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Title: Biochemical and Nutritional Studies of Philippine Indigenous Food and Forage Legumes

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I. Introduction

In recent years, worldwide attention has focused on tropical legumes as a possible major source of animal and human food. Although the protein contents of legumes seeds are usually high, from 20 to 40%, their nutritive values are low due to the limiting sulfur amino acids, cysteine and methionine and the presence of anti-nutritional factors--trypsin inhibitors, hemagglutinins, flatulence factors and tannins. Other toxins may occur in the leaves, pods and seeds of these legumes like cyanogenic glucosides, saponins, alkaloids, non-protein amino acids like mimosine, etc.

This project's funding from USAID started only in January 1984. The studies under this project are:

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

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

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

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

II. Accomplishments

Collection of samples. Mature seeds of different accession of the following food legumes were obtained from the Vegetable Section and National Plant Genetic Resources Laboratory of IPB: sam-samping (Clitoria ternatea), sabawel (Mucuna curanii), batao (Dolichos lablab), sword bean (Canavalia gladiata), jack bean (Canavalia ensiformis) and rice bean (Vigna umbellata). Balatong aso (Cassia occidentalis) (Linn.)) was collected near the Institute.

Seeds of indigenous forage legumes were obtained from the Forage and Pasture Section of IPB. Several species were also collected from different places in Batangas, Laguna and Quezon. These include: Centrosema, Phaseolus lathyroides Calopogonium, Sesbania, Crotolaria.

Planting of materials. To be able to get more seeds and obtain different plant parts, the following species had been planted using agronomic practices recommended by the Institute's Vegetable Section: sword bean (3), batao (4), sabawel (2), sam-samping (1) and jackbean (1). Other samples will be obtained from NPGRL and Forage and Pasture Section.

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

We have initiated this study on mature seeds of sam-samping, sabawel, batao, sword bean, jack bean, rice bean and balatong aso. The protein content of the mature seeds ranged from 17 to 31%. Balatong aso and tapilan had lower protein levels (17-23%) while sabawel, sword bean and sam-samping had the highest (31%). Starch was the predominant substance (37-49%) followed by protein. Fat content was only 2-3.5%. Among these samples, balatong aso exhibited the highest crude fiber of 13% while sword bean and rice bean had relatively lower levels of 3-6%. Tannin content (as % catechin) was highest in a sabawel accession (8.8%) and sam-samping (8.3%). Trypsin inhibitor activity of batao was highest (26-44 units/mg sample), followed by sabawel (16-20). The in vitro digestibility of sabawel was lowest (69%), and balatong aso, the highest (74-77%) as compared to cowpea (74%) and mungbean (80%).

The green pods of sabawel, jack bean and sword bean had protein levels of 11-18%. Carbohydrate was the major constituent (60-71%). Tannin was relatively low (1-6%, as catechin).

Study 2. Investigation of toxic substances

We have standardized the enzymatic procedure for determining the cyanogenic glucoside content. Total HCN potential of mature seeds studied under Study 1 was low (0 to 1.2 μmoles HCN/g fresh weight) and can be considered harmless. Green pods and green seeds also had low HCN potential.

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

We have studied the soluble and tannin-associated proteins of rice bean (Vigna umbellata).

Proximate analysis of three rice bean varieties showed protein content from 17-21%. The rice bean proteins were fractionated into globulins, 56-61%, albumins, 14-17% and a small fraction of prolamines and glutelins.

The gel filtration of albumins resulted into four peaks of molecular weights ranging from greater than 210,000 to 3,000 while the globulins showed three peaks of molecular weights from over 700,000 to 6,000.

Disc gel electrophoresis of the fractions revealed the extreme heterogeneity of the proteins, while SDS-PAGE separated the protein fractions into subunits with molecular weights of 10,000 to 66,000.

Amino acid analysis showed that the meal and the protein fractions have very low chemical scores, with cystine and methionine being the limiting amino acids. In vitro protein digestibility tests showed high digestibility values for the samples with the albumins having the highest digestibility and the globulins being the least digestible.

The dark colored seeds were found to contain higher tannin levels. The tannin-associated proteins gave only three bands in SDS gel electrophoresis corresponding to molecular weights of 74,000 to 78,000. Amino acid analysis of the tannin associated proteins revealed a high level of proline.

II. Plan of Activities

Study 1. a) Proximate chemical analysis is going on for different parts of food and forage legumes.

b) Determination of tannin content, lectins and trypsin inhibitor activity (TIA) are underway.

c) Determination of relative nutritive value (RNV) and *in vitro* protein digestibility (IVPD) will be started this July.

d) Experiments are being set-up for the determination of flatulence factors (oligosaccharides).

e) Amino acid composition of samples will be analyzed as soon as the HPLC amino acid analyzer arrives and is operationalized.

Study 2. a) HCN potential in other samples will be analyzed.

b) Experiments are being set-up for the determination of saponins, alkaloids and phytic acid.

Study 3. a) Experiments have been started to characterize the proteins of *Cassia occidentalis*.

III. Problems Encountered

1. Only a few seeds of each accession of the different species could be given to us. We have solved this lack of sample by planting the seeds ourselves.
2. Many of the biochemicals and supplies we need are not available locally. We could not order them from abroad because of foreign exchange limitation. There is a need for a mechanism for us to be able to purchase small items from the U.S. perhaps once every two months, charge them to the grant and payment to be processed in dollars.