LEAF PROTEIN EXTRACTION FROM TROPICAL PLANTS

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ABSTRACT

A deficiency of animal protein is widespread in developing countries. Any increase in protein supply from animals especially from non ruminants is limited by the shortage of protein-rich ingredients which are not consumed by humans directly. Leaf protein concentrate (LPC), especially from alfalfa in temperate climates, is suggested as a source to replace soybean and fish, products needed by humans for direct consumption. In the present study, several tropical plants mainly legumes and cassava leaves available in Indonesia have been evaluated for protein extraction. After maceration of leaves and squeezing out manually the liquid through cloth, the protein from the extract was precipitated either by heating or by addition of HCl. A protein concentrate with low tannin was obtained from *Sesbania grandiflora* and *Glyricidia maculata* as compared to *Albizzia falcataria, Calliandra callothyrsus* or *Manihot* sp. Some protein precipitated from the extract of plants except *Sesbania grandiflora* without further treatment which may due to the presence of high tannin. Although some plants may have a potential for protein extraction, however choice of appropriate species is severely limited by (a) agronomic and (b) intrinsic chemical factors, mainly interaction of protein and plant tannins.

INTRODUCTION

Protein and calorie malnutrition is widespread in the developing countries including Indonesia. At present growth rate (2.34%), the Indonesian population will double in the next 30 to 40 years. Undoubtedly this population will burden the country in providing enough food supply for the people, and protein in particular. Currently, the major dietary protein sources are from plants and fish. With increasing income of the people, there is a trend to consume more animal protein than plant protein.

However increasing the supply of animal protein is limited by the availability of feed material to support production. Any effort to increase animal production especially for monogastric animals such as chickens and pigs requires feed containing protein which very often compete with potential human food such as soybean and fish. The government has encouraged research to find alternative sources of feed available locally. Several novel sources of protein have been suggested to meet the ever-increasing world demand. Of these, leaf protein concentrate (LPC) has been considered to be among the most promising.

PLANT SELECTION FOR PROTEIN EXTRACTION IN INDONESIA

In a countinuing study of animal feeds in Indonesia, we have collected, identified, and analysed about 150 species of plants known to be fed to village ruminants. The species include crop plants, weeds of cultivation, volunteer plants on post-harvest land, wayside grasses and herbs, cultivated and volunteer tree species. Leaf material was analysed for, *inter alia,* ash, crude protein, fat, fibre (NDF, ADF, cellulose and lignin), tannin and total phenolics. Results are given for those species (23 out of 150 analysed) which had more than 20% crude protein (Table 1).

Table 1. Composition of leaf material from some Indonesian plant species.

Species	Crude protein	Ash	NDF	Phenolics	Tannins
Woody Legumes					
Acacia villosa	26.2	4.3	28	12.6	.6
Albizzia chinensis	31.6	4.3	49.6	6.2	2.2
Albizzia falcataria	23.8	7.5	39.5	7.0	2.0
Bauhinia purpurea	24.8	8.7	44.8	1.4	.1
Calliandra callothyrsus	24.8	4.5	41.2	11.3	13.8
Enterolobium cyclocarpum	33.0	6.1	24.6	1.1	.1
Glyricidia sepium	24.7	10.8	35.1	1.0	.1
Leucaena diversifolia	22.0	5.1	26.4	4.2	3.9
Mimosa pigra	22.0	8.0	39	9.3	8.1
Samanea saman	24.9	6.5	48.1	4.4	.1
Sesbania grandiflora	33.1	7.9	24.7	1.6	.1
Sesbania sesban	27.8	7.9	31.4	.9	.1
Herbaceous Legumes					
Aeschynomene americana	20.5	5.9	37	1.6	.8
Centrosema plumeri	23.0	8.0	35.5	4.5	.4
Centrosema pubescens	21.9	8.0	43.9	—	—
Psophocarpus tetragonolobus	25.9	10.5	33.1	—	—
Woody, Non-legumes					
Maesopsis emenii	25.6	4.9	20.1	2.4	.1
Pisonia sylvestris	25.0	18.7	30.9	1.0	.1
Herbs, Non-legumes					
Amaranthus hybridus	24.6	20.6	27.8	.9	.2
Drynaria cordata	22.0	9.7	44.1	—	—
Ipomea fistulosa	21.5	8.4	21.9	2.6	.1
Manihot utilisima	27.0	5.6	28.6	6.2	2.0
Grasses					
Setaria plicata	23.2	13.4	54.7	.20	1.2

From the results obtained so far it is possible to select candidate species for testing for protein extraction. One looks for high crude protein, low tannin content, moderately low fibre, suitable handling characteristics (lack of thorns, mucilage, etc.) and an indication that productivity and abundance would allow it to be a practical source.

TROPICAL LEGUME TREES AS A SOURCE OF PROTEIN

The production of protein concentrate directly from green leaf material has been studied extensively, but despite major hopes have never led to significant production of food grade protein for human consumption. Protein concentrates for animal feed are however now produced to a limited extent and seem to offer more practical prospects in the tropics.

The potential use of tree leaves as a source of leaf protein concentrate was suggested by Pirie (1968). It has been estimated that the tropics contain 3 million plant species. Working with any particular group of plants, one becomes aware of an amazing number of legumes, forage grasses or green-leaved vegetables.

Telek (1983) has surveyed a large number of tropical plant species as sources of protein concentrate. In general the results are disappointing. The best species are already food crops, and therefore uneconomic for protein extraction. Most species which would be attractive from the agronomic aspect - e.g. vigorously regrowing shrubby species - gave intractable extraction problems due largely to tannin found in the same tissues as the protein. Although grasses can be used as a source of protein, it seems that some tree leaves are more promising. The most potentially attractive species (*Leucaena leucocephala*) was found to be unsuitable because of the tannin content irreversibly degrading protein quality. Although some Leucaena species have high protein content there are limitations on the utilization of leaves directly for human or animal feed. As generally found in plant materials, the fibre content of these leaves is relatively high which is undesirable. Besides that, some leaves contain a toxic compounds which can be dangerous for either human or animals. Mimosine, which can cause loss of hair, has been found in *Leucaena* sp., *Manihot* sp. contain cyanogenic glycosides which yield products that can be goitrogenic or lethal to animals and *Calliandra* contains a large amount of tannin which influences protein quality.

In order to remove the toxic constituents or fibre fraction in the leaves, certain processing is needed so that the value of protein in the leaves can be enhanced or protected. It is suggested that the process to obtain leaf protein concentrate may remove some of the problems while creating new ones. However in the current study, leaf protein from tropical plants was extracted by different methods, either acid precipitation or heat treatment, and some of preliminary results are reported here.

PREPARATION OF LEAF PROTEIN CONCENTRATES

The initial step to extract protein from plant is grinding and pressing the juice from leaf materials. Addition of chilled water is sometimes desirable to increase efficiency. Several approaches to press juice utilization may then be followed and the process suggested by Kohler *et al.* (1983) is shown in Figure 1. This process uses heat treatment to coagulate protein from the juice. Depending upon the temperature, two different LPC can be obtained either for animal feed or human food. In the laboratory scale, the following method can be used to obtain LPC from plant.

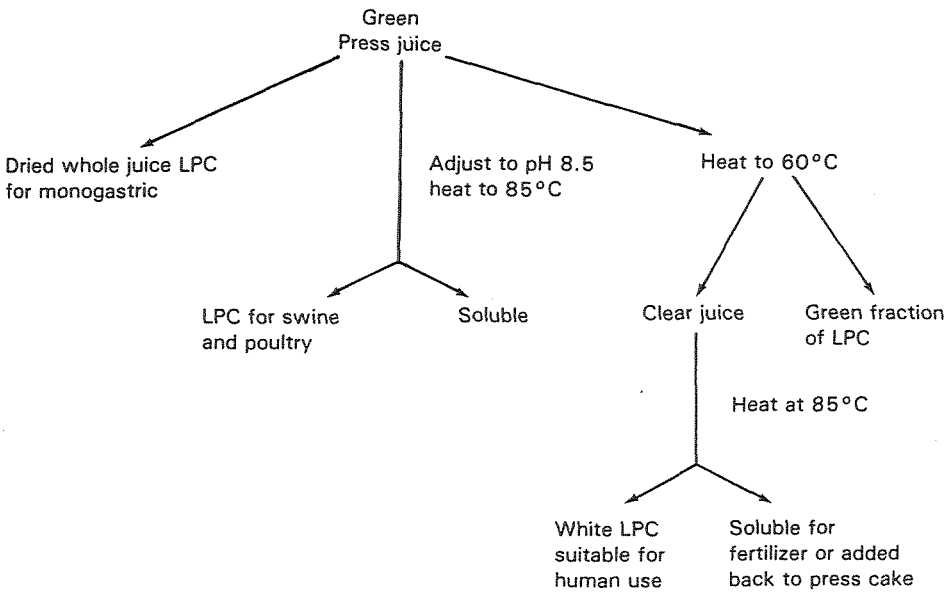


Fig. 1. Heat treatments and uses for the press juice during preparation of LPC. (After Kohler *et al.*, 1983).

Leaves and stems of freshly harvested plants are chopped with a sharp knife to 2—3 cm pieces. For legume leaves this process is hardly necessary. Two hundred grams of chopped plant are extracted in a blender with 600 ml of water. The mixture is blended at high speed for 2—3 min. The slurry is transferred into a filter bag made of fabric and is pressed. The green juice is heated carefully in a flask and agitated slowly at different temperature depending the protein fraction desired. A green coagulum is separated by centrifugation for 10 min. at 3000 rpm. After repeated washing the coagulum

is spread in thin layers on glass plates and dried at room temperature or in an oven or freezedried. The protein yield and content of LPC from various plants available in the villages in West Java is reported in Table 2. Different heating temperatures were used to coagulate protein. Higher protein content in the juice and higher heating temperatures increased the yield of LPC as expected.

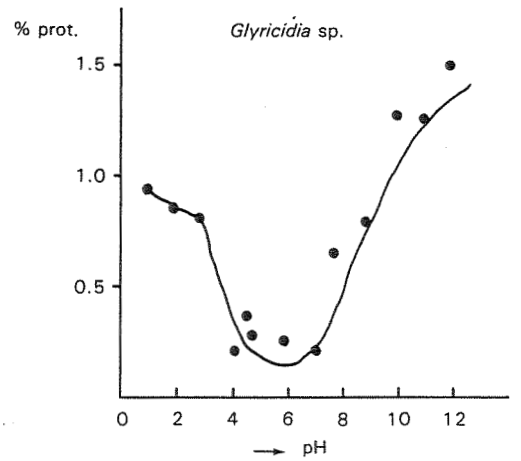
Precipitation of plant juice protein can also be achieved by acid treatment which can be added after juice extraction by alkali or can be obtained by anaerobic fermentation. In the acid precipitation technique, the solubility pattern of leaf protein from a given plant has to be studied so that pH adjustment can be made to isoelectric point in order to obtain maximum protein recoveries. The solubility pattern of protein from some tropical tree legumes is presented in Figure 2. The figure shows that most of protein from plant tested can be precipitated by adjusting pH to around 4.

Table 2. Percent yield and protein content of LPC after different heating obtained from plants available in West Java villages.

Source of LPC	Temperature of heating				
	25°C	45°C	55°C	65°C	75°C
Percent yield (gram/100 grams fresh leaf)					
Manihot sp.	7.2	7.3	8.5	12.5	14.4
Albizzia falcataria	8.0	8.4	7.5	7.6	8.4
Glyricidia maculata	5.4	5.0	4.9	4.5	5.6
Sesbania grandiflora	—	2.3	4.9	9.3	11.0
Calliandra callothyrsus	7.3	7.0	8.0	7.4	8.0
Protein content (g/100 grams DM)					
Manihot sp.	48.7	46.9	55.9	57.7	61.6
Albizzia falcataria	28.8	37.9	37.2	47.6	59.9
Glyricidia maculata	17.1	20.6	25.0	28.3	30.8
Sesbania grandiflora	—	19.1	22.0	24.4	29.3
Calliandra callothyrsus	11.7	12.6	13.2	15.8	20.0

EXTRACTION CHARACTERISTICS OF TROPICAL PLANTS AND POSSIBLE NUTRITIVE VALUE OF LPC

During the extraction of the juice from the plants tested, it was observed that pressed juice of cassava, calliandra and leucaena coagulated at room temperature (Table 1). Spontaneous coagulation of the pressed juice was also reported by Telek and Martin (1983) to occur in several plants. They claimed that no additional protein separated on heating to 55°C.



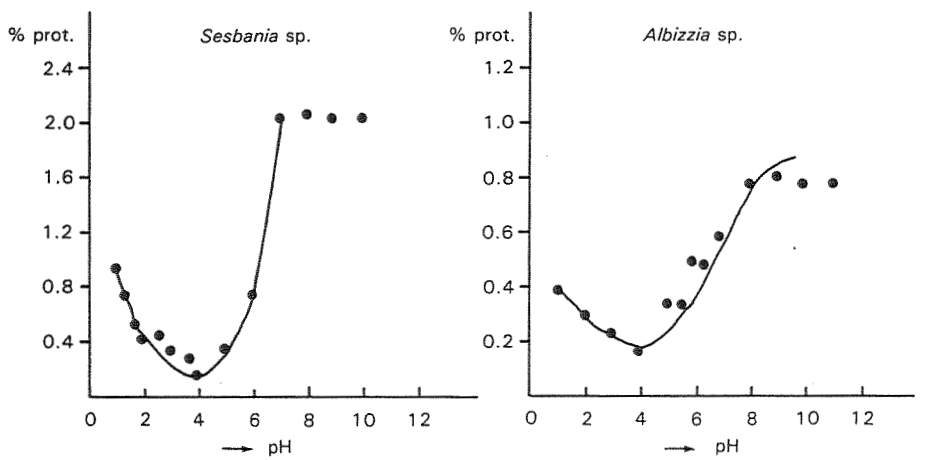


Fig. 2. Protein solubility pattern of three legume tree species.

The spontaneous coagulation can be explained by the reaction that occurred between polyphenols and proteins after they were separated by the disintegration process of their natural tissues. The polyphenol complexes in leaf protein concentrate interfere with the extractibility of protein and with enzyme digestibility *in vitro* and *in vivo* and hence decrease the nutritional value of the protein. Cheeke *et al.* (1980) found poor animal performance in a nutritional evaluation of cassava leaf protein concentrate. Cheeke and Telek (1980) also reported that LPC from leucaena had low Protein Efficiency Ratio (PER) (0.4) when it was fed to rats.

The tannin content of the plants tested and of LPC obtained from them is reported in Table 4. Calliandra was found to have a very high tannin content which may result in self precipitation more easily than in leucaena and cassava. Based on the results of Telek and Martin (1983) it is postulated that Calliandra and also Albizzia will have a poor nutritional value.

Based on the current results, *Glyricidia* and *Sesbania* may have a potential for production of LPC either for animal or human use and further evaluation is warranted for these plants. *Sesbania* is a valued ruminant feed but may not be as productive as *Glyricidia. Glyricidia* however is underutilised for ruminants, extremely productive, and easy to establish. It appears to be the most attractive species for further evaluation.

Table 3. Percent yield, protein and tannin of LPC obtained by acid precipitation technique of some tropical plants.

Source of LPC	% Yield	% Protein	% Tannin
Manihot sp.	8.7	68.9	N.D.
Albizzia falcataria	17.5	66.7	0.9
Glyricidia maculata	7.1	43.6	N.D.
Sesbania grandiflora	8.5	70.1	N.D.
Calliandra callothyrsus	22.3	43.4	4.6

Table 4. Tannin content of LPC from different leaves (gram/100 grams DM).

Source of LPC	Temperature of heating				
	25°C	45°C	55°C	65°C	75°C
Manihot sp.	0.59	0.34	1.39	0.77	0.53
Albizzia falcataria	1.10	1.04	1.02	0.07	1.05
Glyricidia maculata	0.10	0.07	0.07	0.07	0.08
Sesbania grandiflora	–	0.19	0.18	0.27	0.29
Calliandra callothyrsus	15.28	21.48	21.81	12.66	15.70

POSSIBLE USES OF LPC

Protein concentrates recoverable from plant represent a mixture of different type of protein but due to the distinctive dark colour, bitter taste and grassy smell, the unfractionated product is not readily acceptable for direct human consumption (Pirie, 1971). To recover LPC suitable for human use the chloroplast - containing proteins (feed grade) should be separated from chloroplast free protein (white fraction) by either heat or chemical fractionation. The food grade protein from this process may be used as a substitute for certain food products.

The feed grade LPC can be used for chicken or pig feed and it may be used as source of xanthophyll for laying hens. It has been used to replace soybean meal as a source of protein for growing chickens (McKenzie, 1978). The fibre residue after dejuicing can be directly fed to ruminant animals.

Leaf protein concentrates for animal production are manufactured mainly from alfalfa (*Medicago sativa*) in Hungary, France and Denmark, and new plants are under construction in United States and Great Britain.

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