 | 3.42 | 14.76 |
| Mature pods | 74.56 | 1.51 | 4.37 | 1.38 | 3.42 | 14.76 |
| Mature seeds | 6.58 | 5.74 | 17.95 | 3.63 | 9.35 | 56.75 |
| Acc 158 | | | | | | |
| Immature leaves | 80.47 | 1.18 | 5.26 | 1.25 | 1.32 | 10.52 |
| Mature leaves | 75.85 | 2.56 | 6.67 | 2.74 | 2.37 | 9.81 |
| Immature pods | 77.03 | 1.73 | 4.86 | 1.15 | 3.16 | 15.07 |
| Mature pods | 77.03 | 1.73 | 4.86 | 1.15 | 3.16 | 15.07 |
| Mature seeds | 8.67 | 5.29 | 21.42 | 3.61 | 8.00 | 52.02 |

| Legume/Acc/ Part | Moisture (%) | Fat (%) | Protein (%) | Ash (%) | Fiber (%) | NFE (%) |
|---|---|---|---|---|---|---|
| Porpogon | | | | | | |
| Acc BPI-T1 | | | | | | |
| Immature leaves | | | | | | |
| Mature leaves | | | | | | |
| Immature pods | 72.56 | 2.04 | 1.77 | 1.07 | 2.94 | 19.62 |
| Mature pods | 72.34 | 1.66 | 3.91 | 1.49 | 3.77 | 17.38 |
| Mature seeds | 6.75 | 17.05 | 33.10 | 4.22 | 6.55 | 32.33 |

**Table 2. In vitro dry matter digestibility (IVDMD) of selected forage legumes.**

| Sample | Acc | % IVDMD | | | | |
|---|---|---|---|---|---|---|
| | | IL | ML | IP | MP | MS |
| Balatong Aso | BP | 18.91 | 23.51 | 19.58 | 23.68 | |
| Calopogonium | JS-1 | 26.98 | 24.94 | - | - | - |
| | BM-1 | 24.40 | 20.68 | - | - | - |
| Centrosema | BM-1 | 16.29 | 30.91 | 13.31 | 36.22 | |
| | BPI-T1 | 23.84 | 27.94 | 21.68 | 28.23 | |
| | BPTB | 26.68 | 35.14 | 18.67 | 32.60 | |
| | JS-1 | 21.81 | 32.15 | - | - | - |
| Crotolaria | BM-1 | 13.22 | 25.62 | 15.08 | 33.49 | 4.75 |
| | WB PC-1 | 13.45 | 16.41 | 11.53 | 38.14 | 7.25 |
| | NB PC-1 | 38.25 | 25.70 | 18.64 | 29.62 | 7.89 |
| Desmodium | 195 | 21.51 | 28.02 | 28.96 | 29.88 | 19.36 |
| Kudzu | 9P | 27.80 | 20.20 | - | - | - |
| Phaseolus Lathyroides | IPB | - | - | - | 32.47 | 20.66 |
| | 158 | - | - | - | 32.43 | 20.09 |
| Porpogon | BP1-T1 | 20.15 | 26.68 | - | - | - |

IL - Immature Leaves; ML - Mature Leaves; IP - Immature Pods; MP - Mature Pods; MS - Mature Seeds.

Leucaena leucocephala leaves were used as control with IVDMD of 49.72-51.26%.

**Table 3. Crude fiber content and predicted metabolizable energy of several Philippine indigenous forage legumes.**

| Legume/Acc/ Part | Crude Fiber (%) DMB | Crude Fiber (%) DMB | Metabolizable energy Kcal/g DMB |
|---|---|---|---|
| Aeschynomene | | | |
| Acc IPB-1 | | | |
| Immature Leaves | 5.27 | 5.69 | 4.30 |
| Mature Leaves | 3.40 | 5.91 | 4.28 |
| Immature Pods | | | |
| Mature Pods | | | |
| Mature Seeds | 18.98 | 19.76 | 3.42 |
| Acc IPB-1 | | | |
| Immature Leaves | 5.13 | 5.52 | 4.31 |
| Mature Leaves | 5.19 | 5.56 | 4.31 |
| Immature Pods | | | |
| Mature Pods | | | |
| Mature Seeds | 19.93 | 19.73 | 3.42 |
| Balatong Aso (Cassia occidentalis) | | | |
| Acc BF | | | |
| Immature Leaves | 5.41 | 5.93 | 4.28 |
| Mature Leaves | 7.38 | 8.09 | 4.15 |
| Immature Pods | | | |
| Mature Pods | | | |
| Mature Seeds | 8.70 | 9.21 | 4.08 |
| Cassia occidentalis | | | |
| Acc 24 | | | |
| Immature Leaves | 6.04 | 6.59 | 4.24 |
| Mature Leaves | 10.05 | 11.06 | 3.96 |
| Immature Pods | 17.07 | 18.30 | 3.52 |
| Mature Pods | 13.36 | 13.98 | 3.78 |
| Mature Seeds | 12.48 | 13.03 | 3.84 |
| Centrosema | | | |
| Acc BM-1 | | | |
| Immature Leaves | 7.75 | 8.35 | 4.13 |
| Mature Leaves | 12.31 | 13.60 | 3.84 |
| Immature Pods | 11.33 | 11.84 | 3.92 |
| Mature Pods | 6.33 | 6.50 | 4.25 |
| Mature Seeds | 9.50 | 9.75 | 4.05 |

| Legume/Acc/ Part | Crude Fiber (%) DMB | Crude Fiber (%) OMB | Metabolizable Energy (kcal/g) |
|---|---|---|---|
| Acc BP1-T1 | | | |
| Immature Leaves | 10.05 | 11.05 | 3.96 |
| Mature Leaves | 15.81 | 17.11 | 3.59 |
| Immature Pods | 8.85 | 9.25 | 4.08 |
| Mature Pods | 7.81 | 8.05 | 4.15 |
| Mature Seeds | 9.34 | 9.69 | 4.05 |
| Acc JS-1 | | | |
| Immature Leaves | 10.65 | 10.95 | 3.97 |
| Mature Leaves | 9.30 | 10.11 | 4.02 |
| Immature Pods | 11.14 | 11.77 | 3.92 |
| Mature Pods | 7.44 | 7.66 | 4.18 |
| Mature Seeds | 8.83 | 9.06 | 4.09 |
| Acc BP-2 | | | |
| Immature Leaves | 8.41 | 9.11 | 4.09 |
| Mature Leaves | 12.95 | 13.91 | 3.79 |
| Calopogonium | | | |
| Acc JS-1 | | | |
| Immature Leaves | 10.24 | 11.03 | 3.97 |
| Mature Leaves | 8.97 | 9.85 | 4.04 |
| Immature Pods | 10.61 | 11.27 | 3.95 |
| Mature Pods | 9.73 | 10.20 | 4.02 |
| Mature Seeds | 14.24 | 14.90 | 3.73 |
| Crotolaria | | | |
| Acc PC-1 (NB) | | | |
| Immature Leaves | 8.95 | 9.45 | 4.06 |
| Mature Leaves | 10.14 | 10.77 | 3.98 |
| Immature Pods | 12.19 | 12.81 | 3.86 |
| Mature Pods | 9.81 | 10.27 | 4.01 |
| Mature Seeds | 14.15 | 14.75 | 3.74 |
| Acc PC-1 (WB) | | | |
| Immature Leaves | 9.00 | 9.59 | 4.06 |
| Mature Leaves | 9.89 | 10.52 | 4.00 |
| Immature Pods | 11.25 | 11.68 | 3.93 |
| Mature Pods | 9.85 | 10.23 | 4.02 |
| Mature Seeds | 11.16 | 11.61 | 3.93 |
| Acc JS-1 | | | |
| Immature Leaves | 9.55 | 10.22 | 4.02 |
| Mature Leaves | 10.01 | 10.60 | 3.99 |
| Immature Pods | 14.34 | 15.06 | 3.72 |
| Mature Pods | 9.09 | 9.49 | 4.08 |

| Legume/Acc/ Part | Crude Fiber (%) DMB | Crude Fiber (%) OMB | Metabolizable Energy (kcal/g) |
|---|---|---|---|
| Desmodium | | | |
| (Acc DG-1) | | | |
| Immature Leaves | 16.16 | 16.76 | 3.61 |
| Mature Leaves | 12.81 | 14.14 | 3.77 |
| (Acc DG-2) | | | |
| Immature Leaves | 16.28 | 16.88 | 3.60 |
| Mature Leaves | 15.29 | 17.24 | 3.58 |
| Mature Seeds | 6.88 | 7.20 | 4.20 |
| Pueraria | | | |
| Kudzu (Acc DG-1) | | | |
| Immature Leaves | 9.83 | 10.73 | 3.98 |
| Mature Leaves | 9.05 | 9.95 | 4.03 |
| Mature Seeds | 5.49 | 5.82 | 4.29 |
| Phaseolus lathyroides | | | |
| (Acc IPB | | | |
| Immature Leaves | 9.60 | 10.35 | 4.01 |
| Mature Leaves | 8.79 | 9.71 | 4.05 |
| Immature Pods | | | |
| Mature Pods | | | |
| Mature Seeds | 10.01 | 10.41 | 4.01 |
| (Acc 158 | | | |
| Immature Leaves | 6.76 | 7.22 | 4.20 |
| Mature Leaves | 9.81 | 11.07 | 3.96 |
| Immature Pods | | | |
| Mature Pods | | | |
| Mature Seeds | 8.76 | 9.79 | 4.04 |
| Perpogon | | | |
| (Acc BP1-T1 | | | |
| Immature Pods | 10.71 | 11.15 | 3.96 |
| Mature Pods | 10.01 | 10.58 | 3.99 |
| Mature Seeds | 7.02 | 7.36 | 4.19 |

DMD - dry matter basis
OMB - organic matter basis
a
Calculated from the regression equation (Armstrong, 1966)
Y = 4.65 - 0.062 CF.

# SAPONINS, PHYTATE, POLYPHENOLS, OLIGOSACCHARIDES, LECTINS AND CYANOGENIC GLYCOSIDES IN PHILIPPINE INDIGENOUS FORAGE LEGUMES

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## ABSTRACT

Saponins in seeds ranged from 0.26 to 0.92%, highest in Desmodium and Porpogon. All samples were positive to the froth test except for an accession of Calopogonium.

Polyphenols were generally low in the nine species analyzed, both in terms of condensed tannins and flavanols. Only Centrosema and P. lathyroides had relatively high flavanol groups (5.57 to 10.12 mg catechin/g).

Most of the samples had high (>5 mg P/g) phytate-phosphorus. Seeds of Aeschynomene, C. occidentalis and Calopogonium and relatively high levels of oligosaccharides (>5%).

Strong hemagglutinating activity towards bovine red blood cells was observed in seeds of Centrosema (5 accessions) Crotolaria (5 accessions) and Phaseolus lathyroides (1 accession).

Cyanogenic glucosides were very low or nondetectable in all samples tested.

## INTRODUCTION

Aside from proximate composition and dry matter digestibility, the nutritive values of forage can be limited by the presence of deleterious substances. Some are outright toxins causing ill health or death to animals while others do not cause symptoms of ill health but cause reduced production. These dietary-dependent disorders were classified by Reid (1973) into seven groups according to their causes: (1) nutritional mismatching, (2) mineral poisoning, (3) poisoning by naturally occurring substances, (4) disturbances of ruminant physiology or metabolism, (5) interactions of nutrition and noxious agents, (6) poisoning by chemicals introduced by man and (7) physical damage. Other reviews on the subject have been made (Hegarty et al. 1985; Hegarty, 1981).

This study aimed to determine the presence and level of several anti-nutritive factors in seeds and other parts of several Philippine indigenous legumes. Most of the samples used were seeds because earlier studies showed that the anti-nutrients are usually localized in seeds. These anti-nutritive factors are: saponins, phytate, polyphenols, oligosaccharides, lectins and cyanogenic glycosides. Descriptions and effects of these factors are given in other portions of this report. The study on alkaloids is presented in the following paper (Rodriguez and Mendoza, 1988).

## MATERIALS AND METHODS

**Samples.** Seeds of the forage legumes were obtained from the National Plant Genetic Resources Laboratory of IPB and grown in the IPB experimental farms to obtain fresh samples. These legumes were: Aeschynomene, Cassia occidentalis or balatong aso, Calopogonium, Centrosema, Crotolaria, Desmodium, Pueraria, Phaseolus lathyroides and Pueraria phaseoloides.

**Chemicals.** All chemicals used were of analytical grade.

**Preparation of Samples.** Raw mature seeds were dried at 45 C for 48 hr, ground and passed through 60 mesh. Cyanogenic glycosides were determined in fresh samples.

**Analyses.** (1) Saponins were analyzed by the modified Liebermann-Burchard test described by Lubag et al (1988) and by the froth test. (2) Phytate was measured according to the method of Wheeler and Ferrel (1971) and expressed as phytate-phosphorus. (3) Polyphenol content was measured using the modified vanillin assay (Price et al, 1978) and protein precipitation (Hagerman and Butler, 1978). (4) Oligosaccharides were determined by subjecting 70% ethanol extracts to thin layer chromatography using HPTLC plates and subsequent densitometer analysis (Revilleza et al, 1988). (5) Lectins were estimated by means of their hemagglutinating activity (Barroga et al, 1988). (6) Cyanogenic glycosides were determined using both enzymatic (Reay and Conn, 1970) and picric acid method (Revilleza et al, 1988).

## RESULTS AND DISCUSSION

### Saponins

Saponins ranged from 0.26 to 0.92% in 7 species tested (Table 1). These values are much higher than those reported for food legumes (Lubag et al, 1988). Froth test was positive to highly positive. Among forage legumes, alfalfa (Medicago sativa) saponins are best studied and had been shown to cause typical symptoms of bloat in ruminants (Coulson and Davis, 1962). Efforts towards modification of saponin content in alfalfa had been reported (Jones, 1969, as cited by Bray, 1981). The antifungal properties of some saponins have also been implicated in disease resistance of plants. Saponin for alfalfa has been shown to depress growth of the beetle (Tribolium castaneum) larvae (Shany et al, 1970).

### Polyphenols

The seeds of Aeschynomene, Cassia occidentalis, Calopogonium, and Porpogon had low levels of flavanols as measured by vanillin assay (Table 2), about 10 to 30 times less than in mungbean (Barroga et al, 1985). Only Centrosema and C. lathyroides seeds had considerably higher flavanols (5.57 to 10.12 mg catechin/g). Crotolaria had no detectable flavanol group.

Low levels of protein precipitable polyphenols were also obtained in the samples tested (0.13 to 1.81 mg tannic acid/g) (TAE) compared to sorghum (1.03 to 5.66 TAE) (Bullard et al, 1981) and cowpea (1.44 to 4.77 TAE) (Laurena et al, 1983) but higher than for mungbean (0.16 to 0.61 TAE) (Barroga et al,

1985).

These results indicate that these seeds of the species analyzed had low levels of polyphenols of the hydrolyzable type, thus perhaps, do not constitute a nutritional problem. Reviews had earlier concluded the lack of evidence for forage tannins to be harmful in ruminant diets (McLeod, 1974; Price and Butler, 1980). However, in cases where hydrolyzable tannins are hydrolyzed to liberate gallic acid or pyrogallol which is absorbed in such quantity as to overcome the body's detoxification system, kidney neurosis and mortality can occur (Hegarty et al, 1995; Pigeon et al, 1962; McCosker and Hunt, 1966).

Tannins may even be needed in forages. Earlier studies had shown that tannins are not present in the leaves of bloat-inducing temperate legumes, but are present in leaves of legumes which do not cause bloat (Ross and Jones, 1974). Thus, a strategy for reducing/removing bloat producing property of a forage is to increase tannin content in leaves (Bray, 1981). Interestingly, bloat seems not to be a problem with tropical legumes among which only D. lanjab had been reported to induce bloat (Hamilton and Ruth, 1969). However, the absence of bloat was not correlated with tannin content (Hutton and Cooke, 1966).

**Phytate**

Of the samples tested only 4 had lower than 5 mg phytate P/g, all others had higher than 5 with Calopogonium JS-1 and Porpogon BPI-T1 having the highest values of 9.60 and 10.30, respectively (Table 2). Phytate in the seed makes about 50% of

the seed's phosphorus unavailable and it also could complex with other minerals to make them less available.

**Oligosaccharides**

Among the samples analyzed, seeds of Aeschynomene, *C. occidentalis* and Calopogonium had relatively high levels of oligosaccharides (>5%) (Table 3). Aeschynomene and *C. occidentalis* had higher raffinose than stachyose and verbascose combined. The others had similar contents of the above or the reverse, i.e., higher stachyose and verbascose than raffinose. Varietal differences were also quite evident.

Since oligosaccharides are localized in mature seeds and are much lower in the other parts of the plant (Revilleza et al, 1988), they probably are not the major cause of gas production or bloating in livestock. Bloat in cattle has been shown to be caused by soluble leaf proteins which form stable foam in the rumen that prevents the removal of gases produced by microbial fermentation of ingested food.

**Lectins**

Among the forage legumes investigated, Centrosema, Crotolaria and Calopogonium had strong HA to bovine RBC but little or no reaction to rabbit, human A and O RBC (Table 4). *C. lainyoides* accession 158 had a strong hemagglutinating activity against bovine RBC but the accession 178 had none.

Porpogon, Desmodium and *C. occidentalis* had slight to moderate hemagglutinating activity to bovine RBC and mostly no such activity to the other RBC's.

In general, the forage legumes had strong hemagglutinating activity against bovine RBC compared to the strong hemagglutinating of food legumes against rabbit RBC (Barroga et al, 1988) suggesting different composition of their active sites.

**Cyanogenic Glycosides**

The HCN potential of centrosema (5 accessions) Porpogon, Kudzu, and Crotolaria (6 accessions), in immature and mature leaves and immature pods was nil or zero. The picrate method which has the tendency to overestimate cyanide due to its reaction with other volatiles such hydrogen sulfide, aldehydes thiocyanates and nitriles. However, even this method failed to detect cyanide in the samples tested. Cyanogenic glycosides are shown to occur in pasture species such as *Trifolium repens*, *Cynodon dactylon*, *C. aethiopicus* and various *Sorghum* species (Hegarty, 1981).

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**Table 1. Saponin in raw mature seeds of several indigenous forage legumes.**

| Sample | Saponin (%) Liebermann-Burchard Test | Froth Test |
|---|---|---|
| Aeschynomene IPB | 0.56 | ++ |
| Calopogonium BM-1 | 0.48 | ++ |
| JS-1 | 0.48 | - |
| Centrosema JS-1 | 0.26 | ++ |
| BPI-T1 | 0.76 | ++ |
| Crotolaria PC-1 WB | 0.76 | ++ |
| Desmodium DO-1 | 0.52 | + |
| 93 | 0.88 | + |
| Kudzu BP | 0.48 | + |
| Porpogon BPI-T1 | 0.92 | ++ |
| Calauan | 0.46 | ++ |

**Table 2. Polyphenol and phytate content of raw mature seeds of Philippine indigenous legumes.**

| Samples | Polyphenols | | Phytate phosphorus (mg P/g) |
|---|---|---|---|
| | Vanillin Assay (mg catechin/g) | Protein precipitation (mg tannic acid/g) | |
| Aeschynomene | | | |
| IPB | 1.37 | 0.42 | 5.82 |
| Cassia occidetalis | | | |
| Balatong aso | | | |
| BP | 0.1C | 0.75 | 8.24 |
| Calopogonium | | | |
| BM-1 | 0.97 | 0.26 | 5.74 |
| JS-1 | 0.29 | 0.69 | 9.60 |
| Centrosema | | | |
| JS-1 | 5.57 | 0.59 | 4.79 |
| BPI-T | 6.83 | 0.99 | 5.31 |
| BP-TB | 6.92 | 0.51 | |
| Crotolaria | | | |
| NB PC-1 | 0 | 0.42 | 4.01 |
| BM-1 | 0 | 0.91 | 5.76 |
| WB PC-1 | 0 | 0.42 | 5.71 |
| Desmodium | | | |
| DO-1 | nd | 1.81 | 3.37 |
| 93 | nd | 0.38 | 7.06 |
| 195 | nd | 1.01 | 6.61 |
| 158 | nd | nd | 6.88 |
| Porpogon | | | |
| BPI-T | 0.37 | - | 10.30 |
| Cal-Lag | 0.19 | 0.48 | - |
| Phaseolus lathyroides | | | |
| IPB | 7.38 | 0.13 | 5.89 |
| 158 | 10.12 | 0.27 | 6.24 |
| Pueraria phaseoloides | | | |
| Kudzu BP | nd | 0.18 | 4.91 |

**Table 3. Oligosaccharide profile of raw mature seeds of Philippine indigenous forage legumes.**

| Sample | % Raffinose | % Stachyose + verbascose | % Total oligosaccharides |
|---|---|---|---|
| Aeschynomene | 5.04 | 2.92 | 7.96 |
| Cassia occidentalis | 3.78 | 1.58 | 5.36 |
| Calopogonium | | | |
| JS-1 | 3.08 | 2.94 | 6.02 |
| BM-1 | 2.37 | 2.03 | 4.40 |
| Centrosema | | | |
| BPI-T1 | 0.73 | 0.97 | 1.70 |
| JS-1 | 0.73 | 0.84 | 1.57 |
| Crotolaria PC-1 | 1.50 | 2.04 | 3.54 |
| Desmodium 93 | 1.84 | 2.32 | 4.16 |
| DD-1 | 0.78 | 2.75 | 3.53 |
| Phaseolus lathyroides 152 | 1.71 | 2.98 | 4.69 |
| IPB | 1.27 | 2.60 | 3.87 |
| Porpogon BPI-T1 | 1.27 | 2.16 | 3.43 |
| Calauan | 0.17 | 1.02 | 1.19 |

**Table 4. Hemagglutinating activity in several Philippine indigenous forage legumes.**

| Forage legumes | RBC | | | |
|---|---|---|---|---|
| | Rabbit | Bovine | Human A | Human O |
| *Cassia occidentalis* | | | | |
| Balatong aso | - | ++ | - | - |
| Centrosema JS-1 | - | +++ | - | - |
| BPI-T | - | +++ | - | - |
| BPTB | + | +++ | - | - |
| BP | - | ++ | - | - |
| BM-1 | - | +++ | - | - |
| Crotolaria BM-1 | - | +++ | - | - |
| WB-PC-1 | + | +++ | - | - |
| NB PC-1 | + | ++ | - | + |
| BM-1 | + | +++ | - | - |
| JS-1 | + | +++ | - | - |
| Desmodium 93 | + | ++ | - | - |
| 95 | - | - | - | - |
| DQ-1 | - | - | - | - |
| *Phaseolus lathyroides* 159 | + | +++ | - | - |
| IPB | - | - | - | - |
| Porpogon BPI-T | + | + | - | - |
| Calauan | + | ++ | - | - |
| *Pueraria phaseolus* JS-1 | - | - | - | - |
| DQ-1 | - | - | - | - |
| BP | - | - | - | - |

Average of 2 replicates

# ALKALOIDS IN SEVERAL PHILIPPINE INDIGENOUS FORAGE LEGUMES: DETERMINATION AND REMOVAL

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## ABSTRACT

Nine forage legumes indigenous to the Philippine were screened for the presence of alkaloids. These are: Aeschynomene, Cassia occidentalis, Centrosema, Calopogonium, Crotolaria, Desmodium, Pueraria, Phaseolus lathyroides and Porpogon. Immature and mature leaves of Centrosema (4 accessions and Calopogonium (1 accession) had very high levels of alkaloids. Alkaloids were also detected in the leaves of Aeschynomene and in seeds and mature leaves of Crotolaria.

Removal of alkaloids was effected by soaking of mature and immature leaves for 30 to 60 min at 45 to 60 C.

## INTRODUCTION

Among the many types of alkaloids, pyrrolizidines, the quinolizidines, diterpenes, tropanes and steroidal subclasses have deleterious effects on livestock (Everist, 1974). Alkaloids of the pyrrolizidine group are strong hepatotoxins and are found in species of Amsinckia, Crotolaria, Senecio, Heliotropium and other genera which are common grazing areas (Culvenor, 1973). Some of the disorders due to alkaloid toxicity are toxaemic jaundice, reduced liveweight gain and milk production, "sudden death" syndrome, "phalaris staggers", etc (Hegarty, 1981).

This study aimed at screening for the presence of alkaloids in different parts of several Philippine indigenous legumes namely: Aeschynomene, Cassia occidentalis, Centrosema, Calopogonium, Crotolaria, Desmodium, Pueraria, Phaseolus lathyroides and Porpogon.

## MATERIALS AND METHODS

### Materials

Seeds of the above mentioned legumes were obtained from the National Plant Genetic Resources Laboratory of IPB and grown in the IPB experimental farms to obtain fresh samples.

All chemicals used were of analytical grade. Samples of seeds, leaves and pods were dried at 45 C for 18 hr, ground and passed through 40 mesh.

**Analysis for Alkaloids**

Screening for the presence of alkaloids was done according to Hultin and Torsell (1965). Each sample (4 g dried materials, 40 mesh) was incubated with 40 mL of methanol overnight and then warmed for 4 hr at 50 C. The mixture was filtered; the residue was washed with 20 mL of methanol and the combined extracts evaporated *in vacuo*. The residue was suspended in methanol (2 mL) and 12 mL of 1% HCl added. The mixture was shaken and filtered; another 8 mL of 1% HCl was used to wash the residue. The filtrate was made basic with concentrated $NH_3$ and extracted with three 20 mL of chloroform (Fraction A). The aqueous solution was next made half-saturated with sodium sulfate and extracted three times with 20 mL of chloroform: ethanol (3:2 v/v) (fraction B). The organic phases were washed with 5 mL of half-saturated sodium sulfate solution and dried with anhydrous sodium sulfate.

The two extracts were evaporated separately *in vacuo* and 1.0 mL of HCl and 1.0 mL of chloroform were added to each with vigorous shaking. The aqueous phase from each was pipetted off, filtered through cotton and divided into six portions. These samples were tested with the following alkaloid reagents: Mayer's, Wagner's, Dragendorff's, Sonnenschien's silicotungstic, and Hager's reagent. The amounts of precipitate found were compared to those resulting from caffeine solutions.

**Treatments for Removal of Alkaloids**

Two hundred g of dried ground sample was mixed with 250 mL of distilled water. The mixture was placed in a water bath at 30 C for 15 min and centrifuged, and the supernate removed. The residue was recentrifuged and the remaining liquid decanted. The residue was dried and subjected to alkaloid extraction and analysis, according to Hultin and Torsell (1965).

The process was repeated for the following treatments: 30 C, 60 min; 45 C 30 min; and 45 C, 60 min.

## RESULTS AND DISCUSSION

Among the samples analyzed, Centrosema had the highest levels of alkaloids, followed by Calopogonium and then by Crotolaria (Table 1). For Centrosema and Calopogonium, immature and mature leaves but not other parts contained alkaloids. In Crotolaria, mature seeds and leaves had high levels of alkaloids. Alkaloids were also detected in immature and mature leaves in Aeschynomene and Cassia occidentalis. Species which were negative for alkaloids were: Desmodium, Pueraria, P. lathyroides, and Porpogon.

Crotolaria has been reported to contain hepatoxic pyrrolizidine alkaloid and are toxic to both ruminants and non-ruminants. However, feeding of young plants of C. juncea to cattle in Thailand did not cause harmful effects (Wallapon and Pongskul, 1984).

**Detoxification**

For immature leaves, removal of alkaloid was effected by heat treatment at 60 C for 60 min Centrosema, and only 30 min at 60 C was for Aeschynomene and Calopogonium.

For mature leaves, removal of alkaloid was completed by soaking treatment at 60 C for 60 min for Centrosema and 60 C and 30 min for Calopogonium. For Aeschynomene and Crotolaria 45 C - 60 min treatment was required.

These treatments are mild and may not affect nitrogen digestibility and other constituents of the sample. Decrease in nitrogen digestibility had been shown in forages dried at much higher temperature of 180 C (Goering and Waldo, 1979).

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**Table 1. Alkaloid content of the mature seeds, mature and immature pods and mature and leaves of some indigenous forage legumes.**

| Variety | Alkaloid Content | | | | |
|---|---|---|---|---|---|
| | mature seeds | immature pods | mature pods | immature leaves | mature leaves |
| Aeschynomene -1 | - | - | - | + | + |
| 2 | - | - | - | + | + |
| *Cassia occidentalis* | | | | | |
| Balatong aso BP | - | - | - | + | - |
| 7 | - | - | - | - | - |
| 24 | - | - | - | - | - |
| Centrosema BM-1 | - | - | - | +++ | +++ |
| BPI-T1 | - | - | - | +++ | +++ |
| JS-1 | - | - | - | +++ | +++ |
| BPTB | - | nd | nd | +++ | +++ |
| Calopogonium JS-1 | - | - | - | +++ | +++ |
| BM-1 | - | - | - | + | + |
| Crotolaria PC1(NB) | +++ | - | - | - | + |
| PC1(WB) | +++ | - | - | - | +++ |
| BM-1 | + | - | - | - | + |
| Desmodium DQ-1 | - | nd | nd | - | - |
| DQ-2 | - | nd | nd | - | - |
| CT | - | nd | nd | nd | nd |
| 93 | - | nd | nd | nd | nd |
| 195 | - | nd | nd | nd | nd |
| Pueraria Kudzu DQ-1 | - | nd | nd | - | - |
| JS-1 | - | nd | nd | nd | nd |
| BP | - | nd | nd | nd | nd |
| *Phaseolus lathycoides* IPB | - | - | - | - | - |
| 158 | - | - | - | - | - |
| Porpogon BPI | - | - | - | - | - |

[a] High positive, ++; positive, +; negative, -

nd, not determined.

**Table 2. Alkaloid detoxification of immature leaves of selected indigenous forage legumes.**

| Variety | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | 30 C | | 45 C | | 60 C | |
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min |
| Aeschynomene | | | | | | |
| 1 | + | + | + | + | - | - |
| 2 | + | + | + | - | - | - |
| Centrosema | | | | | | |
| BM-1 | +++ | +++ | +++ | + | + | - |
| BPI-T1 | +++ | +++ | +++ | + | + | - |
| JS-1 | +++ | +++ | + | + | + | - |
| BPTB | +++ | +++ | + | + | + | - |
| Calopogonium | | | | | | |
| JS-1 | +++ | +++ | + | + | - | - |

a
High positive, ++; positive, +; negative, -.

**Table 3. Alkaloid detoxification of mature leaves of some indigenous forage legumes.**

| Variety | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | 30 C | | 45 C | | 60 C | |
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min |
| Aeschynomene | | | | | | |
| 1 | + | + | + | - | - | - |
| 2 | + | + | + | - | - | - |
| Centrosema | | | | | | |
| BM-1 | +++ | +++ | + | + | + | - |
| BPI-T1 | +++ | +++ | + | + | + | - |
| JS-1 | +++ | +++ | + | + | + | - |
| BPTB | +++ | +++ | + | + | + | - |
| Calopogonium | | | | | | |
| JS-1 | +++ | +++ | + | + | - | - |
| Crotolaria | | | | | | |
| PC1 (NB) | + | + | + | - | + | - |
| PC1 (WB) | +++ | + | + | - | + | - |
| BM-1 | + | + | + | - | - | - |

a
High positive, ++; positive, +; negative, -.

XVII

# AMINO ACID COMPOSITION, RELATIVE NUTRITIVE VALUE, IN VITRO PROTEIN DIGESTIBILITY OF SEEDS OF SEVERAL PHILIPPINE INDIGENOUS FORAGE LEGUMES

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## ABSTRACT

Methionine was the first limiting amino acid, followed by leucine. In raw mature seeds of 9 forage legumes analysed. High methionine content (>2.0%) was observed in Aeschynomene Acc. IPB (3.1%), Kudzu Acc. BP (4.5%) and Phaseolus lathyroides Acc. IPB (2.8%). Very high lysine (>10%) was also observed in 6 samples.

Relative nutritive value (RNV) of raw mature seeds ranged from 15% (Centrosema) and 77% (Crotolaria NB-PC-1). Cooking the seeds by boiling or roasting increased RNV from 72 to 95%.

In vitro protein digestibility (IVPD) of the raw mature seeds ranged from 73 to 80%.

## INTRODUCTION

The nutritive value of animal feed depends on chemical composition and the digestibility and nature of digested products. Overall pasture/forage quality then depends on nutritive value and intake, the latter being affected by the acceptability (palatability), rate of passage, forage availability per animal per unit time and environmental effects on the animal (Barnes, 1965).

As part of our study to determine the nutritive values of nine Philippine indigenous legumes, the following analyses were made: amino acid composition, relative nutritive value (RNV), and in vitro protein digestibility (IVPD). The proximate composition of these legumes was reported by Rodriguez and Mendoza (1988). The legumes studied were: Aeschynomene, balatong aso (Cassia occidentalis), Calopogonium, Centrosema, Crotolaria, Desmodium, Kudzu (Pueraria), Phaseolus lathyroides and Porpogen.

## MATERIALS AND METHODS

### Materials

Seeds of indigenous forage legumes were obtained from the National Plant Genetic Research Laboratory (NPGRL) of the Institute of Plant Breeding and planted to obtain fresh materials of leaves, pods and seeds for analysis. Tetrahymena pyriformis WATCC 10542 was obtained from the National Institutes of Biotechnology and Applied Microbiology (BIOTECH).

**Preparation of Samples**

Leaves, pods and mature seeds were separately dried at 45 C in a forced draft oven for 48 hr. Afterwards, they were ground in a Willey Mill and passed through 60 mesh sieve.

**Amino Acid Analysis**

Defatted, ground samples containing 6.25 mg protein were heated in 6 N HCl under vacuum for 24 h at 110 C. After sample clean up, the acid hydrolysates were dried under vacuum and redissolved in citrate buffer pH 3.2. The amino acid analysis was done using a Waters HPLC system with a cation exchange resin a post column reaction unit using orthoaldehyde and hypochlorite as derivatizing reagents and a fluorescence detector (Millipore-Waters Assoc. 1983).

For determination of cysteine and methionine, samples were subjected to performic acid oxidation.

**Relative Nutritive Value** (RNV)

The RNV assay procedure of Stott et al (1963) with minor modifications was used.

**In Vitro Protein Digestibility**(IVPD)

The multienzyme technique of Hsu et al for IVPD (1977) was followed. The IVPD was calculated according to the regression equation $Y = 210.464 - 18.103\ x$ where Y = IVPD (%) and X = pH of the sample suspension after 10 min digestion with the multienzyme solution.

**Preparation of Samples for RNV**

Samples of seeds were boiled or cooked until cooked.

## RESULTS AND DISCUSSION

### Amino Acid Composition

Table 1 summarizes the amino acid pattern of the raw mature seeds of forage legumes studied. As expected, methionine was the prevailing first limiting amino acid with leucine as second limiting amino acid.

Notably, several samples were identified to have high met content (> 2.0%), namely: Aeschynomene IPB (3.1%), Kudzu BP (4.5%), Phaseolus IPB (2.8%). Lysine was very high (> 10%) in the following: Aeschynomene IPB (11.5%), Calopogonium (11.5%), Crotolaria PC-INB (14.3%), Crotolaria PC-IWB (11.3%), Cassia (12.7%) and Phaseolus lathyroides (10.5%).

Variability in amino acid content among accessions was also observed (Table 1).

### Relative Nutritive Values

RNV of the raw samples ranged from 15% (Centrosema BM-1) to Crotolaria NB-1 (79%) (Table 2). Crotolaria had the highest RNV (52 to 79%). A large variation was seen with Centrosema (15 to 59%).

Cooking the seeds by boiling or roasting increased RNV from 72 to 95%. This indicates that some heat labile toxic constituents which could be protein in nature in the seeds were inactivated or removed by heat treatment. The presence of toxins forages limits animal production from pastures (Hegarty, 1981). The toxin in Cassia occidentalis causes degeneration of skeletal and cardiac muscles but has not been identified (Hebert et al,

1983). The presence of heat stable toxins like alkaloids and saponins has been reported.

**In Vitro Protein Digestibility**

IVPD of seed proteins of the indigenous forage legumes ranged from 73 to 80% (Table 3). Crotolaria had the highest levels (79 to 80%). Centrosema had the lowest levels (73%).

## ACKNOWLEDGEMENT

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**Table 1. Amino acid composition of raw mature seeds of indigenous forage legumes.**

| Legume Sample | Protein content % N x 6.25 | Amino Acid ( % amino acid or g amino acid/ 100 sample) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cys* | Met* | Trp** | Met | Thr | Phe | Lys | Val | Leu | Ile | Tyr | Gly | Ala | Pro | His | Arg | Asp | Glu | Ser |
| Aeschynomene IPB | 24.77 | 1.6 | 3.1 | 0.21 | 2.3 | 3.5 | 4.5 | 11.5 | 4.2 | 3.6 | 4.5 | 3.8 | 5.2 | 4.5 | 1.6 | 1.9 | 13.8 | 9.9 | 18.1 | 5.4 |
| Calopogonium JB-1 | 33.28 | 1.8 | 1.1 | 0.19 | 2.5 | 4.8 | 3.2 | 4.4 | 3.7 | 2.4 | 3.8 | 3.1 | 4.0 | 3.8 | 2.2 | 3.3 | 5.2 | 10.6 | 15.3 | 4.7 |
| Calopogonium BM-1 | 30.44 | 1.8 | 1.0 | 0.20 | 0.9 | 2.9 | 4.0 | 11.5 | 4.2 | 3.3 | 3.7 | 2.5 | 3.6 | 3.3 | 2.4 | 1.8 | 5.1 | 7.3 | 13.1 | 3.2 |
| Cassia BP | 21.08 | 1.2 | 1.1 | 0.10 | 1.2 | 3.3 | 4.5 | 12.7 | 4.0 | 5.4 | 9.8 | 3.5 | 5.2 | 4.5 | 3.6 | 1.7 | 8.7 | 7.7 | 12.1 | 4.6 |
| Centrosema BM-1 | 22.73 | | | 0.16 | 0.7 | 3.0 | 6.3 | - | 7.0 | 3.4 | 4.2 | 5.2 | 4.0 | 3.2 | 7.6 | 6.9 | 5.4 | 8.6 | 12.8 | 3.8 |
| Centrosema JB-1 | 19.72 | | | 0.16 | 3.9 | 4.8 | 7.2 | 6.2 | 5.9 | 6.2 | 6.2 | 7.4 | 4.3 | 3.5 | 3.9 | 2.2 | 6.4 | 9.8 | 14.9 | 5.0 |
| Centrosema BPT-TI | 18.45 | | | 0.21 | 0.9 | 4.5 | 4.9 | 3.9 | 6.1 | 5.6 | 2.1 | 3.9 | 5.7 | 4.6 | 1.5 | 3.2 | 6.4 | 11.9 | 16.5 | 4.5 |
| Crotolaria PC-1NB | 26.78 | | | 0.12 | 1.8 | 2.7 | 2.6 | 14.3 | 2.9 | 2.8 | 4.0 | 2.8 | 5.1 | 3.4 | 2.3 | 2.8 | 9.7 | 7.0 | 14.5 | 3.9 |
| Crotolaria PC-1WB | 28.08 | 1.2 | 1.1 | 0.06 | 1.2 | 3.2 | 3.4 | 11.3 | 3.5 | 3.4 | 4.5 | 2.4 | 4.2 | 3.8 | 3.0 | 1.1 | 7.7 | 10.1 | 21.2 | 4.8 |
| Crotolaria BM-1 | 30.81 | 1.9 | 0.8 | 0.09 | 0.8 | 2.7 | 2.9 | 8.1 | 2.9 | 2.9 | 3.7 | 2.1 | 3.5 | 3.1 | 3.9 | 1.3 | 7.2 | 9.1 | 19.6 | 4.0 |
| Desmodium DB-1 | 30.75 | | | 0.19 | 0.9 | 2.7 | 3.1 | 2.8 | 3.9 | 5.2 | 3.8 | 2.9 | 3.4 | 3.2 | 1.5 | 1.5 | 6.7 | 9.7 | 12.2 | 2.7 |
| Desmodium 93 | 29.96 | | | 0.27 | 0.6 | 4.0 | 5.8 | 5.1 | 4.9 | 3.9 | 4.5 | 4.3 | 4.3 | 5.1 | 1.9 | 2.3 | 4.3 | 14.0 | 18.3 | 6.5 |
| Desmodium 195 | 30.97 | | | 0.12 | 1.0 | 3.6 | 5.0 | 6.0 | 4.6 | 3.1 | 3.9 | 3.4 | 4.2 | 4.3 | 2.9 | 6.0 | 7.1 | 11.1 | 15.5 | 4.6 |
| Kudzu BP | 30.46 | 1.1 | 4.5 | 0.32 | 1.5 | 2.6 | 3.5 | 5.6 | 3.6 | 4.6 | 3.3 | 3.8 | 2.6 | 2.8 | 1.2 | 1.1 | 5.3 | 7.8 | 13.2 | 3.9 |
| Kudzu BB-1 | 27.43 | | | 0.11 | 0.8 | 3.3 | 4.2 | 2.1 | 3.9 | 3.8 | 3.6 | 3.9 | 4.5 | 4.1 | 1.1 | 2.0 | 5.4 | 9.6 | 15.3 | 4.4 |

| Legume Sample | Proximate content % N x 6.25 | Amino Acid (% amino acid or g amino acid/100 sample) Cys* | Met* | Trp** | Met | Thr | Phe | Lys | Val | Leu | Ile | Tyr | Gly | Ala | Pro | His | Arg | Asp | Glu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kudzu JS-1 | 33.68 | | | 0.12 | 0.5 | 3.2 | 5.0 | 8.9 | 3.9 | 3.5 | 4.9 | 5.3 | 3.5 | 3.9 | 2.5 | 5.2 | 5.0 | 9.9 | 17.0 |
| Phaseolus 158 | 23.24 | | | 0.17 | 3.9 | 3.4 | 2.9 | 9.0 | 7.0 | 3.8 | 4.8 | 3.7 | 3.6 | 3.9 | 6.2 | 2.4 | 5.6 | 9.3 | 13.6 |
| Phaseolus 198 | 22.50 | 0.9 | 2.8 | 0.10 | 1.2 | 2.9 | 4.4 | 10.5 | 5.0 | 3.8 | 4.2 | 2.5 | 3.5 | 3.8 | 4.1 | 1.1 | 9.0 | 8.2 | 13.5 |
| Porpogon Cal | 33.10 | 1.3 | 1.1 | | 2.0 | 4.8 | 7.0 | 5.7 | 6.7 | 4.6 | 5.8 | 6.1 | 6.0 | 5.9 | 2.4 | 2.9 | 7.2 | 14.0 | 23.3 |
| Porpogon BPI-Ti | 29.66 | | | | 1.3 | 4.4 | 6.4 | 4.6 | 6.4 | 4.0 | 5.7 | 4.0 | 6.5 | 5.7 | 3.0 | 3.3 | 7.0 | 11.6 | 21.7 |
| Bov A-casein*** | 95.10 | | | | 2.5 | 3.8 | 4.4 | 7.7 | 5.4 | 8.3 | 4.9 | 6.1 | 1.6 | 2.9 | 7.5 | 2.0 | 3.7 | 7.8 | 21.5 |

*
Analysis of Met and Cys by performic acid oxidation.

**
Analysis is colorimetric method.

***
Given for comparison

...roasted matured seeds of several Philippine indigenous forage legumes.

| Sample | Relative Nutritive Value | | |
|---|---|---|---|
| | raw | boiled | roasted |
| **Cassia occidentalis** | | | |
| balatong aso BP | 55.58 ± 1.29 | 80.80 ± 3.78 | 64.78 ± 3.36 |
| Calopogonium | | | |
| JS-1 | 52.56 ± 1.33 | 90.96 ± 3.69 | 92.77 ± 1.40 |
| BM-1 | 33.41 ± 4.99 | 76.13 ± 1.44 | 88.26 ± 5.20 |
| Centrosema | | | |
| BPTB | 59.07 ± 2.27 | 75.09 ± 1.77 | 67.00 ± 6.85 |
| BM-1 | 14.93 ± 2.02 | 82.84 ± 3.96 | 86.26 ± 5.20 |
| JS-1 | 39.26 ± 3.42 | 72.56 ± 2.61 | 95.67 ± 2.42 |
| BPi-T1 | 45.55 ± 2.86 | 76.28 ± 1.97 | 51.99 ± 2.48 |
| Crotolaria | | | |
| BM-1 | 59.21 ± 2.77 | 85.60 ± 1.57 | 70.22 ± 4.72 |
| NB-PC-1 | 79.93 ± 2.08 | 89.79 ± 4.08 | 86.71 ± 4.06 |
| WB-PC-1 | 51.79 ± 9. | 82.42 ± 5.14 | 68.90 ± 6.90 |
| Desmodium | | | |
| 93 | 44.61 ± 1.67 | 85.77 ± 1.86 | 59.49 ± 5.24 |
| 195 | 56.61 ± 2.67 | 93.11 ± 2.12 | 73.29 ± 4.87 |
| Pueraria phaseoloides | | | |
| Kudzu BP | 41.17 ± 6.41 | 86.84 ± 1.86 | 75.24 ± 3.74 |
| JS-1 | 49.36 ± 9.93 | 95.64 ± 2.00 | 81.25 ± 3.33 |
| Phaseolus lathyroides | | | |
| 158 | 54.84 ± 1.29 | 74.07 ± 0.00 | 76.32 ± 3.03 |
| IPB | 55.87 ± 3.09 | 73.58 ± 3.82 | 73.77 ± 5.24 |
| Pordogon | | | |
| BPi-T1 | 20.06 ± 4.86 | 89.95 ± 4.93 | 74.20 ± 3.25 |
| Cal. Lag. | 51.14 ± 7.32 | 89.03 ± 4.99 | 72.39 ± 6.38 |

**Table 3. In vitro protein digestibility of raw mature seeds of several Philippine indigenous forage legumes.**

| Sample | IVPD (%) |
|---|---|
| Aeschynomene IPS | 77.87 |
| *Cassia occidentalis* | |
| BP | 77.29 |
| Calopogonium | |
| BM-1 | 73.93 |
| JS-1 | 73.22 |
| Centrosema | |
| BM-1 | 73.54 |
| JS-1 | 73.00 |
| BPTB | 72.95 |
| Crotolaria | |
| BM-1 | 79.61 |
| NB-PC-1 | 80.00 |
| NB-PC-1 | 78.50 |
| Desmodium | |
| 93 | 78.19 |
| 195 | 78.56 |
| DO-1 | 79.54 |
| *Pueraria phaseoloides* | |
| Kudzu | |
| BP | 74.12 |
| JS-1 | 73.67 |
| DO-1 | 77.42 |
| *Phaseolus lathyroides* | |
| 158 | 76.31 |
| IPB | 77.55 |
| Porpogon | |
| BP1-T1 | 79.86 |
| Cal. Laguna | 78.49 |

XVIII

# SURVEY ON USES OF SEVEN PHILIPPINE INDIGENOUS LEGUMES: A PRELIMINARY REPORT

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## ABSTRACT

Partial results of a survey on uses of indigenous legumes in the Philippines revealed that hyacinth bean (batao) and lima bean (patani) had the highest rates of usage, 78% and 59%, respectively, followed by rice bean (22%), sabawel (15%), jack bean (11%) sam-samping (11%) and sword bean (4%).

Mature seeds are primarily used for "ginisa" dishes while immature pods and immature leaves are used as vegetable in vegetable dishes like salad, "pinakbet", "lumpia", "ginataan" and as vegetable component of meat dishes such as "sinigang" and "nilaga". Hyacinth bean, lima bean, jack bean and sabawel were also noted to be used as animal feed. Sabawel seeds are roasted and used as substitute for coffee.

## INTRODUCTION

This survey was conducted due to lack of written information on the various uses of the indigenous legumes and the extent of their utilization in the country. Although this is not covered by the objectives of the project, the information obtained from such survey can help in planning how research results can be disseminated and can be made useful to as many people as possible.

The results presented and discussed herein are still partial but could already reflect how these indigenous legumes are utilized in the country. The legumes covered by this survey are: jack bean (*Canavalia ensiformis*), sword bean (*Canavalia gladiata*) sabawel (*Mucuna pruriens* or *cochinchinensis*), batao (*Dolichos lablab*), sam-samping (*Clitoria ternatea*), lima bean (*Phaseolus lunatus*) and rice bean (*Vigna umbellata*).

## METHODOLOGY

Survey forms and photographs of the legumes accompanied by a short note, (Appendix-A) were given to possible respondents in the Los Baños area. The target respondents were 10 individuals from each of the regions of the Philippines. As of this writing, only 46 accomplished survey forms have been collected. Difficulty was met in getting respondents who come from the Visayas and Mindanao regions. Table 1 shows the profile of respondents.

The map of the Philippines showing the 12 regions is given in Fig. 1.

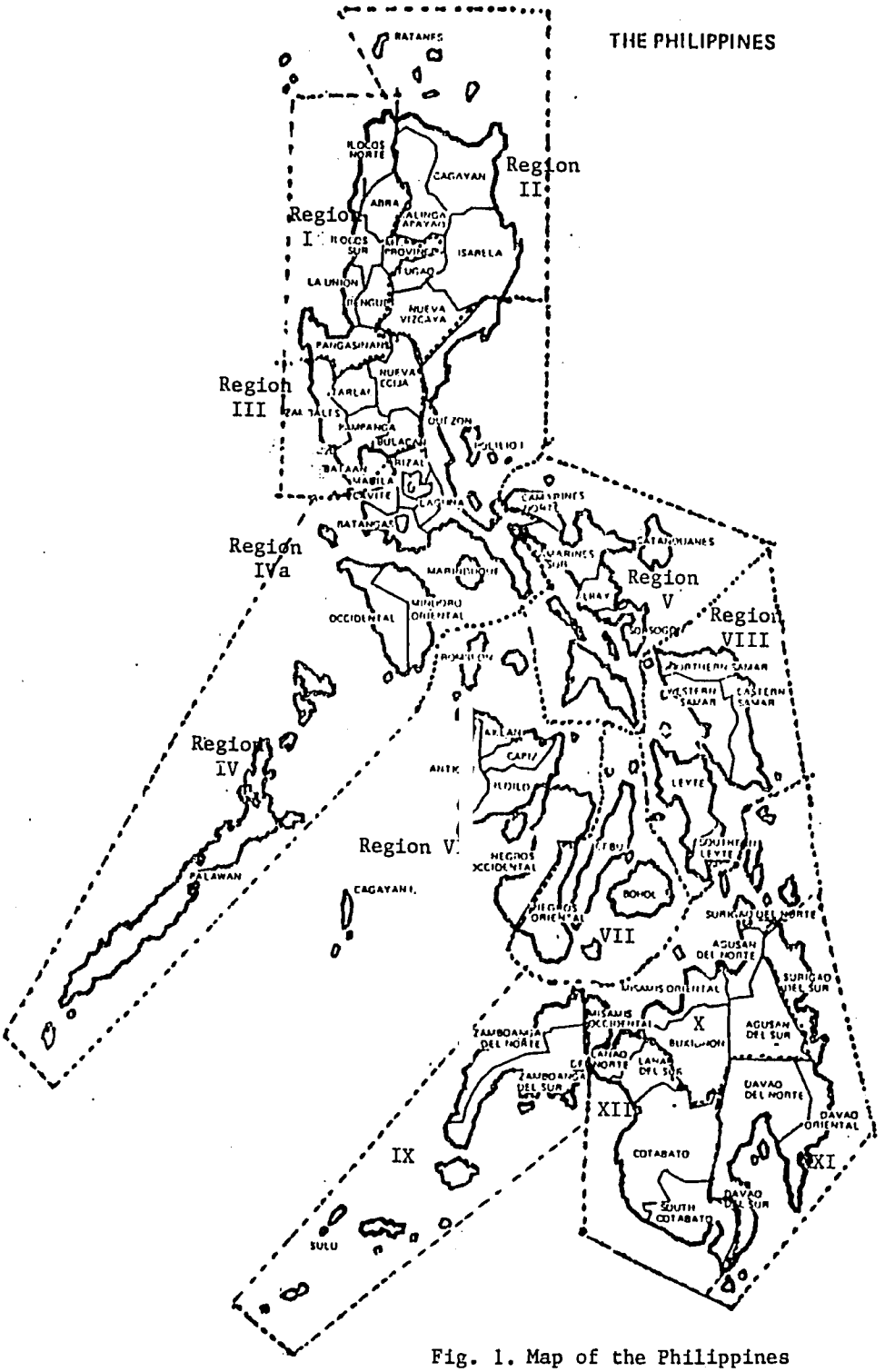


Fig. 1. Map of the Philippines

## RESULTS AND DISCUSSION

Extent of Use. Among the 7 legumes, hyacinth bean and lima bean had the highest recognition/usage rates of 78% and 59% (Table 1). These are followed by rice bean (22%), sam-samping (11%) and last was sword bean (4%). Respondents from Region I reported use of all seven legumes included in the survey. Notably, only respondents from Region I reported usage of sam-samping. So far, jack bean's usage had been reported only by respondents from Regions I, II and III. However, jack bean, also known in Visayas as "Lambajong" and in Tagalog as "Pataning Dagat" was the subject of studies by science clubs in Southern Leyte (Infoscience, 1981) and is perhaps also utilized by people in the area. The accurate picture of extent of utilization of these legumes has to wait until this survey is completed.

Uses of Legumes. The food uses of the seven legumes are quite extensive (Table 2). A common way of preparing the mature seeds of the legumes seems to be "ginisa". Immature pods and immature leaves are used in different vegetable dishes and as component in meat dishes such as "sinigang" and "nilaga" and noodle dishes. Boiled mature seeds specifically of tapilan may also be used as dessert, eg, as component of "halo-halo". Mature seeds of sabawel are also roasted and used as substitute for coffee as similarly noted by Basuel and Valdez (1989). A brief description of the dishes is in Appendix-B.

Among the legumes studied, hyacinth bean and lima bean are used in the most number of dishes. Their varied uses also reflect regional dish preferences. For example, "pinakbet," "bulanglang" or "dinengdeng" are favorite dishes in Northern Luzon and make use of edible parts of both hyacinth bean and lima bean, as well as other legumes in the study. On the other hand, "ginataan" is the more popular way of using these legumes in the Bicol region. Jack bean known as "Lambajong" in the Visayas and "Pataning Dagat" in Tagalog was the subject of studies by science clubs in Southern Leyte (Lugatiman and Ruiz, 1981). The plant, reported to be a favorite feed for rabbits, was shown to be a source of various products such as substitute meat, flour, vegetable, beverage, jelly etc. From its stems, fibers were obtained for home decors, slippers, mats, rugs, bags and other. In this survey, only jack bean and sword bean were noted to cause dizziness when eaten raw or not well cooked. This could be due to high level of the lectin concanavalin A present in jackbean.

A few respondents noted that some of the legumes in the study, ie, hyacinth bean, rice bean, sabawel and jackbean, are used as animal feed. Basuel and Valdez (1989) observed that sabawel leaf meal incorporated in broiler diet at 5 to 10% resulted in body weight, gain in weight feed conversion and return above feed cost which were comparable to control. Higher levels of 15% and 20% produced lighter birds. The potential use of these legumes as animal feed certainly deserves careful investigation.

Availability. Whereas hyacinth bean, lima bean and rice bean were noted be available in the market and usually grown in the backyard for home consumption, the rest were not available in the market. Several commented that the less availability of these legumes was a major factor for their playing minor legumes to mung bean.

Limitations of Study. This study has certain limitations. Firstly, the respondents have probably been residing in or near Los Baños, for a number of years. Many of them originally come from different parts of the country. Therefore, their answers to the survey questions may be based on past experiences. Some, of course, may have visited their home provinces in recent years; and thus, their responses could be based on recent experiments. Secondly, even if the quota of 10 respondents per region is met, their answers may still not be reflective of the total situation in the various provinces of each region.

Nonetheless, the survey has brought out a wealth of information which may be useful in increasing the utilization of these legumes.

## ACKNOWLEDGEMENT

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Lugatiman, MAA and C. Ruiz, Jr. 1981. Food uses of "Lambajong." Infoscience (Phil) 1: 16-18.

**Table 1. Survey of usage of seven Philippine indigenous legumes.**

| Region | Number of respondents | Number of respondents reporting use of: Jack bean | Sword bean | Sam-samping | Hyacinth bean | Sabawel | Lima bean | Rice bean |
|---|---|---|---|---|---|---|---|---|
| I | 8 | 1 | 1 | 5 | 7 | 3 | 5 | 2 |
| II | 6 | 2 | | | 4 | 1 | 4 | |
| III | 5 | 1 | 1 | | 4 | | 3 | 1 |
| IV | 12 | 1 | | | 10 | 1 | 6 | 4 |
| V | 5 | | | | 4 | 1 | 5 | |
| VI | 2 | | | | | | 1 | 2 |
| VII | | | | | | | | |
| VIII | 2 | | | | 2 | | | 1 |
| IX | 3 | | | | 3 | | 1 | |
| X | | | | | | | | |
| XI | 3 | | | | 2 | 1 | 2 | |
| XII | | | | | | | | |
| | 46 | 5 | 2 | 5 | 36 | 7 | 27 | 10 |
| % of Total | | 11 | 4 | 11 | 78 | 15 | 59 | 22 |

| Legume | Parts | Food Uses |
|---|---|---|
| Hyacinth bean<br>*Dolichos lablab*<br>(Batao, bulay, parda, harabilla, kadyos) | mature seeds | ginisa |
| | immature pods | vegetable dish; vegetable component of sinigang, nilaga, lumpia, pansit, pinakbet |
| | immature leaves | vegetable component of sinigang, nilaga, lumpia and other vegetable dishes |
| Lima bean<br>*Phaseolus lunatus*<br>(Patani, palaminko, betsuelas, utung, perkules, pataning dagat) | immature seeds | ginisa; cooked with coconut (ginataan) |
| | mature | boiled; ginisa; vegetable component of pinakbet; halo-halo |
| | immature pods | vegetable dish; vegetable component of sinigang, nilaga, ginataan, pinakbet; may be added to all types of dishes as vegetable |
| Rice bean<br>*Vigna umbellata*<br>(Tapilan, munggo) | mature seeds | boiled beans; ginisa; component of halo-halo |
| | immature leaves | vegetable component of sinigang, nilaga or tinola |
| | immature pods | vegetable component of sinigang, salad, pansit |
| Sabawel<br>*Mucuna pruriens or cochinchinensis*<br>(sabawel, cocoa) | mature seeds | ginisa; roasted as coffee |
| Jack bean<br>*Canavalia ensiformis*<br>(Pataning espada; | mature seeds | ginisa |
| | immature leaves | vegetable component of sinigang, nilaga, dinengdeng, pinakbet, ginataan |

| Legume | Parts | Food Uses |
|---|---|---|
| Sam-samping<br>*Clitoria ternatea*<br>(Sam-samping; kum-kumpitis; competes) | mature seeds | ginisa; mixed with pinakbet |
| | immature leaves | blanched; added to vegetable dishes |
| | immature | added to pinakbet, sinigang, nilaga, salad, other vegetable dishes |
| Sword bean<br>*Canavalia gladiata*<br>(Pataning espada) | mature seeds | |
| | immature leaves | added to sinigang, nilaga, salad |
| | immature pods | added to ginisa, sinigang |

Local names in parenthesis.

Appendix-A

**INSTITUTE OF PLANT BREEDING**
CA-UPLB

June 28, 1989

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We have done extensive studies on the biochemistry and nutritional quality of Philippine indigenous legumes. However, we have noted the lack of written information on their uses, both as food and non-food. We request you to take a look at the photographs, see if you recognize any of them and to accomplish the survey form.

We will include the information obtained from this survey in the report we are preapring on the indigenous legumes.

Thank you for your cooperation.

EVELYN MAE T. MENDOZA, PhD
Researcher (Biochemist)

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Respondent's Name : _____

Affiliation : _____

Present Address : _____

_____

Original Home Address: _____

_____

## SURVEY ON USES OF PHILIPPINE INDIGENOUS LEGUMES

A. Which of the legumes shown in the photographs have you used? Check.

____ a) Jackbean (*Canavalia ensiformis*)
____ b) Sword bean (*Canavalia gladiata*)
____ c) Sam-samping (*Clitoria ternatea*)
____ d) Batao (*Dolichos lablab*)
____ e) Sabawel (*Mucuna pruriens or cochinchinensis*)
____ f) Lima bean (*Phaseolus lunatus*)
____ g) Rice bean (*Vigna umbellata*)

B. If you have encircled one or more of the legumes, kindly accomplish this questionnaire for each of them.

Name of Legume: _____

1. What part of the country (province) did/do you use this legume as food?

2. Where is the legume usually obtained?
_____ Market ____ Backyard ____ Other (specify) _____

3. How it is usually called? _____ _____

4. What parts of the legume are used for food?
_____ Mature seeds; _____ Immature leaves; _____ Immature pods

5. How is the legume cooked?
For seeds _____ boil ____ ginisa (as in ginisang mungo)
_____ roast
For immature leaves _____ blanched
_____ added to "sinigang" or "nilaga"
_____ other (specify _____)
For immature pods _____ vegetable dish
_____ added to "sinigang" or "nilaga"
_____ other (specify _____)

6. Do you know of other food uses of this legume? Please describe briefy. _____
_____

7. Do you know of nonfood uses of this legume. Please describe briefly.
_____
_____

8. Is this a widely utilized legume in your province? _____

How does it compare with mungbean? _____
_____

**Description of dishes/ingredients used in the text**

"Bagoong" is fermented fish or small shrimp.

"Sinigang" is basically boiled meat (pork, beef, chicken, shrimp or milkfish) in a water with tomato and onion. When the meat is done, vegetables are added followed by fruit that will make the broth sour. This can be tamarind, calamansi juice, green mango or kamias.

Boiled beans ("nilagang munggo") refers to boiled beans served with its broth, sugar to taste and perhaps milk; a favorite breakfast or snack food.

"Ginisa" is a basic cooking method of sauteeing in oil the following ingredients: pressed garlic, onion and tomato. A small amount of sliced meat (pork, beef or chicken) is added after which the boiled beans, broth and leafy vegetables are added. A popular variation is the addition of shrimp and shrimp sauce to the dish. Pepper and salt are added to taste.

"Ginataan" is basically sauteed dish to which coconut milk ("gata") is added.

"Halo-halo" means mixture, refers to a snack food or dessert consisting of a mixture of beans cooked in syrup like navy beans, red beans (cowpea or rice bean) chick pea, plus jackfruit, to which shaved ice milk and sugar are added. Other types of fruits and delicacies may be added.

"Lumpia" (egg roll) is mixture of finely shredded vegetables sauteed with small bits of meat and wrapped with egg roll wrapper.

"Nilaga" is similar to sinigang except the souring agent is not added.

"Pansit" is a general term for noodle dish.

"Pinakbet", "dinengdeng", "bulanglang" are vegetable dishes originating from Northern Luzon. Basically, leafy vegetables and other types of vegetables (like okra, pole sitao, ampalaya) are cooked together with cooked meat (fried or boiled fish, boiled or adobo pork), tomato, onion and bagoong, over low fire.

## SUMMARIES AND HIGHLIGHTS OF RESEARCH ACCOMPLISHMENTS

### Biochemical and Nutritional Studies of Philippine Indigenous Food and Forage Legumes (AID/SCI 2N-04; AID-936-5542-G-00-1347-00)

### 1. Proximate Chemical Composition of Several Phillipine Indigenous Food Legumes

Evelyn Mae T. Mendoza, Felicito M. Rodriquez and Ma. Jamela R. Revilleza

Biochemistry Laboratory, Institute of Plant Breeding College of Agriculture, UPLB, College, Laguna 4031 Philippines

**Abstract**

Protein (18 to 30%) and carbohydrates (50 to 60%) are the major constituents of the mature seeds of 33 samples comprising seven legume species. *Vigna umbellata* had the lowest protein content of 17.42 to 17.56% while *Canavalia ensiformis*, *Canavalia gladiata*, *Mucuna pruriens* or *M. corbinchinensis* and *Clitoria ternatea* had similar protein contents of 28 to 30%. Fat content ranged for 1.2 to 3.7% *Dolichos lablab*, *Phaseolus lunatus* and *Canavalia gladiata* had the largest (5-6%) difference in protein and carbohydrate contents.

The proximate compositions of the immature and mature leaves and pods were also obtained: Moisture (70-90%), carbohydrates (15-18%) proteins (3-10%), fibers (2%), fat (2-4%) ash (0.5-4%).

### 2. AMINO ACID COMPOSITION, RELATIVE NUTRITIVE VALUES AND IN VITRO PROTEIN DIGESTIBILITY OF SEVERAL PHILIPPINE INDIGENOUS FOOD LEGUMES

Antonio C. Laurena, Evelyn Mae T. Mendoza, Noel G. Sabino, Agnes F. Zamora, and Felicito M. Rodriguez

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**ABSTRACT**

This study demonstrates variability in amino acid composition among accessions of several Philippine indigenous legumes. Moreover, two accessions of *D. lablab* were identified to have unusually high level of methionine (> 2%) and could be

possible sources of methionine rich proteins.

The IVPDs of the legumes under study ranged from > 70 to 79%. Raw mature seeds had relatively low RNVs of 11-68% which increased to 68 to 94% and 51 to 89% after boiling and roasting, respectively, suggesting the presence of heat labile toxic constituents in the raw seeds and their removal/inactivation upon cooking.

## 3. POLYPHENOLS, PHYTATE, CYANOGENIC GLYCOSIDES AND TRYPSIN INHIBITOR ACTIVITY OF SEVERAL PHILIPPINE INDIGENOUS FOOD LEGUMES

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### ABSTRACT

Low levels of polyphenols with flavanol groups and those capable of precipitating proteins were observed in raw mature seeds of six legumes analyzed.

Seeds of batao or hyacinth bean, jack bean and lima bean had relatively high levels of phytate phosphorus ranging from 6 to 11.6 mg phytate P/g sample. Lower values (<5) were obtained for sabawel and sword bean as well as some varieties of mungbean used as control.

None of the indigenous legumes had potentially toxic levels of cyanide (<50 ppm) in the seeds, immature and mature leaves and immature pods. The lima bean samples had low HCN potential (0-10 ppm).

Among the legumes investigated, batao had the highest trypsin inhibitor activity (TIA) ranging from 14.25 to 27.05 units/mg sample for 4 accessions. Accessions 6-1 of sabawel had 19.8 units/mg sword bean and jack bean had low levels of 1.20 to 4.95 units/mg except for one jack bean accession (A-1) which had 1.45 units/mg. Rice bean also had low levels of TIA, 5-7 units/mg.

## 4. Oligosaccharides in Several Philippine Indigenous Food Legumes: Determination, Localization and Removal

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**Key words:** oligosaccharides, raffinose, stachyose, verbascose, legumes, lima bean (Phaseolus lunatus L.), sword bean (Canavalia gladiata (Jacq.) DC.), jack bean (Canavalia ensiformis (L.) DC.), sabawel (Mucuna pruriens or cochinchinensis), batao or hyacinth bean (Dolichos lablab L.) and rice bean (Vigna umbellata Ohashi.)).

**Abstract.** The oligosaccharide profile of raw mature seeds of seven different legumes indigenous to the Philippines was measured in 70% ethanol extracts of the seeds by thin layer chromatography using HPTLC plates and quantified by a densitometer. Based on the results, the legumes could be ranked according to decreasing oligosaccharide content or flatulence potential as follows: Sam-samping (Clitoria ternatea) > Batao (Dolichos lablab) > Sabawel (Mucuna pruriens) > Lima (Phaseolus lunatus) > Swordbean (Canavalia gladiata) > rice bean (Vigna umbellata) > Jack bean (Canavalia ensiformis). Sam-samping had 4.79% total oligosaccharides and batao, 3.78%. A jack bean accession had 1.79% oligosaccharides.

Simple processing methods were tested to detoxify the oligosaccharides. Soaking of batao seeds had no effect while boiling even resulted in a net 23-31% increase in the levels of raffinose, stachyose and verbascose. On the other hand, two min of dry roasting resulted in complete removal of oligosaccharides whereas germination resulted in about 30-40% decrease after 1 and 2 days, respectively.

b
Part of MS thesis of the senior author.

## 5. Changes in Oligosaccharides, Soluble Sugars and Hydrolyzable Polysaccharides in Developing Seeds of Batao (*Dolichos lablab*) and Sabawel (*Mucuna pruriens* or *cochinchinensis*)

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**Keywords:** oligosaccharides, raffinose, stachyose, verbascose, batao or hyacinth bean (*Dolichos lablab*), sabawel (*Mucuna pruriens* or *cochinchinensis*), ontogeny, hydrolyzable polysaccharides.

**Abstract:** During seed maturation of batao and sabawel, hydrolyzable polysaccharide increased in the cotyledon while it decreased in the pod wall. As disaccharides decreased in the cotyledon, the oligosaccharides raffinose and stachyose started to increase. Verbascose was detected one week later in sabawel and even later in batao. The oligosaccharides were very low or nondetectable in the podwall and seed coat of both batao and sabawel. Monosaccharides were detected in the podwall and seed coat but not in the cotyledon.

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[a] Part of MS thesis of the senior author.

[b] To whom correspondence should be addressed

## 6. DETERMINATION OF SAPONINS IN PHILIPPINE INDIGENOUS FOOD LEGUMES

Angelo J. M. Lubag, Jr., Felicito M. Rodriguez, Ana Gina T. Sembrano and Evelyn Mae T. Mendoza

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### ABSTRACT

Saponin content in mature seeds of seven legumes ranged from 0.09 to 0.44%. Sam-samping had the highest content of 0.44%. In mature pods, saponin ranged from 0.17 to 1.63%. Batao pods had high levels (1.00 to 1.63%) in four accessions. No correlation was found between results of the froth test and Liebermann-Burchard colorimetric test for saponins.

## 7. ALKALOIDS OF SEVERAL PHILIPPINE INDIGENOUS FOOD LEGUMES: DETERMINATION AND REMOVAL

Felicito M. Rodriguez and Evelyn Mae T. Mendoza

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### ABSTRACT

Eight legumes indigenous to the Philippines were screened for the presence of alkaloids. These are: batao or hyacinth bean (*Dolichos lablab*), jack bean (*Canavalia ensiformis*), sword bean (*C. gladiata*), sam-samping (*Mucuna pruriens* or *cochinchinensis*), sabawel (*Clitoria ternatea*), pigeon pea (*Cajanus cajan*), lima bean (*Phaseolus lunatus*) and rice bean (*Vigna umbellata*). High to very high levels of alkaloids were detected only in mature and immature leaves of pigeon pea, jack bean and sword bean. Soaking the leaves in water at 60 C for 60 min reduced completely the alkaloids. Soaking at 30 C for 30 to 45 min and at 45 C for 30 min did not remove the alkaloids while soaking at 45 C for 60 min and at 60 C for 30 min partially removed the alkaloids.

## 8. Lectins in Philippine Indigenous Food Legumes. I. Screening

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### ABSTRACT

Several accessions each of seven different legumes were screened for the presence of hemagglutinins or lectins. Jackbean, sword bean and batao had consistently high levels of hemagglutininins while lima bean had the least amount. Batao had a strong hemagglutinating activity against human type A RBC while others had strong reaction with either or both rabbit and human type O RBC. Varietal differences were also observed.

**Keywords:** lectins, phytohemagglutinins, legumes, lima bean (*Phaseolus lunatus* L.), sword bean (*Canavalia gladiata* (Jacq.) DC.), jack bean (*Canavalia ensiformis* (L.) DC.), sabawel (*Mucuna pruriens* or *conchinchinensis*), batao or hyacinth bean (*Dolichos lablab* L.) and rice bean (*Vigna umbellata* Ohashi.).

## 9. Lectins in Philippine Indigenous Food Legumes. II. Sword bean Lectins: Removal and/or Inactivation

**Ana Gina T. Sembrano and Evelyn Mae T. Mendoza**

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### ABSTRACT

Soaking up to 36 hr did not reduce the lectins of mature green and mature dry seeds of sword bean as measured by hemagglutination and double immunodiffusion (Ouchterlony) technique. Fifteen min boiling of mature green seeds already removed lectins although 45 min were needed to completely cook the seeds. Mature dry seeds of swordbean A-4 took 45 min to remove the lectins although another accession (A-9) took only 15 min. Cooking by boiling of whole seeds (without seed coat) cut into 4 and minced or granulated resulted in removal of lectin as determined by immunological method. However, hemagglutinating activity was still detected in the whole and quartered seeds.

Cooking for 45 min in water , 0.9% NaCl, coconut milk with water and coconut milk without water resulted in removal of lectin immunologically but hemagglutinating activity was maintained/increased in the second and third media. Autoclaving and roasting removed lectins as measured immunologically but not the hemagglutinating activity.

**Keywords:** sword bean (*Canavalia gladiata*), lectins, phytohemagglutinin, hemagglutinin, detoxification, immunodiffusion, Ouchterlony technique.

## 10. Isolation and Characterization of Soluble Proteins from Rice Bean [*Vigna umbellata* (Thumb.) Ohwi Ohashi]

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### ABSTRACT

Globulins comprise the largest fraction in rice bean seed proteins and accounted for 56-60% of total proteins, with albumin (17-26%), prolamin (1.5-1.8%) and glutelin (3.2 to 4.2%).

When fractionated on Sepharose 48, globulins separated into three peaks with molecular weights of approximately >700,000, for the first peak. 175,000 and 18,000 for Tapilan 28; 138,000 and 7,000 for Tapilan 46 and 60,000 and 3,000 for Tapilan PROC 1. Albumins exhibited 3 major peaks and a minor one. The major peak had a molecular weight >210,000. The other peaks had: 75,000, 3,900 and 1,700 for Tapilan 28; 144,000, 14,800 and 2,000 for Tapilan 26 and 56,000, 7,800 and 3,400 for Tapilan PROC 1. Both albumins and globulins exhibited heterogeneity showing 7-10 bands on polyacrylamide gel electrophoresis.

The most limiting amino acids were cysteine and methionine with chemical scores of 38 to 59% only.

In vitro protein digestibility (IVPD) ranged for 82-86% for the seed meal, 86-88.5% for the albumins and 75.9 to 83.3% for the globulins.

[1] Part of MS thesis of the senior author.

[2] Present address: Institute of Chemistry, College of Arts and Science, University of the Philippines Los Baños, College, Laguna 4031.

## 11. CHARACTERIZATION OF SEED PROTEINS FROM SABAWEL (MUCUNA PRURIENS) AND SAM-SAMPING (CLITORIA TERNATEA)

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### ABSTRACT

Seed proteins of sabawel (Mucuna pruriens or cochinchinensis) and sam-samping (Clitoria ternatea) were fractionated according to solubility. Globulins were the major fraction (73-76%), with albumin and glutelin having similar proportion (8-12%) and prolamin at 0.6% only.

Albumins of sabawel consist of proteins of molecular weight (mw) from 21,100 to 152,000 and a polypeptide of low mw (6,600). Albumins of sam-samping had larger mw constituents: 7,700, 26,500, 73,100 and 430,000. Globulins had larger proteins, 37,600 to >700,000 for the two legumes.

Electrophoresis of the fractions revealed that the fractions and sub-fractions were mostly heterogeneous. Methionine contents of the factions were determined.

## 12. Isolation, Fractionation and Characterization of Seed Proteins from Sword bean (Canavalia gladiata) and Jack bean (Canavalia ensiformis)

Joseph L. Samonte, Antonio C. Laurena and Evelyn Mae T. Mendoza

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College, Laguna, Philipoines 4031

### ABSTRACT

Based on solubility, sword bean has 79% globulin, 12% albumin, 6% prolamin and 0.3% glutelin. Jackbean has 66% glutelin, 14% jackbean, 14% prolamin and 0.8% glutelin.

A major portion of jackbean albumin fractionated into 40-60% saturated $(NH_4)_2SO_4$ while its globulin fractionated into 40-60 and 60-80%. Sword bean albumin and globulin fractionated into 60-80% and 40-60 and 60-80%, respectively.

The albumin and globulin of both sword bean globulin were eluted on Biogel A50m and SP-Sephadex to produce two to three peaks. These peaks were shown to be heterogenous by electrophoresis.

## 13. PROXIMATE ANALYSES AND IN VITRO DRY MATTER DIGESTIBILITY OF SEVERAL PHILIPPINE INDIGENOUS FORAGE LEGUMES

Felicito M. Rodriguez, Eureka M. Ocampo and Evelyn Mae T. Mendoza

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### ABSTRACT

The proximate composition of the mature and immature leaves, mature and immature pods and mature seeds of nine forage legumes was determined. These legumes include: Aeschynomene, Cassia occidentalis, Centrosema, Calopogonium, Crotolaria, Desmodium, Pueraria, Phaseolus lahyroides and Porpogon.

In vitro dry matter digestibility (IVDMD) ranged from 16 to 38% for leaves, and 13 to 38% for pods. Seeds of two samples had IVDMD of 8 and 21%.

Metabolizable energy (ME) predicted from crude fiber contents ranged from 3.60 to 4.30 kcal/g for leaves, 3.50 to 4.25 kcal/g for pods and 3.42 to 4.29 Kcal/g for seeds.

## 14. SAPONINS, PHYTATE, POLYPHENOLS, OLIGOSACCHARIDES, LECTINS AND CYANOGENIC GLYCOSIDES IN PHILIPPINE INDIGENOUS FORAGE LEGUMES

Evelyn Mae T. Mendoza, Felicito M. Rodriguez, Antonio C. Laurena, Ana Gina T. Sembrano, Ma. Jamela R. Revilleza, Angelo J.M. Lubag, Jr. and Charlene F. Barroga

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### ABSTRACT

Saponins in seeds ranged from 0.26 to 0.92%, highest in Desmodium and Porpogon. All samples were positive to the froth test except for an accession of Calopogonium.

Polyphenols were generally low in the nine species analyzed, both in terms of condensed tannins and flavanols. Only Centrosema and P. lathyroides had relatively high flavanol groups (5.57 to 10.12 mg catechin/g).

Most of the samples had high (>5 mg P/g) phytate-phosphorus. Seeds of Aeschynomene, C. occidentalis and Calopogonium and relatively high levels of oligosaccharides (>5%).

Strong hemagglutinating activity towards bovine red blood cells was observed in seeds of Centrosema (5 accessions) Crotolaria (5 accessions) and Phaseolus lathyroides (1 accession).

Cyanogenic glucosides were very low or nondetectable in all samples tested.

## 15. ALKALOIDS IN SEVERAL PHILIPPINE INDIGENOUS FORAGE LEGUMES: DETERMINATION AND REMOVAL

Felicito M. Rodriguez and Evelyn Mae T. Mendoza

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### ABSTRACT

Nine forage legumes indigenous to the Philipoine were screened for the presence of alkaloids. These are: Aeschynomene, Cassia occidentalis, Centrosema, Calopogonium, Crotolaria, Desmodium, Pueraria, Phaseolus lathyroides and Porpogon. Immature and mature leaves of Centrosema (4 accessions and Calopogonium (1 accession) had very high levels of alkaloids. Alkaloids were also detected in the leaves of Aeschynomene and in seeds and mature leaves of Crotolaria.

Removal of alkaloids was effected by soaking of mature and immature leaves for 30 to 60 min at 45 to 60 C.

## 17. AMINO ACID COMPOSITION, RELATIVE NUTRITIVE VALUE, IN VITRO PROTEIN DIGESTIBILITY OF SEEDS OF SEVERAL PHILIPPINE INDIGENOUS FORAGE LEGUMES

Antonio C. Laurena, Evelyn Mae T. Mendoza, Noel Sabino, Agnes F. Zamora and Felicito M. Rodriguez

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### ABSTRACT

Methionine was the first limiting amino acid, followed by leucine, in raw mature seeds of 9 forage legumes analysed. High methionine content (>2.0%) was observed in Aeschynomene Acc. IPB (3.1%), Kudzu Acc. BP (4.5%) and Phaseolus lathyroides Acc. IPB (2.8%). Very high lysine (>10%) was also observed in 6 samples.

Relative nutritive value (RNV) of raw mature seeds ranged from 15% (Centrosema) and 77% (Crotolaria NB-PC-1). Cooking the seeds by boiling or roasting increased RNV from 72 to 95%.

In vitro protein digestibility (IVPD) of the raw mature seeds ranged from 73 to 80%.

## 18. SURVEY ON USES OF SEVEN PHILIPPINE INDIGENOUS LEGUMES: A PRELIMINARY REPORT

EVELYN MAE T. MENDOZA

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### ABSTRACT

Partial results of a survey on uses of indigenous legumes in the Philippines revealed that hyacinth bean (batao) and lima bean (patani) had the highest rates of usage, 78% and 59%, respectively, followed by rice bean (22%), sabawel (15%), jack bean (11%) sam-samping (11%) and sword bean (4%).

Mature seeds are primarily used for "ginisa" dishes while immature pods and immature leaves are used as vegetable in vegetable dishes like salad, "pinakbet", "lumpia", "ginataan" and as vegetable component of meat dishes such as "sinigang" and "nilaga". Hyacinth bean, lima bean, jack bean and sabawel were also noted to be used as animal feed. Sabawel seeds are roasted and used as substitute for coffee.

## Possible Research Projects Arising from Philippine Indigenous Legumes Project

1. Isolation, characterization and cloning of methionine rich proteins from batao (Dolichos lablab).

2. Investigation of the nature and practical applications of the galactomannan, lectins, alkaloids and saponins in selected Philippine indigenous legumes.

3. Physiological and nutritional studies of Philippine indigenous legumes for livestock feed.

4. Book and article-writing project to disseminate the information obtained from project.

## Comments and Constraints/Problems on Project Implementation

1. In general, the administration of the project funds by the local USAID mission was very good. Obtaining and closing each advances took about 4-5 weeks except for two or three ocassions.

2. The procurement of the first order of equipment, and supplies worth about $60,000 in 1984 took only 8 months. Connel Bros. did a good job in handling the order. It also consolidated the sending of the many items in only 3 shipments.

   The second and last order worth $7,000 February 1987 arrived only in March to May 1989. The order consisted of items that can be obtained from 3 sources which sent the items separately. Thus, for such a small order, so much difficulty and expenses were met by us in getting them from

the Custom.

3. In general the administration of Project funds by the UPLB Foundation was good.

4. Delays in experiments and repair of instruments were primarily due to the fact that most of supplies and parts needed had to be ordered from outside the Philippines.

5. IPB allowed the full use of its laboratory greenhouses and field facilities for the implementation of the project. However, the major problem was the absence of a working generator for use during electrical failures.