Leaf Protein Extraction From Tropical Plants

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ABSTRACT

Protein and calorie malnutrition is widespread in the less developed countries. Importing high-grade animal products, cereal grains, and animal feeds puts a strain on these countries' economies. Leaf protein concentrates (LPCs) should be seriously considered as additional protein sources. Leaf protein concentrates from alfalfa are prepared on a large commercial scale in Europe and the United States. Unfortunately, alfalfa has not been grown successfully in the humid tropics, and a suitable tropical replacement is needed for leaf protein extraction.

At least 500 introductions of tropical plants have been tested at the U.S. Department of Agriculture's (USDA's) Tropical Agriculture Research Station in Puerto Rico. Potential plants for LPC production have been evaluated and selected for further research, Machinery has been developed which may be suitable for laboratory, on-farm, village level, and commercial extraction. Further research is needed in agronomy and leaf protein extraction and use. On-farm use is the most economical system for the tropics, It is suggested that crude leaf protein concentrate be used as human food only in extreme emergencies.

Tropical Plants for Loaf Protein Extraction

At the present growth rate, world population will double in the next 30 to 40 years. This population increase mainly will burden the lesser developed countries in the humid lowland tropics, where the current annual population growth rate is 2.3 percent and yearly increase in food production remains low. The demand for food of plant origin will continue to increase, and the supply of meat will decrease because yields of cereal grains in the tropics generally are low and local production is consumed by humans. This ever-widening food shortage cannot be alleviated by conventional agriculture alone. As an additional source of protein, leaf protein concentrates (LPCs) should be given serious attention because leaves are abundant year-round in the tropics and many have high protein content. With suitable plant material, the yield per hectare per year of leaf proteins can be at least four times higher than that of seed proteins.

Leaf protein concentrates for animal feed currently are manufactured from alfalfa in Europe, and a new processing plant was started recently in the United States. Because efforts to adapt alfalfa to the tropics have been only marginally successful in a few areas, possible tropical plant sources have been investigated.

In 1978, a broad research program to find suitable tropical plants for leaf protein fractionation was organized at the Tropical Agriculture Research Station, USDA (Science and Education) in Mayaguez, Puerto Rico. At least 500 introductions were planted and critically evaluated as potential sources for LPC extraction (77) (table 1). The following criteria were used in selecting plants suitable for LPC production: high protein and dry matter (DM) content, good protein extractability when freshly cut, and good regrowth potential. The plants should fix nitrogen, be erect and easily harvested mechanically, and be nontoxic and low in antinutritional factors,

Freshly harvested plants were extracted in a blender with 600 ml ice water at high speed for 5 to 10 minutes and filtered in a bag made of closely woven fabric. The pressed green juice was heated carefully in a 2-liter Erlenmeyer flask immersed in boiling water and agitated with a slow motion stirrer. At 550 C, a green coagulum formed and was separated by centrifugation, washed several times, and finally spread in thin layers on glass plates and dried, The supernatant from the centrifugation was heated carefully to 64° and the white curd coagulum that formed was separated by centrifugation, washed with acetone, and dried in a rotary evaporator. The liquid was further heated to 820. After cooling, a light tan precipitate formed and was processed as the 640 fraction (see fig. 1),

The spontaneous coagulation of protein in the juice extracted from some plants was observed during the survey of tropical plants. This phenomenon occurred during extraction of leaves of cassava, *Leucaena leucocephala;* some *Indigoferas, Desmodium, and Mimosa* species; and many of the tree legumes of Mimosa, Cassia, and Pea subfamilies. These plants have been classified as Type I (table 1). Another group of plants yielded a green

Table 1.—Crude Protein Content of Tropical Plants

Plant	Origin	Percent		Type[a]
		Dry matter	Crude protein	
AMARANTHACEAE				
Amarantlius anclancaluius	Hungary	12.3	26.6	Iv
A. caudatus	Sweden	13.0	27.7	Iv
A. cruentus	Taiwan	14.6	28.3	IV
A. gangeticus	Hungary	14.5	24.4	Ii'
A. hypochondriacus	Sweden	11.5	27.9	Iv
A. mantegazzianus	Sweden	16.2	30.0	IV
COMPOSITAE				
Helian thus uniflorus	Hungary	26.6	29.9	11
H . annuus	Sweden	24.6	25.4	11
CRUCIFERAE				
Brassica alba	Hungary	22.5	39.8	III
B . campestris	Pakistan	14.2	19.1	III
	Guatemala	10.8	21.0	III
B. hirta	Yugoslavia	10.4	30.0	III
	Poland	12.6	30.8	111
B. napus	Hungary	11.8	21.1	111
	France	11.6	26.4	III
	China	14.4	24.4	III
Brassica juncea	India	13.8	27.1	III
	Cuba	12.2	22.3	III
	Nepal	14.2	24.8	III
B. nigra	India	10.2	26.4	III
	Turkey	10.8	24.0	III
	Creece	14.1	20.6	III
B. oleracea	United States	14.0	19.4	III
B. " var. gongyloides	United States	12.6	19.4	III
Lepidium sativum	Puerto Rico	12.3	17.6	III
Nasturtium officinale	Puerto Rico	18.2	22.6	IV
CUCURBITACEAE				
Benincasa hispida	India	18.0	18.0	11
Lagenaria siceraria	S. Africa	11.4	26.3	II
Luffa cylindrical	India	19.3	26.3	II
EUPHORBIACEAE				
Cnidoscolus chayamansa	Mexico	18.8	26.3	11
Manihot esculenta	Colombia	20.8	25.5	I
LECUMINOSAE				
Aeschynomene falcata	Brazil	20.2	13.5	III
A. scabra	Mexico	20.8	15.5	III
A. indica	India	20.8	16.9	III

Alysicarpus vaginal is	India	**20.1**	20.0	*111*
	Ceylon	**18.6**	20.3	III
Cajanus cajan	India	**24.0**	22.5	111
	Mexico	**2 .0**	20.6	111
Calopogonium **muconoides**	Indonesia	**20.0**	19.7	111
Canavalia ensiformis	India	**21.5**	18.4	111
	Brazil	**19.8**	22.1	111
C. gladiata	Philippines	**20.9**	21.9	111
Centrosema **pubescens**	India	**23.5**	18.9	111
	Philippines	**25.0**	23.2	111
	Ivory Coast	**20.0**	19.4	111
Clitoria ternatea	Brazil	**16.1**	23.6	111
	Cuba	**20.6**	23.0	111
	Australia	**19.2**	24.3	III
Crotalaria alata	India	**18.6**	19.9	II
C. argyrolobioloides	Kenya	**20.4**	23.9	II
C. brachystachya	Brazil	**19.2**	27.4	11
C. incana	Argentina	**19.3**	22.9	II
C. juncea	India	**21.4**	25.8	11
	USSR	**24.0**	26.3	II
Cyamopsis tetragonoloba	India	**14.2**	19.2	III
Desmodium canum	Brazil	**20.8**	18.9	I.
D. distortum	Hawaii	**16.8**	17.8	111
D. intortum	Spain	**25.0**	18.7	III
	Brazil	**25.0**	23.5	111
D. perplexum	Brazil	**20.0**	16.2	11
D. sandwicense	Australia	**21.7**	23.0	1
Glycine wightii	S. Africa	**19.2**	19.6	III
Indigofera arrecta	Chana	**31.2**	15.1	II
I. brevipes	Costa Rica	**18.3**	26.8	II
I. cireinella	Korea	**17.6**	26.2	I
	S. Africa	**16.4**	28.1	I.
I. colutea	Australia	**20.2**	28.7	III
I. confusa	Indonesia	**19.8**	14.6	11
I. cryptantha	S. Africa	**19.8**	24.0	111
I. echinata	Tanzania	**21.0**	21.4	II
X. hirsuta	Nigeria	**22.1**	24.2	11
	Brazil	**21.0**	27.9	II
	Rhodesia	**23.4**	30.6	II
I. **hochstetteri**	Rhodesia	**18.3**	21.2	II
I. microcarpa	Argentina	**34.9**	22.3	II
I. mucronata	Peru	**20.8**	22.4	II
	Brazil	**23.7**	24.4	11
I. recroflexa	Kenya	**21.8**	21.2	11
I. schimperi	Africa	**14.6**	23.3	11
I. semitijuga	India	**31.8**	17.2	II
I. spicata	Tanzania	**16.5**	35.7	11
I. subulata	Kenya	**27.2**	12.3	II
	Cuba	**26.4**	10.8	II
I. suffruticosa	Brazil	**21.4**	25.3	11
	Mexico	**20.7**	32.4	11
I. sumatrana	Australia	**21.8**	28.2	II
I. tetlensis	Africa	**20.2**	20.5	III
I. Ceysmannii	Malaya	**21.6**	31.0	11
I. tinctoria	Dem. Republic	**20.6**	15.2	11

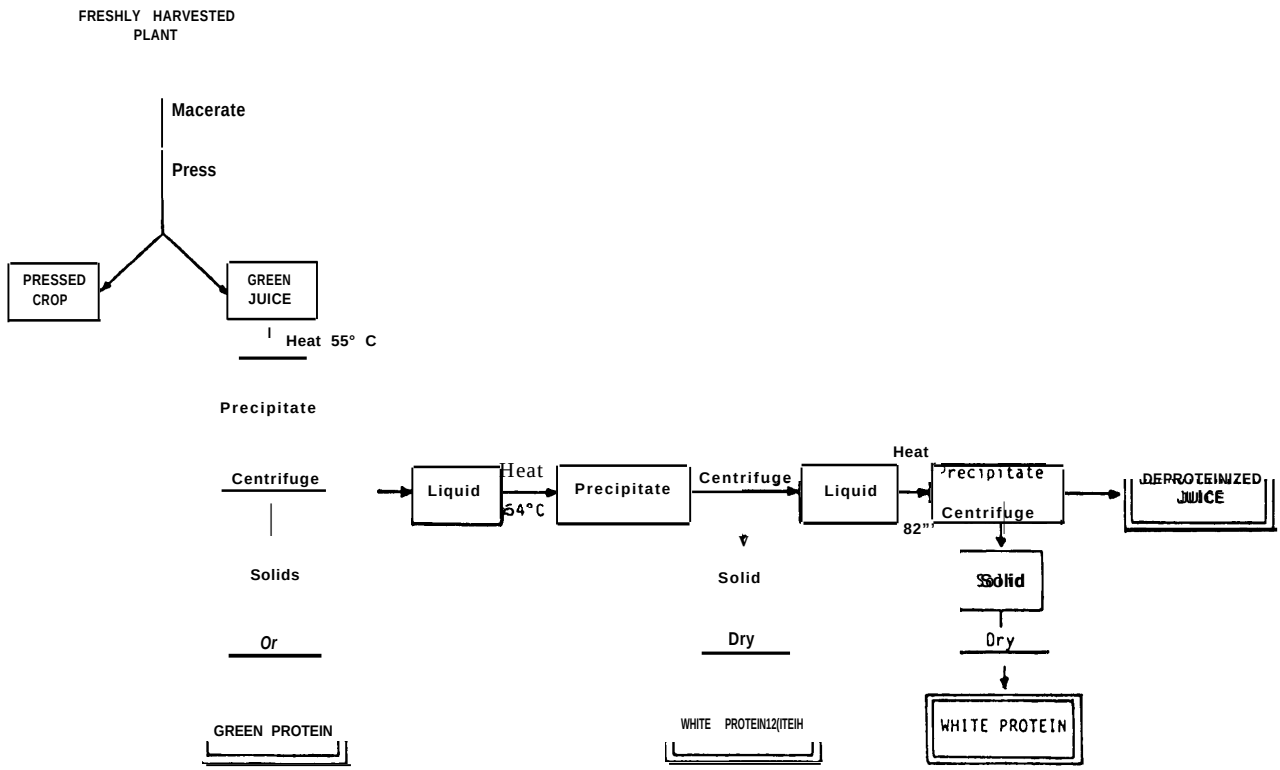
Lablab purpureus	Malaysia	21.5	27.7	III
Lupinus albus	Turkey	18.8	19.5	111
L. angustifolius	Hungary	20.4	18.8	111
	S. Africa	22.6	16.6	III
L. luteus	Hungary	22.2	16.1	III
	Spain	20.4	19.2	III
L. hispanicus	Portugal	20.6	19.8	III
	Spain	20.2	19.2	111
Macroptilium lathyroides	Australia	18.8	19.5	III
Macrocyloma uniflorum	Puerto Rico	18.6	26.4	III
	South Africa	21.0	21.3	III
Mucuna deeringiana	Mozambique	17.2	32.6	11
Phaseolus acontifolius	India	18.8	18.3	111
	Afghanistan	22.2	15.8	111
P. calcaratus	Ivory Coast	19.7	20.8	111
	Honduras	20.1	23.7	111
Psophocarpus tetragonolobus	Puerto Rico	20.6	22.0	III
Sesbania arabica	Turkey	21.8	29.9	111
	Afghanistan	22.2	30.0	111
S: cannabina	India	19.8	21.4	111
S. exasperate'	Brazil	20.4	20.9	111
	Argentina	20.6	20.6	III
S. macrocarpa	Australia	22.0	19.2	III
	Mexico	19.8	19.9	111
S. sesban	India	20.6	27.6	111
	China	19.4	29.4	111
Stizolobium aterrimum	Mexico	20.6	27.4	111
Stylosanthes gracilis	Paraguay	21.2	22.3	IV
S. humilis	Australia	22.0	20.9	IV
	Brazil	21.8	18.9	Iv
Tephrosia adunea	Venezuela	22.2	18.0	11
T. cinerea	Uruguay	24.2	14.0	11
T. incana '	Kenya	23.8	19.3	II
	Indonesia	24.4	21.4	II
T. noctiflora	Brazil	23.6	13.2	II
T. vogelii	Puerto Rico	20.4	12.3	II
Vigna mungo	Pakistan	19.3	23.6	III
	China	21.0	24.0	111
	Iran	19.3	16.9	111
V. radiata	Turkey	18.9	22.9	111
	India	21.1	22.2	III
V. unguiculata	Guatemala	18.8	19.5	111
	Mexico	19.0	19.4	111
Zornia brasiliensis	Brazil	20.2	15.8	III
Z. diphylla	Brazil	19.8	16.8	111
Z. latifolia	Brazil	20.0	15.8	111
MALVACEAE				
Abelmoschus manihot	Japan	14.6	14.0	111
SOLANACEAE				
Capsicum annuum	Yugoslavia	20.0	***22.4***	111

Capsicum annuum	Ecuador	12.5	19.9	III
	Israel	20.0	21.8	111
C. chinense	Ceorgia-USA	15.3	20.0	111
	Guatemala	15.3	23,.4	111
	Colombia	16.7	19.9	111
C. pendulum	Mexico	18.0	19.8	111
	Chile	22.2	21.0	111
	California-USA	16.0	20.2	111
Solarium melongena	India"	21.0	21.2	III
	China	21.4	21.4	111

[a] Yield of different, fractions after extraction: I. Only one green fraction coagulated at room temperature: II. One green fraction on heating to 55°C and one minute amount of light tan fraction at 82"; III. One green fraction at 55°. one white fraction at 64" and another light can fraction at 82°; IV. No distinct separable coagulum by heat fractionation.

SOURCE: Telek(77)

Figure 1.— Laboratory Method for Preparation of Protein Concentrate From Tropical Plants



SOURCE:Telek(77)

protein coagulum after the extracted green plant juice was heated to 55°C and yielded a very small quantity of a light tan precipitateat820 C. This first was observed with leaf protein extract of sorghum-sudan grass hybrids and other grasses. Plants of this group have been classified as Type II. Type III is the most important group of plants. Careful heat fractionation of aqueous leaf extracts yielded three distinct protein fractions: a greencoagulumat550 C, a copious white protein precipitate at 64° C, and a smaller amount of a light tan precipitate at 820 C. The most desirable plants for further studies were selected from this group (table 2). A final group, designated as Type IV, includes plants in

Table 2.—Selected Plants for Leaf Protein Concentrates Production

Plants	Dry metter yield Mg/he/year	% Protein of dry matter	% protein extractability	Biological nitrogen fixation	Regrowth
Brassica napus	28	25.4	62.4		Poor
Centrosema pubescens	12	18.8	37.2	+	Good
Clitoria ternatea	28	24.0	59.8	+	Good
Desmodium distortum	11	18.0	47.9	.+	Good
Lablab purpureus	19	28.4	58.1	+	Good
Macroptilium lathyroides	12	26.0	59.0	+	Good
Psophocarpus cetragonolobus	20	22.2	53.6	+	Fair
Sesbania sesban	20	28.6	40.2	+	Fair
Vigna radiata	16	22.9	47.8	+	Fair
V. unguiculata	25	19.5	52.0	+	Fair

SOURCE:Telek(77).

which the proteins in the aqueous extracts do not precipitate either spontaneously or after heat treatmerit. This was observed in extractions of Stylosan*thes gracilis, S. humilis, Nasturtium officinale, and Amaranths* spp.

Pilot Plant Scale Preparation and Nutritional Evacuation

Leaf protein concentrates from the tropical legumes *Leucaena Zeucocephala, Vigna unguiculata, Clitoria ternatea, Desmodium distortum, Psophocarpus tetragonolobus, Macroptilium lathyroides, Phaseolus calcaratus, Brassica napus,* and *Manihot* esculenta were prepared on a pilot plant scale. The plants were harvested in the vegetative stage of growth, stored in a freezer, transported in a frozen state, and processed in the pilot plant of the Food Technology Laboratory, University of Puerto Rico. Processing consisted of chopping to 2-cm pieces followed by soaking in 2-percent sodium metabisulfite, The soaked material was disintegrated in a hammer mill and pressed in a single-screw press. The expressed juice was heated with steam to 820 C until a protein coagulum appeared. The hot coagulum was collected in a basket centrifuge, pressed in a canvas bag under a hydraulic press, spread in a thin layer on glass plates, and dried in an air-conditioned, dehumidified room. The pressed green juice of *Leucaena leucocephala* and cassava was left for 20 hours for self-precipitation of proteins at ambient temperatures (290 to 310 **C).** The settled precipitates were processed as outlined above.

The protein quality of the LPC was evaluated by using rats (17). The tropical legumes *Vigna unguiculata, Desmodium distortum, Phaseolus calcaratus,* and *Psophocarpus tetragonolobus* gave excellent results, comparable to those obtainable from alfalfa LPC. These plants have low polyphenol contents. Another legume low in phenols, *Macroptilium* lathyroides, gave relatively poor results [table 3) due to its high saponin content. The differences in rat growth probably are due to different amino acid availabilities, as influenced by polyphenols and other compounds that may react with protein to form indigestible complexes.

The LPCs from the tropical legumes tested were found to have amino acid contents similar to each other and to reported values for alfalfa LPC and soybean meal (table 4).

Cassava and *Leucaena* were included in this nutritional evaluation, alongside the selected plant sources, to support our classification of plants based on the number of protein fractions. It was suspected that the spontaneously precipitated protein concentrate from Type I plants would have less nutritional value. Rats fed LPC from cassava and *Leucaena* grew poorly. The data showed that protein concentrates from these plants cannot be produced by the accepted LPC processes.

About two decades ago, the use of tree leaves as a potential source of leaf protein concentrate was suggested (60,64), and their possible use is still being mentioned in literature, The presence of phenolic substances negatively affects the nutritional value of the proteins prepared from tree leaves. After our studies of *Leucaena leucocephala, we ex*tended our investigations to the other tree legumes located in Puerto Rico. The Leguminosae family is huge (14,000 species) and extremely diverse, ranging from forest trees to shrubs to herbaceous annuals.

The results of our investigations, given in table 5, clearly indicated that, with current methods,

Table 3.—Protein Content of Tropical Plant LPC, Diet Composition, and Rat Growth Performance

Source of LPC	Diet Composition: Crude Protein (%) of LPC[1]	% LPC	% Corn	% Soybean Heal	Rat Performance: Avg. Daily Gain[2] 9)	Avg. Daily Gain as % of Control	Avg. Daily Feed Intake[2] 9)	Protein Efficiency Ratio
Corn-soy control	----	----	69	22	7.2±.4[f]	100	16.411.5	2.7
Leucaena leucocephala	31.3	*31.9*	57	2.1	0.9±.4[h]	12.5	11.0±1.8	0.4
L. leucocephala (acetone-washed)	38.2	*26.1*	64.5	*0.4*	0.72.1[h]	9.7	11.6±1.9	0.4
L. leucocephala (acid-washed)	28.2	35.5	52.7	*2.8*	1.0±.9[gh]	13.9	9.7±1.4	0.5
Manihot esculenta[3]	36.2	27.6	62.6	*0.8*	1.8±.2[gh]	25.0	11.7± .9	0.9
M. esculenta[4]	32.1	31.2	58.1	1.7	2.0±.5[gh]	27.8	11 .121.2	0.8
M. esculenta[5]	33.3	30.0	59.6	1.4	1.2±.3[gh]	16.7	11 .721.9	0.6
M. esculenta (acetone-washed)	41.4	24.2	66.8	---	2.0±.4[gh]	27.8	14.811.7	0.8
Vigna unguiculata	51.9	19.3	71.7	---	5.8±.9[f]	8 0 . 6	18.2±1.8	2.0
Cl i toria ternata	59.3	16.9	74.1	---	6.7±.4[f]	93.1	17.7±1.6	2.4
Desmodium distortum	36.5	27.4	62.8	0.8	5.4±1.0[f]	75.0	13.0±1.1	2.0
D. distortum (acetone-washed)	47.0	21.3	69.7	---	6.6±.9[f]	91.7	16.6±2.0	2.3
Psophocarpus tetragonolobus	51.9	19.3	71.7	---	6.0±1.0[f]	83.3	17.2=1.3	2.2
Macroptilium lathyroides	44.6	22.4	68.6	---	3.02.57	41.7	14.9±2.0	1.3
Phaseolus calcaratus	38.0	26.3	64.2	0.5	6.0±.6[f]	83.3	13.5±1.2	2.1
Brassica napus cv. Early Giant	40.4	24.8	66.1	0.1	6.1±.9[f]	84.7	17.4±1.5	2.1

[1] N X 6.25
[2] Mean ± standard deviation.
Means followed by different letters differ at p 0.01.
[3] Dried in microwave oven.
● Dried in air-conditioned room.

SOURCE: Cheeke (17).

good quality leaf protein concentrates for nonruminants could not be prepared from many of the leguminous tree leaves, especially those of Type I plants. However, some of the leaves of the Leguminosae definitely could be used as feed for ruminants after careful analysis for toxic ingredients.

Stylosanthes humilis and S. *guianensis offered* promise for leaf protein extraction. These valuable pasture plants are perennial legumes with high DM yield and a protein content that is as high as that of alfalfa. However, during maceration of these plants, a thick emulsion formed which could not be separated from the fibrous material either by centrifuging or by pressing. On heating, the emulsion thickened and separation became even more difficult. After unsuccessful processing experimerits with 10 different cultivars, further research was abandoned.

The lush vegetation of the humid tropics is often considered to have a high potential for animal production. The grasses are an extremely large family of more than 10,000 species, Tropical grasses have a capacity for photosynthetic high rates, grow year round, and show excellent regrowth after repetitive cutting. However, the natural nitrogen (N) content of grasses is relatively low and heavy nitrogen fertilization is required for high DM production.

Our experiments with forage sorghum (Millo Blanco) and sudan hybrids showed encouraging yields (71), However, the extractions were disappointingly low: 28.6 percent extractable protein compared with 52.4 percent for legumes. Investigations of tropical grasses as sources for leaf protein extraction were suspended. It was concluded that the ever-increasing cost of nitrogen fertilizer, the higher processing costs due to lower crude protein content, low extractability of tough fibrous plant material, higher energy requirement of the disintegrator, and the lower quality of protein would make the process uneconomical. With a low initial N content, the extraction of protein from grasses could reach the point where the pressed residue, the most important byproduct, could not be used by ruminants.

Our relatively short systematic investigation of possible tropical plant sources for leaf protein extraction and fractionation produced the following valuable results:

1, Plants equivalent to alfalfa in yield, extractability, and nutritional quality were selected,
2. It was recognized that in the Tropics a single crop cannot be used for year-round production; a pattern of different plantings has to be formulated for rainy and dry seasons. Hello

Table 4.—Essential Amino Acid Composition of LPC From Tropical Species

Source of LPC	gm amino acid/100 g recovered amino acids									
	Arg	Hist	Iso l	Leu	Lys	Meth	Phe	Thr	Val	1/2 cyst
Leucaena leucocephala	6.4	2.2	5.0	9.1	6.3	2.4	5.9	4.8	6.0	0,7
Manihot esculenta[a]	6.3	2.4	5,4	4,3	6.5	2.5	7.0	4,9	6.0	0.8
Manihot esculenta[b]	6.0	2,4	5.2	9.1	6,4	2.4	6,8	5.2	5.7	0.7
Vigna unguiculata	6.7	2,4	5.4	9,4	5,8	2.7	7*5	5.3	6,4	0.6
Clitoria ternatea	6.1	2,2	5.8	9.1	6.1	2.4	7.2	5.2	6.2	2.0
Desmodium distortum	6.1	2.6	5*5	9.4	6.5	2.6	7.3	5.0	6.0	0.6
Psophocarputs tetragonologus	6.2	2.6	5.4	9.3	6.4	2.5	7.1	5.2	6.4	0.6
Macroptilium lathyroides	6.6	2.5	5.6	9.7	5.4	2.7	7.5	5.0	6.4	0.6
Phaseolus calcaratus	6.0	2.5	5.4	9.2	6,4	3.0	7.1	5.1	6.2	0.7
Brassica napus	6.2	2.6	5.3	9".3	5.8	2.6	7.5	5.3	6.4	0.7
Medicago astiva[c]	6.5	2.3	5.6	9.3	5.9	2.3	5.9	5.1	6.3	0.6

[a] Dried in air conditioned room.
[b] Dried in microwave oven.
[c] Kuzmicky and Kohler (1977).

SOURCE: Cheeke (17),

and Koch's (32) statement that "When it is possible to use the raw material of one or only a few plant cultures—e. g., in tropical regions, the technological problems are not as great" now seems inaccurate.

3. Our studies seriously question the popular belief that cassava, *Leucaena leucocephala,* other tropical tree leaves, and tropical grasses are potential sources of LPC.
4. The protein pattern, or number of protein fractions obtained by heat fractionation, was discovered to be a rapid preliminary method to screen plants for protein extraction potential. Type I plants have a low probability of yielding good LPC.

Machinery of Leaf Protein Fractionation

The development of economical equipment for successful farm-size leaf protein processing is a major task. Hjalmar Bruhn (14), a leading authority in the United States on farm machinery designed especially for protein extraction wrote: "I don't see much hope for any appreciable protein production unless there are well engineered machines that are commercially available at farm machinery prices."

The separation of plant juice from the fiber is a two-step process: The rupture of the plant cells by maceration and the separation of the juice and fiber. Cell rupture is the most energy intensive process in leaf protein extraction.

Macerating

Initially, macerating sugarcane rollers were used, but such equipment proved to be ineffective, The overall capacity was low when operating under higher pressure, the power requirement was high, and the machine was very heavy for portable farm use.

Varying degrees of cell disintegration can be accomplished by hammer mills of different construction. These should be used when a high percentage of protein is to be extracted, when the initial plant material has a high protein content, or when the pressed crop will not be used as forage for cattle.

The evolution of small equipment designed in Rothamsted Experimental Station in England

Table 5.—Protein Content of Leaves of Tropical Legume Trees

	% Protein	Plant type
1 Cassia Subfamily (Caesalpinioideae)		
Bauhinia alba	17.43	I
Bauhinia candida	13.20	
Bauhinia galpini	16.22	I
Bauhinia purpurea	8.92	I
Bauhinia reticulata	12.59	I
Bauhinia violacea	11.80	III
Brownea grandiceps	6.47	
Brownea macrophylla	8.58	I
Cassia moschata	14.24	III
Cassia nodosa	22.85	I
Cassia spectabilis	25.90	III
Delonix regia	10.57	I
Libidibia punctata	14.89	I
Tamarinds indica	10.03	I
2. Mimosa Subfamily (blimosoideae)		
Albizia adinocephala	20.80	III
Calliandra inaequilatera	14.77	I
Calliandra surnamensis	16.60	I
Enterolobium cyclocarpum	26.58	I
Inga laurina	18.34	I
Inga Vera	21.44	I
Leucaena leucocephala	26.80	I
Parkia biglandulosa	12.85	I
Pithecellobium dulce	18.43	III
Samanea sampan	17.41	III
3. Pea Subfamily (Faboideae)		
Dalbergia sissoo	12.91	III
Erythrina poeppigiana	19.13	III
Erythrina variegata orientalis	17.94	III
Myrospermum frutescent	19.42	III

SOURCE: Telek, unpublished.

before 1960 has been published by Davys and Pirie (19). Later, to standardize processing methods, new equiment was built and evaluated in several countries. The new pulper contains 58 fixed beaters with 2 mm clearance from the drum. The rotor is driven by a s-horsepower (HP) motor. Field experimentation has shown that units can be mounted on a Landrover whose engine drives the pulper. The standard model takes 1 kg crop/rein, but could be increased to 6 kg/rein. The capacity to macerate 360 kg/hr makes this pulper useful at the village production level (61).

The large-scale fractionation machinery at the National Institute of Research on Dairying (NIRD), Shinefield, England, was developed from a design of Davys and Pirie (20). The pulper has 32 arms that rotate in a cylinder and are driven at 1,100 rpm by a 32.3 HP motor. The total power requirement is 6 to 8 kilowatt hours per metric ton (kWh/MT) of initial crop. More powerful disintegrators of this type were built for the large industrial process. These machines will not be discussed in detail, since they are not related yet to tropical production.

The most energy efficient way to macerate at high capacities is by using extrusion, where the plant material is forced through an orifice (50). High capacity rotary extrusion macerators have been designed by Basken (5) and Nelson, et al. (56). These macerators consist of an internal roller operating against a die ring perforated by radial orifice holes. One of these experimental macerators operated with 22.1 MT/hr capacity at a power input of about 1.7 kWh/MT (fig. 2).

Figure 2.— Rotary Extrusion Macerator

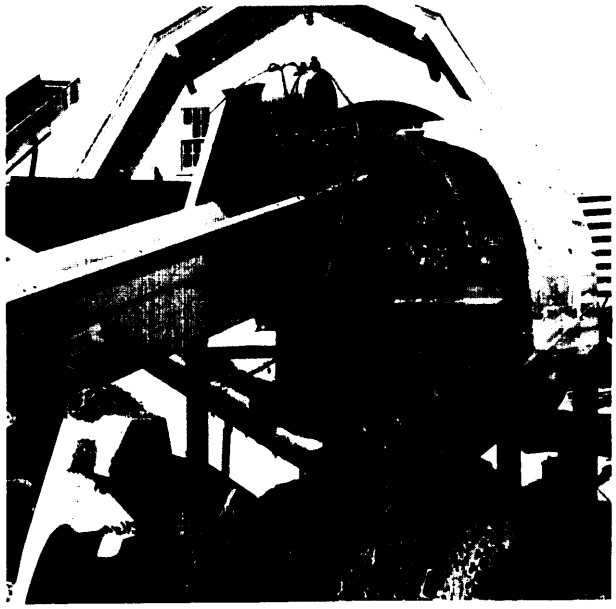


Photo credit: Courtesy of H. D. Bruhn and R. J. Koegel

Figure 3.— MINIPRESS MI IB.

Photo credit: Courtesy of N W Price

Pressing

The performances of the double screw, single screw, and belt presses used in leaf protein preparation in British research and a commercial crop drying plant were evaluated by Shepperson, et al. (68). Screw presses are effective machines for removing juice from the macerated forage, but their energy consumption is higher than that of some other press designs due to rubbing and shearing action in the press. industrial screw presses are expensive and less suitable for mobile installation (fig. 3). Platen presses have been used in small or medium installations and can have a capacity of 1.8 to 3.6 MT/hr. The power consumption is low. Belt presses were found to have limitations in the forage dewatering process because maximum pressure is limited by the nature of belt tensions. If new synthetic belts could be produced, this system could be built as relatively light equipment. Several presses for LPC production were described by Pirie (61) (fig. 4). A commercially available cone press was evaluated by Koegel, et. as. (51) and gave satisfactory results for final moisture content and energy requirement. A new press was designed with a capacity to press 16.5 MT/hr of freshly macerated material to a final moisture content of 65 per cent or less. The weight of the press is 3.1 kg. The results of the evaluation of the cone press for forage fractionation were reported by Straub and Koegel (73), who suggested some changes in cone rotation speed. The average total energy required for the press was low; 0.95 KWh/MT. The sum of energy requirements for macerating alfalfa and pressing it in the cone press is about 3.25 kWh/MT (Nelson et al, 1981). Energy

Figure 4.—Cone Press

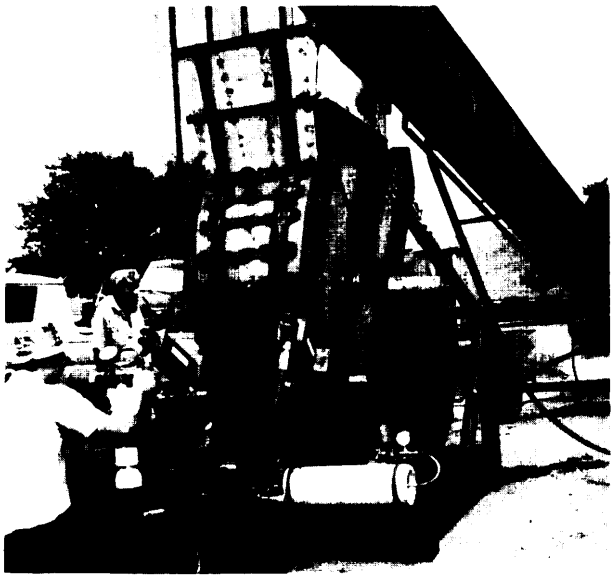


Photo credit: Courtesy of H. D. Bruhn and R. J. Koegel

requirements for maceration and pressing of freshly harvested forage are compared in table 6.

Professor Bruhn (14) converted meat grinders into good, medium, and miniature size systems for laboratory evaluations to replace Waring blenders (fig. 5). The high-speed electric blenders do not duplicate industrial crushers; they chop the fibers into small particles, which will be mixed into the green leaf protein concentrate during the heat-coagulation process.

Separation of Protein Concentrate

Juice, if not directly fed to animals, should be processed without extensive delay. Separation of protein is accomplished by several methods (table 7); however, the most convenient is coagulation by steam injection. An automatic system was developed by Straub, et al. (75), to coagulate the juice and separate the coagulated protein from the brown juice. The incoming juice was preheated by a heat

Table 6.—Energy Requirements for Producing Leaf Protein Green Juice

Process	kJ/kg	hp/h/ton	Reference
Field harvesting			
Direct cut and chop	3 - 7*5	1 - 2.5	ASAE Yearbook, 1975
Maceration			
Extrusion	7.5 - 30	2.5 - 10	Basken et al., 1975
Roll crushing	15 - 90	5 - 30	"
Hammer milling	42 - 150	15 - 50	"
Hammer milling	16.5	5.5	Carroad et al., 1980
Pressing			
Screw press	6- 30	2 - 10	Basken et al., 1975
Roll press	15 -30	5 -10	"
High-cycle platen	15 - 30	0.5 - 1	"
Cone	---	1.14	Bruhn and Koegel, 1982

SOURCE: Compiled by Telek, 1983

Figure 5.— Rebuilt Meat Grinder as Miniature Screw Press

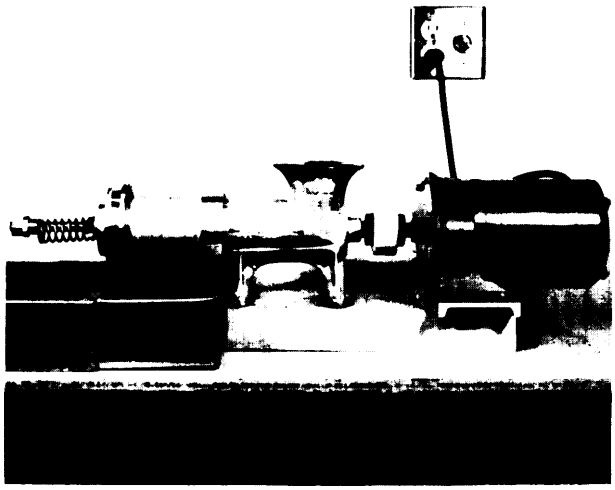


Photo credit: Courtesy of H. D. Bruhn

exchanger, salvaging the heat from the brown juice. The generally accepted energy requirement is 50 kg of steam/MT green crop.

Low-cost and simple technology systems for leaf protein separation and recovery using on-farm level operations have been reviewed by Straub, et al. (74). A farm-scale centrifuge designed to separate suspended solids from animal waste was evaluated. It had a slower than expected acceleration rate and the through-put rate was low: 5 kg/rein. Other systems were designed based on flotation, consisting of a stainless steel tank (0.25 m x 0.36 m x 2.64 m) with a working capacity of 211 liters and a built-in steam injector. The flow rate was 42 l/rein with a hold time of approximately 5 minutes for flocculation in the tank. The mechanical skimmer was rotated at a speed of 2.3 m/min, and the paddles were immersed in the tank to 5 cm below the spillway (fig. 6).

The belt filtration system consisted of an 8.5-m long continuous woven polyester belt with a vari-

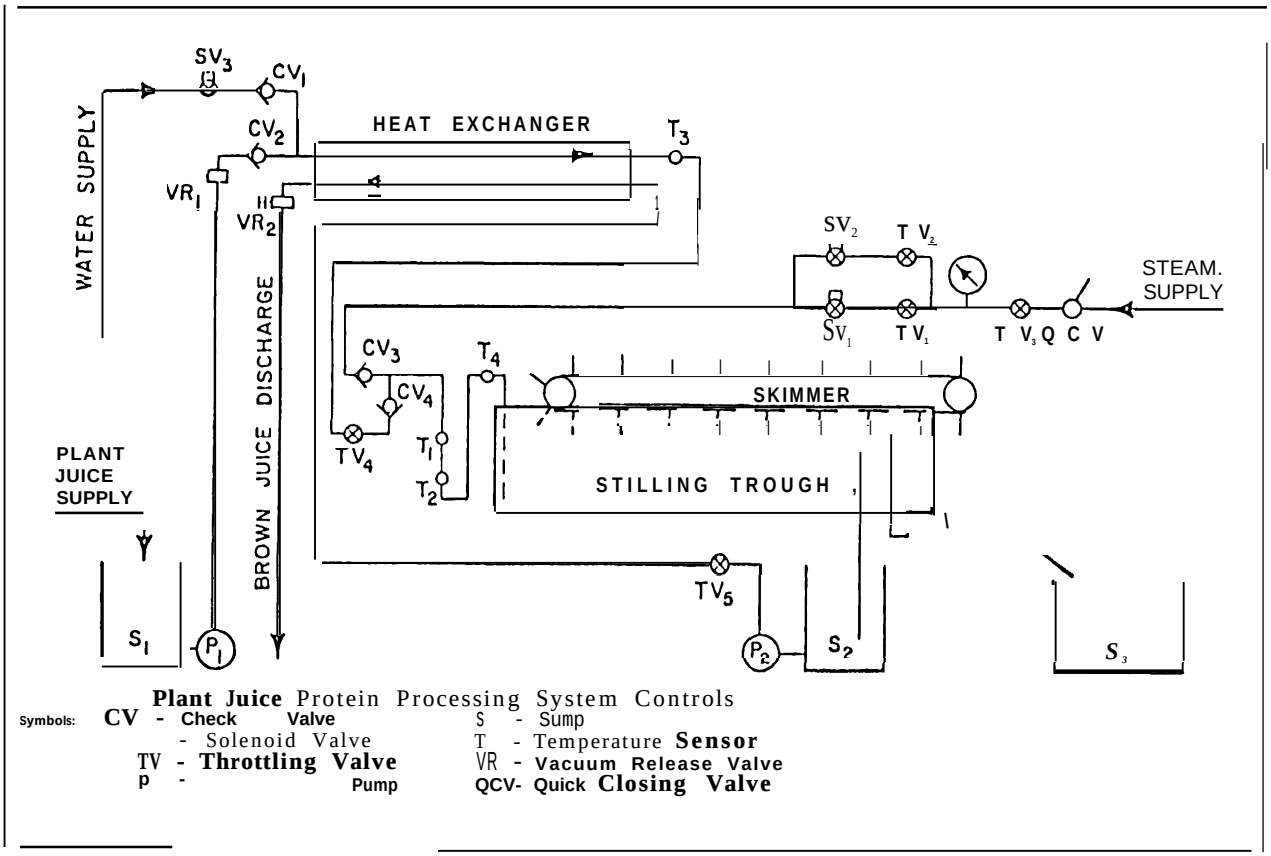
Table 7.—Separation Methods of LPC From Green Juice

	Treatment	Fractions	Reference
HEATING	not measured	green and white	Rouelle, 1773
	80°-840 C	green	Pirie, 1971
	60°and 84°	green and white	Edwards et al., 197!
	55° , 64° , 82°	green and two whites	Telek, 1979
ACID		green	Pirie, 1971
ORGANIC SOLVENTS	ethanol acetone	green	Huang, 1971
	n butanol	green	Allison, 1973 in Hove and Bailey, 1975
ANAEROBIC FERMENTATION		green	Stahman, 1978
FLOCCULANTS		green	Anelli et al., 1977 Knuckles, 1980

EEN JUICE

SOURCE: Compiled by Telek, 1983

Figure 6.— Flotation Separator



SOURCE: Straub, et al, (75).

able moving speed of 3.0 to 15.2 m/min. (fig. 7). The belt filter was fitted with a 0.25-m long x O, S-m x 0.3-m tank to allow for flocculation of the juice protein. This provided an average 1- minute hold time for the heat treated juice prior to being spilled onto the traveling belt by a paddle wheel assembly which rotated at a rate of 20 rpm. There was an initial free drainage section on the belt. This was followed by a vaccuum box dewatering section. The material then was scraped off to pans by a spring-loaded doctor blade (fig. 8). The evaluation of this process showed that flotation provides good recovery. However, the protein concentrate was dilute; it was less than 12 percent solids. The filtration provided moderate levels of solids, but had poor recovery rates. Use of flotation as pretreatment to belt filtration provided improved recovery and moderate solid concentration. Mean solid levels of proteins separated by various methods are shown in table 8. The results using this relatively complex machinery are somewhat disappointing, and further refinement is needed. In an on-farm operation, such as hog raising where a wet product can be used, this solid level would be acceptable. The protein concentrate could be mixed with barley or cassava chips for immediate use or partly extruded and sun-dried for short duration storage.

LPC Extraction at Village Level

The most basic application of leaf protein fractionation is at the village level. The operation is simple. Production should be geared to consumption by farm animals to avoid preservation and storage problems, The system, using the pulper and press developed at Rothamsted Experiment Station, England, and purchased by donors, has been studied in Pakistan (66), India, and Sri Lanka.

In a rural resettlement of people from urban slums in Pakistan, a trial has been suggested to test-market LPC produced by this machinery for dairy

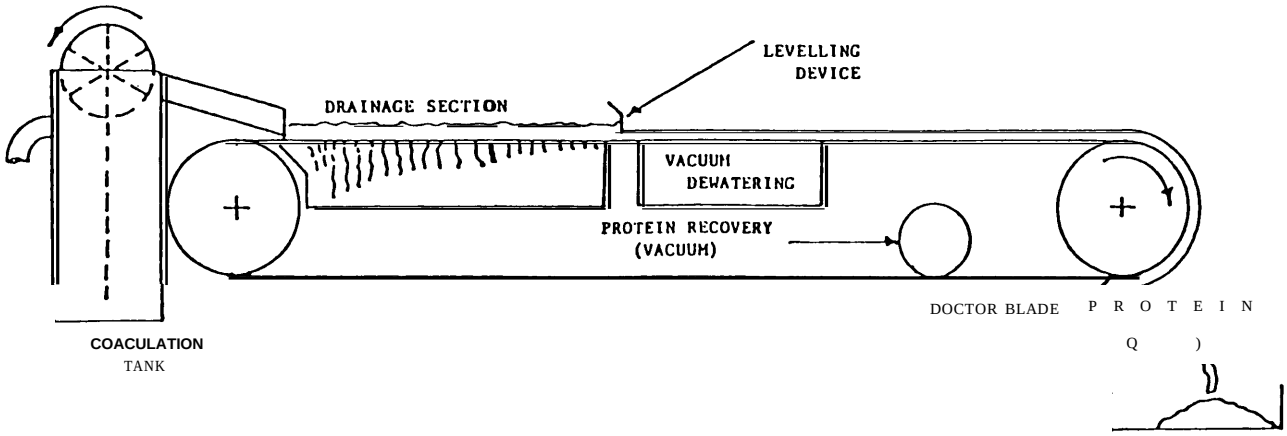
Table 8.—Mass Distributions, Suspended Solid Distribution and Resulting Protein Solid Levels for Flotation, Belt Filtration, and Combinations

	Separation Process*				
Mass Distribution (% of total)	Decant	Drainage	Dewatering	Recovery	Protein
Floatation & belt filtration	42.5	8.9[a]	20.4	3.0	25.2[a]
Floatation & belt drainage	42.5	8.9[a]	-	-	48.6[b]
Floatation	42.5	-	-	-	57.5[c]
Belt filtration	-	46.1[b]	23.1	3.3	27.7[a]
Recoverable Solids (% of total(% absolute)					
Floatation & belt filtration	0.5(0.05)	0.7(0.37)	1.5(0.37)	5.7	91.6[a]
Floatation & belt drainage	0.4(0.05)	0.6 0.37	-	-	99.1[b]
Floatation	0.4(0.05)	0.6(0.37)	-	-	99.6[b]
Belt filtration	-	1.1(0.15)	0.9(0.23	5.5	92.5[a]
Solid Content (%)					
Floatation & belt filtration	-	-	-	9.7	18.4[a]
Floatation & belt drainage	-	-	-	-	12.4[b]
Floatation	-	-	-	-	11.4[b]
Belt filtration	-	-	-	10.5	20.5[a]

* Differing superscripts indicate statistical difference of 95% C.L.

SOURCE: Straub, et al. (75).

Figure 7.—Belt Filtration



SOURCE: Straub, et al. (74)

Figure 8.—Belt Filtration

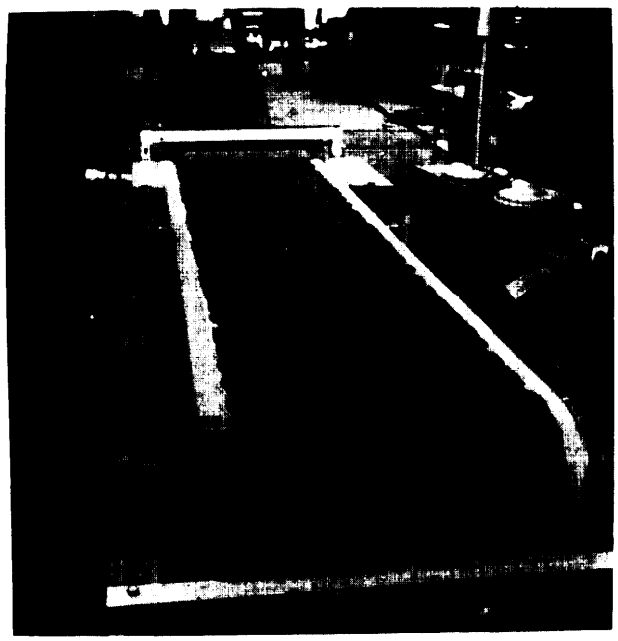


Photo credit Courtesy of F/. D. Bruhn and R. J. Koegel

cattle and green LPC for local sale. The labor force would be recruited from the resettled families under the supervision of trained personnel.

Indian and Pakistani scientists are cooperating in the leaf protein work. Dr. Shah of the Pakistan Council of Scientific and Industrial Research visited Mysore, Coimbatore, and Aurangabad to learn of the Indian progress in implementing village-level LPC production.

The work in Aurangabad, India, is the most relevant to practical application of any in progress. It attempts to establish a commercially viable LPC production unit at a village farm, using a simplified screw press that Pirie (62) has been developing for a number of years (fig. 9).

This press accomplishes both cell rupture and juice expression in a single press. A similar unit was built in the workshop of Marathwada University. The press is driven by a 3-HP motor. The juice is drained over a vibrating screen, then precipitated in a thermostatically controlled oil bath. The coagulum is filtered through cloth stockings (fig. 10). The locally constructed equipment could be scaled up according to need (18).

Joshi, et al, (42), reviewed the prospects and problems of leaf protein production on a small farm in Bidkin, an Indian village about 25 km from Aurangabad. The green protein concentrate made from alfalfa was used as a milk replacer for calves, as poultry feed, and as human food. The pressed crop was remixed with solubles and fed immediately to cows. They accepted the material willingly, however, they rejected it when offered it the next morning. Equipment and a dairy unit of 5 to 6 cows would cost at least $4,450 (Rs 35,500*), an excessive expenditure for a small farmer. Joshi suggested that, in the immediate future, both of the products of green crop fractionation be sold in the market. This project was supported by the Meals for Millions Foundation, and its economics were evaluated by Bray (13).

A flow chart for the process is shown in figure 11. After the crop is pulped, it is pressed to yield green juice and fiber. Heating the juice yielded 25

*The exchange rate of the Indian rupee (Rs) is calculated at Rs 7.8 per $U. S.

Figure 9.-Simplified Screw Press

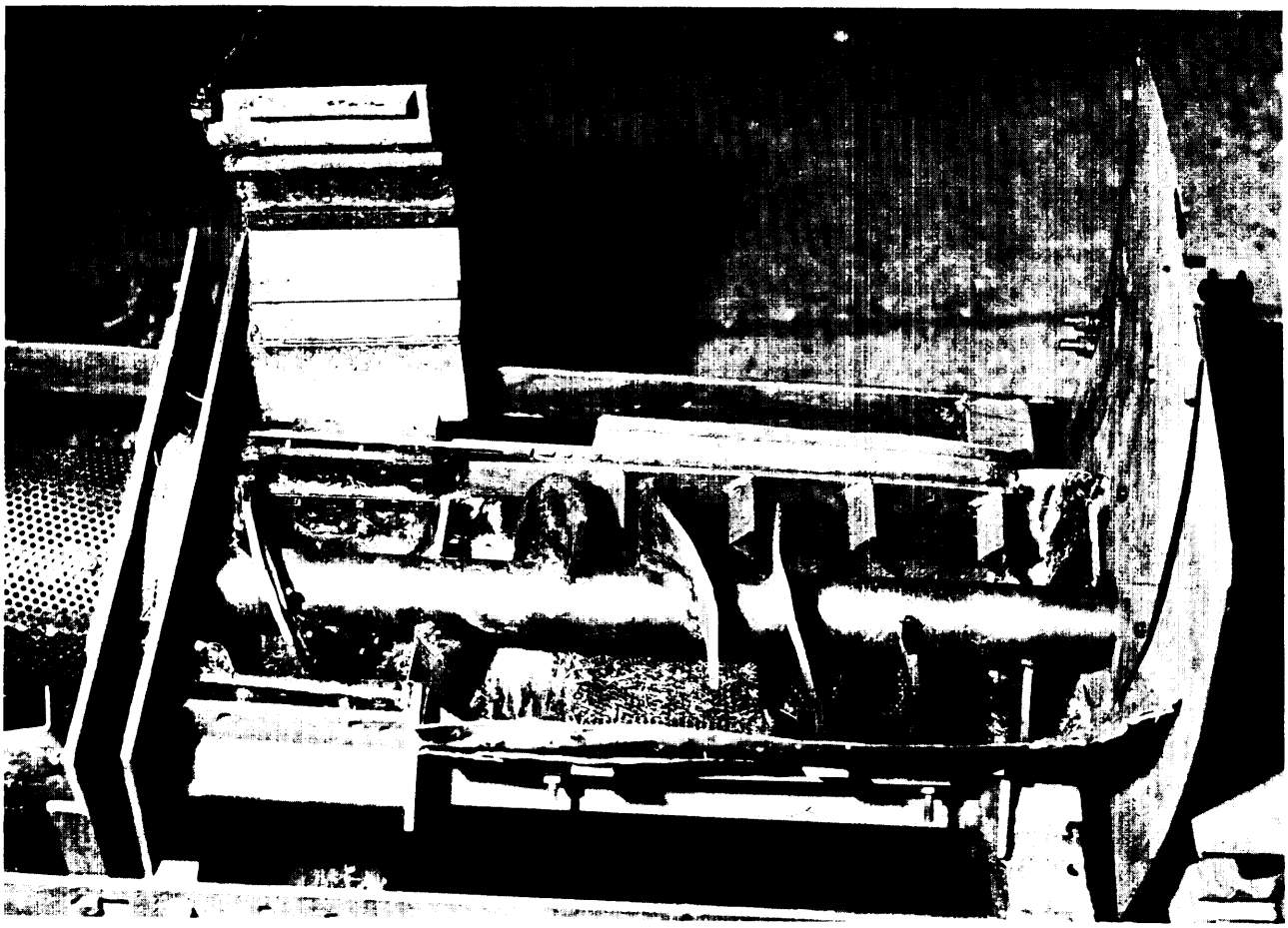
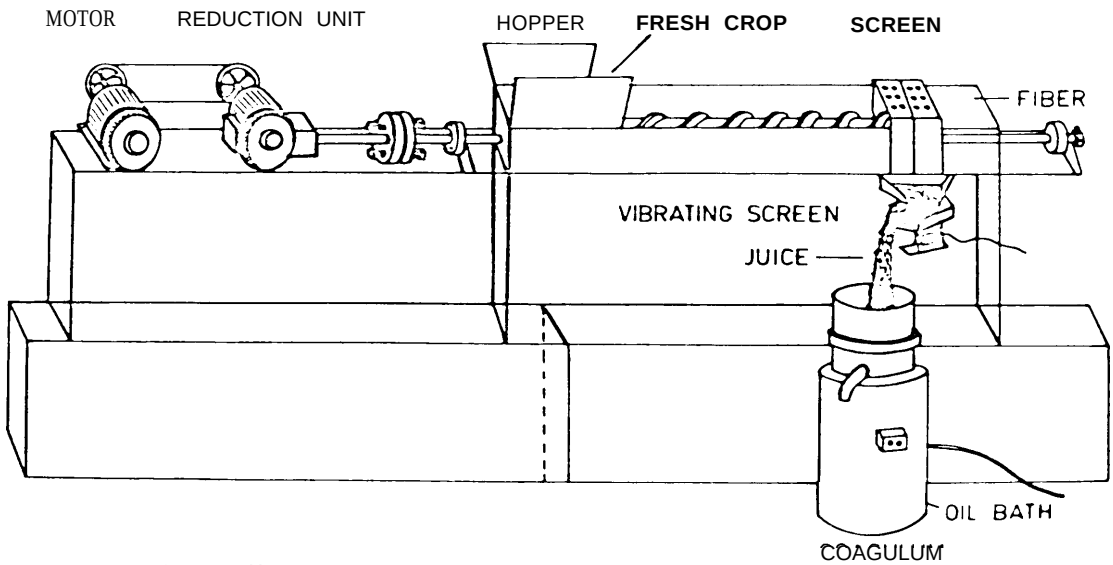


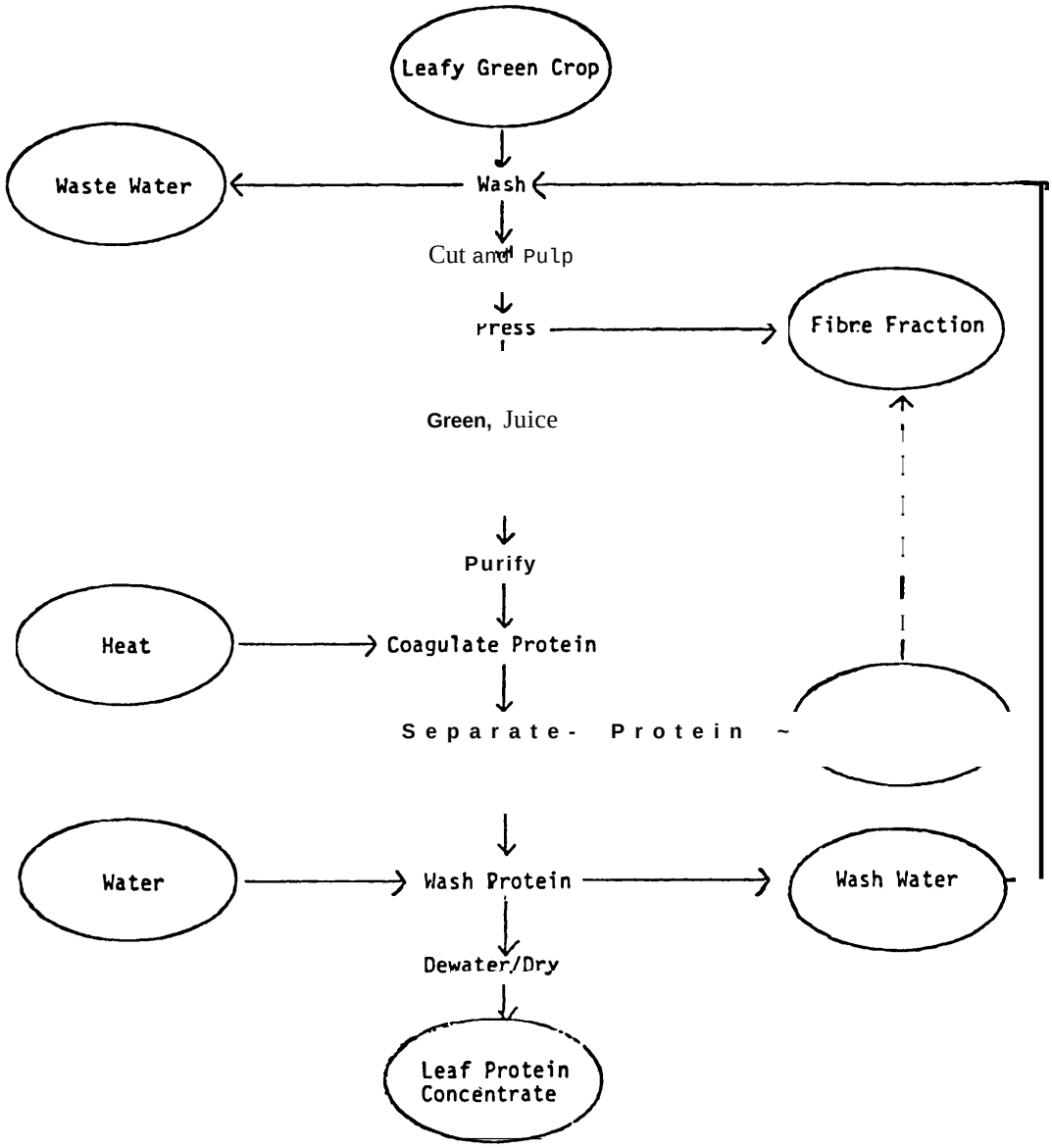
Photo credit: Courtesy of N W. Pirie

Figure 10.-Screw Press Designed and Built in Aurangabad, India



SOURCE Courtesy of R. N. Joshi

Figure 11.— Process Flow Chart of Leaf Protein Fractionation on Village Level in India



SOURCE: Bray (13)

kg LPC from about 1 MT of freshly cut alfalfa. In a-variation of this process, the pulped crop is extracted with the solubles from the protein precipitation phase, to yield an enriched fiber fraction for feeding ruminant animals. The leaf protein concentrate—containing 60 to 65 percent protein, 22 to 24 percent lipids, and carotenoids (pro-vitamin A)– could be consumed by people or nonruminant anireals, or used as a milk replacer for calves. The input requirements for this process are indicated below.

Equipment Costs

The cost for both processes was calculated at about $3,200 (table 9).

Land

Since the extraction process requires about 1 MT of fresh alfalfa per day, the produce from about 2 ha would be needed to keep it operating continuously.

Table 9.— Equipment Costs for LPC Preparation on Village Level in India

Wash Tank	**Rs 1500**
Cutter	**1540**
Pulper	**5500**
Dewatering press	**4500**
Juice pump	**1160**
Liquid Cyclone	**350**
Waste Receiver	**80**
Holding Tank	160
Heater/Coagulator	1700
Curd Filter	100
Beam Press	**900**
Holding Tank	320
Mixing/Wash Tanks	160
Beam Press	**900**
Total Cost	Rs **18870** **($2,359.)**
Installation 25%	4720
Contingency 10%	1890
	Rs 25480 ($3 ,185.)

SOURCE: Bray (13).

Crop Cost and Income

Current costs for alfalfa vary widely. With an expected yield of about 100 MT of fresh alfalfa/ha/yr, a farmer would receive $1,600, an income higher than he would get raising other crops.

Labor Requirements and Salaries

For an 8-hr day, the salary of the supervisor is $1.50, $0.99 for operators, and $0.75 for helpers.

Electric Power

The power required is **5.6** kW for pulping and **6.75** kW for pressing at $0.034 per kWh.

Fuel

The fuel is low quality coal that would cost about $0.002/kg LPC. The production cost per kg LPC is summarized in table 10.

Suggested retail price for 1 kg LPC would range

Table 10.—Leaf Protein Production Cost Summary at Village Level in India

	Process 1 Pressing)
Raw material (net cost)	Rs 1.02/kg LPC
Labor and supervision	1.72
Power	0.58
Fuel	0.13
Maintenance	0.66
Supplies, etc.	0.29
	Rs 4.40 = 55¢
Depreciation	0.91
Interest expense	0.60
Other fixed charges	0.25
Total fixed costs	Rs 1.76 = 22¢
Total production cost	Rs 6.16/kg LPC = 77¢

SOURCE Bray(13)

from $l,19 (Process 2) to $0.79 (Process l) Leaf protein probably would be incorporated into a final food product, then sold. For example, in the Coimbatore feeding program, LPC was mixed with cassava flour and sugar and fed to children as a soft sweet mixture called a laddu. If a similar product containing leaf nutrient concentrate were to be sold in the market, it could be priced at $0.50 per kg. For only $0.025 to $0,03 per day, a child could obtain 50 percent of his daily protein, iron, and calcium and 100 percent of his vitamin A from such a product.

Conclusions

The following conclusions are drawn from this study (13):

1. A green crop fractionation/leaf protein unit could be easily operated in a village.
2. The cost of the equipment is low enough to be affordable by village cooperatives.
3. On a nutritional basis, leaf protein would be much less expensive than most of the protein from grain legumes consumed in the area.
4. An LPC-containing product that would provide 50 percent of the daily protein requirement of a child would be affordable by the majority of the Indian poor.

In Coimbatore, Friesian, and Jersey cows are consuming the pressed crop, and in preschool nurseries children are getting the LPC in laddu, a food item developed by Dr. Devadas, which consists of a mixture of leaf protein with jaggery (a crude sugar made from the sap of a palm tree), cassava flour, pearl millet flour, and sesame seed molded into soft balls. It was fed to the children as a snack. The object of her research was to evaluate the nutritive value of LPC through feeding programs for 600 preschool children for a period of 3 years.

On-Farm Use of LPC

The basic principle of leaf protein fractionation is that some plants, mostly the Leguminosae, contain much higher levels of protein than are necessary for ruminant nutrition. This protein can be removed without negatively affecting the growth of animals. On the other hand, nonruminants are unable to digest high contents of cellulose and cannot consume the amount of dry matter required to satisfy their protein requirements. Using protein fractionation, plant material can be separated into one product suitable for nonruminants and another suitable for ruminants. Experimental proof indicates that the process can double meat production in a given area **(35).**

The concept of on-farm use of leaf protein fractionation is that a weather-independent system can be devised in which the processing takes place on a farm and at least one of the products is used at the production site. The ideal situation would be a combined dairy and hog production farm using all of the products grown onsite, thus reducing transportation expenses, storage costs, and spoilage. This approach has been researched in Britain, Australia, and the United States.

The research team of the University of Wisconsin, under the leadership of Professor Bruhn, contributed the most in developing new concepts in machinery designs for this system (14). Research at the University of Wisconsin at Madison has concentrated on development of a weather-independent, on-farm forage harvesting system using a protein fractionation process. After harvesting, the main product is pressed forage, which can be preserved directly as silage. The prime objective is a quick reduction in the moisture content of the fresh forage from approximately 80 to 65 percent, a desirable moisture concentration for proper fermentation in a silo (31). By this process, field losses can be minimized to about 2 percent, a reduction from **32** percent or higher when the crop is preserved as baled hay. The pressed residue contains **50 to 80** percent of the dry matter of the original green crop. It retains 70 to 80 percent of its original protein content, which is substantially higher than that of sun-dried hays.

Use of Pressed Residue

Use of the pressed residue is the key factor in leaf protein fractionation. The maceration will make the fibers more digestible by ruminants. In sheep feeding trials, the pressed crop from either alfalfa or ryegrass was equally effective when fed freshly pressed or as silage (80,81). In steers fed a perennial ryegrass and Italian ryegrass mixture, the mean intake of whole and pressed crops was equal, and there was no significant difference in weight gain (40). The liveweight gains in cattle fed the pressed crop of perennial ryegrass were found to be significantly higher than those of cattle grazed on whole ryegrass *ad libitum (35).* Other research concluded that pressed residual can be fed directly to ruminants with as good results as the original nonfractionated plant (59).

Use of the Juice

The DM content of the green juice is low (8 to 10 percent). The isolation of green protein is an expensive process. The most economical use would be to feed it directly to hogs to minimize storage and preservation expenses. The green juice contains proteins, carbohydrates, and lipids. Enzymes present in juice degrade proteins rapidly, especially during warm days (69), and the soluble carbohydrate fraction ferments in 24 hours (4). Therefore, the process must be geared to the feeding time of the animals. Houseman and Connell (34) effectively replaced separated LPC and conventional seed proteins and dried LPCs by direct feeding of grass juice.

According to Braude, et al. (11), grass and alfalfa juice can replace half of the protein supplement (soybean or fish meal) in the feed of growing pigs. However, feeding experiments carried out in Wisconsin gave disappointing results. Pigs did not consume alfalfa juice even when their drinking water was withheld.

The most economical method for isolating proteins from green juice would be by anaerobic fermentation *(1,49,72).* Bacteria normally present on leaves fermented juice samples of many different plants (alfalfa; corn; oats; pangola, elephant, brome, and sudan grasses) in sealed containers. The initial pH of 5.5 to **6.0** dropped to 4.5 after 48-hour fermentation. Amino acid analyses of the fermented and spray-dried juice protein showed that it contained 40 percent more cystine and 12 percent more methionine, The protein yield was *11* percent lower than that prepared by heat precipitation. However, pigs' acceptance of feed containing the fermented product, especially in high proportions, was always low. An important need exists for animal nutritionists to convert the fermented LPC product into a palatable swine feed.

Industrial Production of LPC

In contrast to the village use concept stressed for LPC research, a serious investigation of process development for commercial LPC production was initiated in 1967 at the Western Regional Research Center of USDA in Berkeley, California. A large number of papers have been published on every phase, including use of the different products. A highly mechanized process evolved which is covered by several patents (8,10), Several reviews describe the development of the Pro-Xan process, the commercial production method for obtaining leaf protein concentrate from alfalfa (22,23,52). Figure 12 describes the flow sheet of the process.

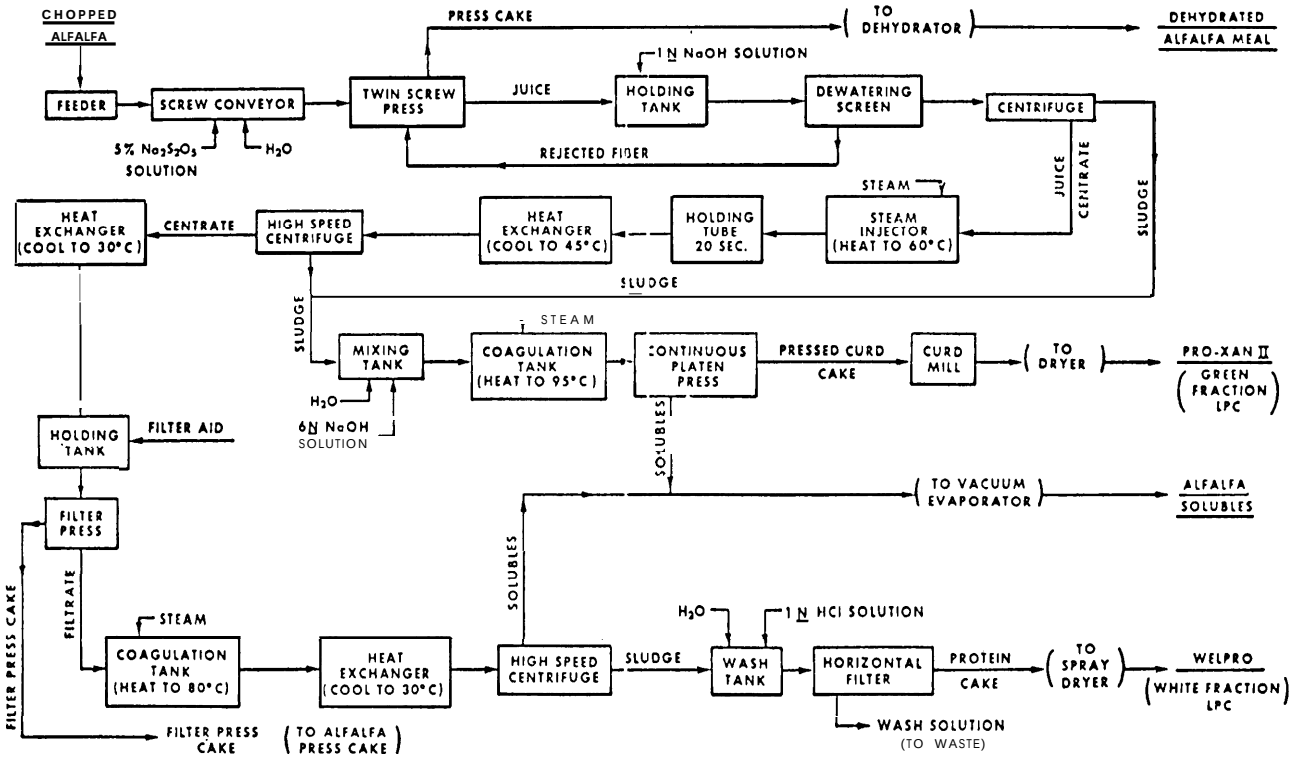
The original process has undergone several changes. Energy saving and pollution reduction were the main targets of process research; improved machinery was selected to accomplish these goals. The economies of producing Pro-Xan were studied by the USDA Economic Research Service and updated after major process changes (28,82).

The first commercial application of the Pro-Xan process was by France Lucerne, the largest producer of alfalfa dehydrators in Europe. The first pilot plant was constructed next to a dehydrator and produced 2 MT/day of green protein concentrate. In this process the pressed residue is added to the pelleted, dehydrated alfalfa. Also, the concentrated solubles are recycled to the pelleting operation. The success of the operation lies in the central location of the plant in Champagne, the largest alfalfa growing region in France; its well-organized sales operations; and the barge canal, rail, and highway connections available for shipping its products economically. A larger plant was completed in 1981 and 7,000 MT of green protein concentrate Pro-Xan are being produced yearly. France annually produces 900,000 MT of dehydrated alfalfa. Sixty percent of this is produced and marketed by France Lucerne in Champagne.

Alfa-Laval and France Lucerne agreed to sell two 600 MT/day production plants to the Soviet Union. The process is based on Pro-Xan technology modified by France Lucerne using alfalfa as plant material (3).

In conventional dehydration, alfalfa containing 20 to 22 percent dry matter is chopped, transported immediately to the plant, and dehydrated to 90 to

Figure 12.—The Pro-Xan Process used by the Valley Dehydrating Co.



SOURCE: Edwards, et al (25)

92 percent dry matter in the rotary drier. This process generally produces a product higher in protein content than hay or silage produced from the same field, without the losses occurring during hay making and ensiling. Dehydration is less weather dependent than the other forage conservation techniques and is highly mechanized. The final dehydrated product is generally in the form of pellets which can be easily handled.

In the United States, the production of dehydrated alfalfa increased from 285 MT in 1944-45 to over 1,542,650 MT in 1969-70. In early 1970, a sudden decline in production occurred primarily due to competition of foreign producers. Later, the steady increase of energy cost and new air pollution standards decreased the profit margin in dehydrating. Many plants were closed and production fell below 1,181,818 MT.

Wet fractionation of freshly harvested crops can reduce the energy requirement because the pressed forage contains about 50 percent less water to be evaporated per MT of final produce. The latent heat from the dryers' exhaust gases was recycled for use in the evaporation processes, resulting in less air pollution.

The Valley Dehydrating Company (VDC), Sterling, Colo. (fig. 13) converted one of their existing plants to Pro-Xan production. The Department of Energy (DOE) supported the conversion as a means of demonstrating the efficient energy conservation possibilities in alfalfa dehydrators. With this funding, the research efforts of the USDA Western Regional Research Center, U.S. Department of Agriculture at Berkeley, Calif., could be realized in a new LPC production plant in the United States. The process equipment and operating parameters have been described in several publications (23,24).

The results of a detailed study of this company by Edwards, et al. (25), were published as a DOE report. The exceptionally high level of technology associated with this industry can be seen in table 11, which shows the considerable cost investment for conversion of a conventional 18 MT/hr dehydration plant to a 36 MT/hr Pro-Xan plant. Because of this high investment, it would be prohibitive to construct a similar factory in the humid tropics without previous extensive studies of available plant material at the on-farm level,

During the experimental period, the plant operated at 13.6 to 21.8 MT/hr, and produced an average

Figure 13.—Aerial View of the Valley Dehydrating Co., Sterling, Colo.

Photo credit: Courtesy of R. H. Edwards

LPC yield of 12.8 percent (dry basis). The VDC plant consumed 25 percent less total energy. Based on the experience at VDC, future LPC plants are projected to reduce overall energy consumption by 35 percent. The VDC products have been marketed readily; the press cake has been sold to cattle feeders at a price equivalent to dehydrated alfalfa, and the LPC to a broiler producer at prices varying from $391 to $530/MT. Animal performance trials using VDC produced products were highly satisfactory. Projected current cost of a new LPC plant processing 36 MT of chopped alfalfa per hour is $4.7 million (table 12); the cost of converting an existing 18 MT/hr dehydration plant to a 36 MT/hr LPC plant is estimated at $3.6 million. The calculated rate of return on investment for the new plant was 12.0, 26.2, and 40,4 percent for operating seasons of 130, 180, and 230 days, respectively (table 13).

The Vepex process was the first large-scale direct production method for LPC. It was built as a separate industrial unit not associated with a dehydrating industry. Its primary purpose was to maximize production of plant protein both for the fodder industry and for human nutrition. The process can also use raw material other than alfalfa.

Another essential feature is use of the deproteinized brown juice (33). Some of the nitrogen compounds present in the green juice are not precipitated by heat treatment, The soluble N in the deproteinized residual brown juice can amount to 30 to 40 percent of the total N content in the green juice. This can be used as substrate for feed grade yeast production.

A flow sheet of the Vepex process is shown in figure 14. Vepex plants are located in Denmark and Hungary. The plant in Tamasi in southwest Hungary is temporarily closed to make energy-saving improvements,

In Britain, the BOCM-Silcock Co, studied the preparation of dry leaf protein concentrates on a pilot plant level with the aim of creating a system to provide reasonably priced protein for feed in the form of storable products. The dry matter and pressed crop had to be at least 14 percent, a limit set by the European Common Market (78). The flow

Table 11.— Basic Equipment Requirements for a Modified Pro-Xan Plant Processing 36.3 Mg (40 t) of Fresh Alfalfa Per Hour

j@ Item	Specifications	No. req'd	Total Connected power, kW (hp)
Forage harvester/chopper .,	self-propelled , 12 ft header	4	(1/
Truck	22 ton, tandem axle, 34 ft bed , diesel powered.	4	1/
Truck	3/4 ton pickup	2	1/
Truck scale	60 ton	1	
Feeder	40 tons/hr		29.8 (40)
Wet grinder	10 tons/hr	1	298.4 (400)
Screw press	single screw, 20 tons/hr	4	179.0 (240)
Hydrasieve	6 ft wide	1	— --
Steam injector	3 in. dia.	2	
Centrifuge	decanter type , 80 gal/rein.	2	85.8 (115)
Drier, Pro-Xan with recycle system	triple pass, 6,000 lb H_2O/hr	1	44.8 (60)
Pellet mill, Pro-Xan	1-1/2 torls/hr	1	30.6 (41)
Bucket elevator, Pro-Xan	1-1/2 tons/hr	1	1.5 (2)
Pellet cooler, Pro-Xan	1-1/2 tons/hr	1	11.7 (15-3/4)
Scalper screen, Pro-Xan	1-1/2 tons/hr	1	0.4 (1/2)
Inventory scale, Pro-Xan	1-1/2 tons/hr	1	— --
Bucket elevator, Pro-Xan	1-1/2 tons/hr	1	1.5 (2)
bad-out bin, Pro-Xan	1280 cubic feet	2	— --
Waste heat evaporator, (3 stage, 2 effect) with cooling tower	40,000 lb H_2O/hr	1	173.1 (232)
Drier, press cake with recycle system	30,000 lb H_2O; 185°F	1	122.3 (164)
Grinder, dried press cake	9 tons/hr	1	224.9 (301-1/2)
Bag filter, press cake	9 tons/hr	1	23.5 (31-1/2)
Pellet mill, press cake	9 tons/hr	1	233.1 (312-1/2)
Bucket ● levator, press cake	9 tons/hr	1	2.2 (3)
Pellet cooler, press cake	9 tons/hr	1	30.6 (41)
Scalper screen. press cake	9 tons/hr	1	0.4 (1/2)
Inventory scale, press cake	9 tons/hr	1	23.5 (31-1/2)
Pneumatic transfer system, press cake	9 tons/hr	1	
Load-out bin, press cake	3328 cubic feet	4	
Boiler, with economizer	400 boiler horsepower	1	41.0 (55)
Air compressor	36 SCFM	1	7.5 (lo)
Pumps 2/	various , to 200 gpm	6	31.7 (42-1/2)
Conveyors , wet product	various, to 40 tons/hr	12	55.6 (74-1/2)
Tanks, with agitators	various , to 10,000 gal.	6	3.4 (4-1/2)
Well	250 gal/rein	1	14.9 (20)
Heat exchanger.	600 sq . ft. , shell and tube	1	
Waste water treament	unspecified	. .	37.3 (50)
		Tot al	1708.5 (2290.25)

~/ Not applicable.
~/ Does not inclued pumps *in* evaporator installation.

SOURCE: Edwards, et al. (25).

Table 12.—Investment Costs for a Revised Pro-Xan Plant with a Capacity of 36.3 Mg (40 t) of Chopped Alfalfa Per Hour

Item	Investment Cost, dollars
Equipment, harvesting and hauling	504,400.
Equipment process plant	2,793,349"
Buildings	282,500
Land[3]	46,800
Engineering and installation cost[4]	1,117,340
Total	4,744,389

1 Based on May 1982 prices.
2 Buildings include space for all operations except long term bulk storage which is treated separately.
3 Six acres
4 Assumes 40 percent of cost of process plant equipment

SOURCE. Edwards, et al (25)

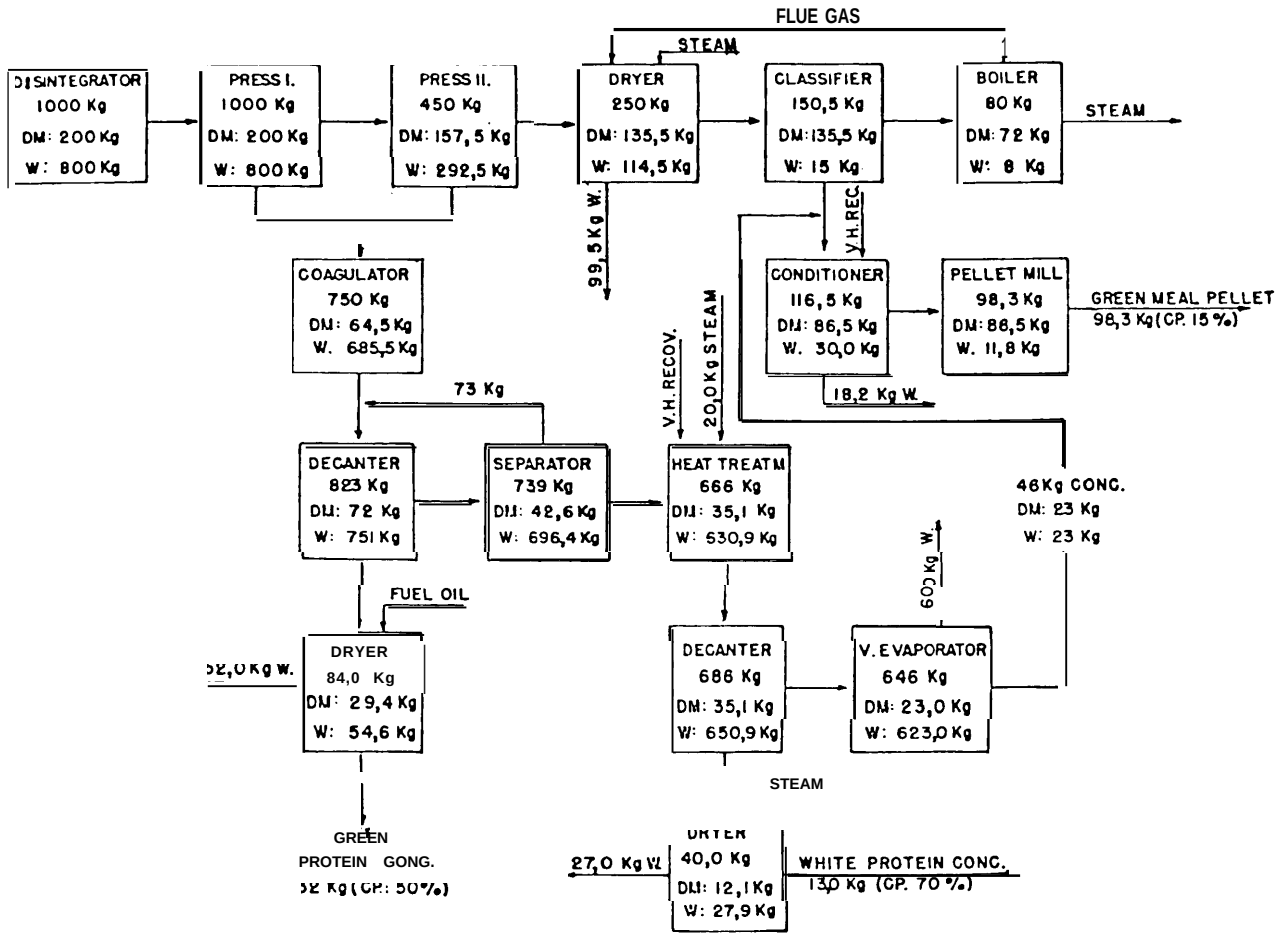
Table 13.—Annual Operating Costs, Revenues, and Return on Investment for the Revised Model Pro-Xan Plant

Item	cost (dollars) at season length (days) 130	180	230
Annual Revenues			
Dehydrated press cake	**2,645,261**	**3,662,669**	4,580,078
Pro-Xan	**1,803,023**	**2,496,494**	3,189,964
Total Revenues	**4,448,284**	**6,159,163**	7,370,042
Annual Costs			
Alfalfa, raw material	766,480	**1,061,280**	1,356,080
Chemicals	60,126	**83,251**	106,377
Natural gas	516,663	**715,377**	914,094
Electricity	177,663	**245,995**	314,327
Fuel and Oil	131,560	**182,160**	232,760
Maintenance and repairs	407,923	**451,923**	495,923
Labor	182,520	**252,720**	322,920
Administration	71,100	71,100	71,100
Property taxes	19,926	19,926	19,926
Insurance	54,676	54,676	54,676
Interest	499,491	537,027	574,563
Depreciation	336,317	336,317	336,317
Storage costs	143,162	198,225	253,287
Marketing costs	73,626	101,944	130,261
Transportation costs	436,304	604,113	771,922
Total costs	3,877,537	4,916,034	5,954,533
Total **earnings**	**570,747**	1,243,129	1,915,509
Total Investment	**4,744,389**	4,744,389	4,744,389
Annual return on investment (%).	12.0	26.2	40.4

1 Plant capacity 36.3 Mg (40 *tons*) chopped alfalfa (22 percent dry matter) *per* hour.

SOURCE:Edwards, et al. (25)

Figure 24.—Material Flow in Vepex Process



SOURCE: Koch (48).

sheet of this LP fractionation process is shown in figure 15.

'When the full-scale plant was built, it required a growing area of **480** ha within an 8-km haulage radius. To operate at full capacity, the plant had anticipated producing lye-treated straw in the idle season. However, the straw feed was not acceptable in Britain, and the new plant was shut down after a brief interval of operation because the short production period made it inefficient to operate (79).

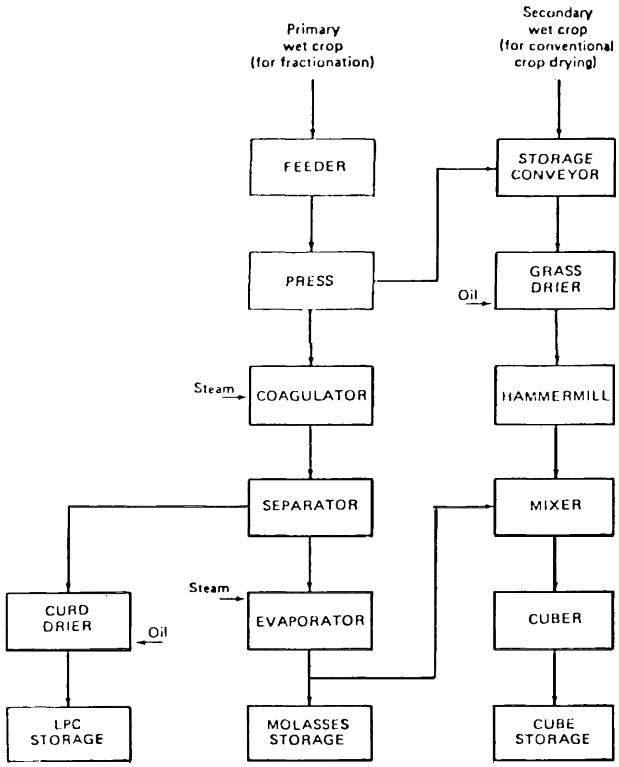
In New Zealand, Alex Harvey Industries, Ltd., in cooperation with the Ministry of Agriculture and Fisheries and the Broadlands Lucerne Company, is planning to establish a plant to produce a leaf protein concentrate with 47 to 50 percent crude protein content and high levels of xanthophyll and carotene pigments. This will be used for poultry feeding, A high fiber pellet for ruminant stock will also be produced. The plant will have a capacity of 10 MT/hr of green alfalfa and be operated by geothermal steam (67).

Two pilot plants managed by agricultural cooperatives began operation in Japan in *1981.* Each processes 2.7 MT/hr of fresh herbage. One of them has facilities to separate LPC by centrifugation, condense green juice by reverse osmosis, condense brown juice by heating, cultivate yeast using brown juice, and make water from fibrous residue (58).

Preparation of Colorless Edible Proteins From Alfalfa

The processing of LPC to obtain a food-grade product was reviewed by Bray (12). Green LPC can be produced economically. However, some problems exist in human acceptance of protein concentrate prepared by heat precipitation at 820 C: its green color, grassy flavor, and low volubility,

Figure 15.—The BBOCM-Slicock Green Crop Fractionation Process



SOURCE Thrlng (78)

A step in preparing improved protein in larger quantities by solvent extraction has been reported. Ineritei (38) found that freshly prepared coagulum could be decolonized with an acetone and isopropanol mixture. The treatment improves flavor and texture and gives a prolonged shelf life to the light cream-colored product by removing the lipids. The lipid distribution in green LPCs prepared from four tropical plants was analyzed by Nagy, et al. (55). The sale of lipid components, especially xanthophyll and B-carotene, and solvent recovery with solar power in the tropics could decrease the cost of this process.

Solvent extraction does little to improve the nutritional value of LPC. The tannin-damaged leaf protein concentrates are not or are only slightly improved nutritionally, Only a small amount of adsorbed tannins can be removed (Ii').

In the heat fractionation process, after isolation of the green protein at 55° C, in many plants two additional white protein fractions can be separated: one at 64° C and another at 820 C (77). The white protein fraction prepared from alfalfa is nutritious, with a protein efficiency ratio (PER) similar to that of casein (9).

The heat coagulated proteins are practically insoluble. It was suggested that they could be used in soups, gravies, cheese, and cookies (7). Food technologists for industrial application require certain functional properties for proteins. If leaf proteins could be processed to possess the desired properties, they would have wider use in the food industry.

Since solvent extraction of undried green protein has been costly and only partially effective and the white protein prepared by heat precipitation is insoluble, radically different and more complex procedures for producing soluble white and bland-tasting protein have been initiated.

In diafiltration, water is added to clarified alfalfa juice during ultrafiltration so that small molecular weight components can be washed through a membrane. On a laboratory scale, diafiltration, after a mild heat treatment and centrifugation for clarification, resulted in a freeze-dried product that was cream-colored and highly water soluble (46).

Pilot-scale ultrafilter units were tested by Knuckles, et al. (44) for concentrating and purifying soluble alfalfa leaf protein solutions after coagulating the protein at 60" C. Operating temperature was generally maintained at a low 100 C to avoid microbial growth and precipitation of the heat labile protein. The clarified alfalfa juice was concentrated to one-tenth of original volume, producing protein concentrates containing about 50 percent crude protein and 10 percent ash. Using diafiltration, water was added to the concentrated alfalfa juice until the permeate volume was 10 times the original sample volume. This method resulted in material containing 70 to 76 percent protein, Dried protein products were tan colored despite the removal of more than 86 percent of the ortho dihy - droxy phenolic compounds.

The ultrafiltration systems tested by Knuckles and his coworkers cannot produce light cream-colored protein concentrates of greater than 90 percent purity. Because of their high cost and ineffectiveness, they are not viable methods for large-scale purification of alfalfa protein.

Flocculants are used to remove suspended solids from solution, Knuckles, et al. (47) reported their work with 54 commercial flocculants tested to improve the separation of the green chloroplastic protein fraction from alfalfa juice. With a l-percent level of cationic flocculent, the chloroplastic fraction was separated by continuous high-speed centrifugation. Residual sediment was less than 0.5 percent; however, the processing rate was low (11.41/min). This technique also proved to be effective as pretreatment in membrane filtration. The

treated juice yielded greater soluble protein content than the untreated control,

Knuckles and Kohler (43) prepared soluble leaf protein concentrates (light tan colored). According to the authors, these should be acceptable as a food source. However, no cost data is given. Gel filtration was previously used for fractionation of LPC by Fishman and Burdick (29) in characterization of protein of Coastal Bermuda grass proteins. The use of filter gels such as Sephadex is costly in even analytical processes. Freeze-drying is also a very expensive process because the special equipment is expensive and requires high energy input. Special proteins such as active enzymes may be prepared by this method for biochemical or medicinal purposes. Comparing quality and price, the proteins separated by gel filtration cannot compete with the Fraction I protein of tobacco, which can be prepared by crystallization and is water soluble. Dr. Wildman will discuss this unique protein in complete detail.

Environmental and Cultural Aspects

Nature and tradition have created richly variable cultures in tropical areas. It is difficult to design a general plan for a leaf protein extraction system for the entire tropical zone. It is evident that the whole system has to be geared to the natural and cultural local environment: the climate, physical location, soil fertility, and local cultural habits. Two-product use is a key to the viability of the LPC process. Ruminants must remain in the chain of protein production to use the pressed residue. In countries such as India, dairy animals or goats will be used on a smaller scale operation than that involving beef cattle in developed countries. The green LPC could be fed to humans or to calves as a milk replacement, saving milk for human consumption. In Islamic lands, cattle, lambs, goats, and rabbits could be fed the pressed residue, and chickens and ducks the green concentrate. In Latin America, the green juice could be fed to pigs and chickens, and the pressed residue to beef or dairy cattle.

The increasing protein shortage in the tropics cannot be alleviated effectively by village scale production of LPC, especially if it is used only for infant feeding. At the First International Conference on Leaf Protein Research held recently in Aurangabad, India, there were arguments regarding infant feeding trials between the representative of the bilateral and multilateral donor agencies and the recipient of grants for a children's feeding trial on one side and a highly respected local scientist on the other. The scientist claimed that "There is no doubt that leaf protein is good for protein and carotene nutrition. But its use in children feeding trials is considered unethical, purposeless, and unscientific. It is argued that the scope of the pigmented leaf protein in food is limited to the individual family or to communities of no social and economic disparities, Its production and use as a means for overcoming the protein and carotene deficiency in human nutrition is not a practical proposition" (70).

Advocates of infant feeding claim that infants respond favorably to feeding formulas containing alfalfa LPC. This claim could be contested. A protein-depleted infant would respond rapidly to any proteinous feeding. The effect of prolonged infant or child feeding of crude alfalfa green protein has not been properly evaluated. The following facts are disturbing. It is well documented that saponins of alfalfa impede the growth of chicks *(16).* Its tannin-phenol complexes are not digestible; their interaction with digesting enzymes of infants can be damaging, The biologically active coumestrol present in alfalfa was found in leaf protein concentrate (45). In the growth of children, the possibility of ill effects caused by the physiologically active ingredients of alfalfa green protein after prolonged feeding must be recognized. The dietetical value of the touted laddu, a product of high sugar content, also remains questionable. The large sum of research money spent did not produce basic data on possible long-term undesirable effects of the use of alfalfa LPC and did not reduce substantially the ever-increasing number of ill-fed children,

LPC as an emergency food for humans, as was suggested by Pirie for England during World War II, should be considered, Experimentally, it has proven to be nutritious; however, the author is against human use of LPC, especially that of alfalfa, because of incomplete testing and the possible presence of antinutritional factors. The small-scale production of LPC should not be rejected entirely. In extreme poverty, it could be incorporated as a protein source into native dishes. However, for human consumption of LPC, plants should be selected from local green leafy vegetables such as collard, mustard green, and other brassicas.

Pirie (63) suggested at the Belo Horizonte meeting in Brazil: "Except for infant feeding, nothing could be gained by extracting protein from leaves that can be eaten as green vegetables. The best way to use LP to improve the nutrition of unweaned infants is to give it to the mother rather than the infant, A broad-minded approach to bot-

any is needed; the present occupation with alfalfa is unfortunate. ”

Advocates for LPC feeding for children cite the importance of carotenoids in diets of ill-fed children. LPC could supply these but carrots, tomatoes, and green leafy vegetables could also supply children with the needed carotene.

It is obvious that a farm’s topography and size will be decisive factors in determining its ability to produce LPC. Small hamlets and subsistence farms will not be able to participate in LPC production, On small farms in the mountainous areas of the tropics, pods of winged beans and leafy vegetables such as collards, mustard greens, amaranthus, or even spinach could be additional sources of proteins and carotenes to supplement the family’s daily consumption of beans and corn. Bananas or plantains, cassava, or yams are staples in such areas. It is difficult to believe that the wife of a struggling farmer in the Tropics will harvest leaves, pound them, filter the juice, and prepare a coagulum for food. Alternate sources of protein might be more acceptable to the low-income farmer. For example, the breeding of rabbits could be popularized on small farms. Harris, et al. (30), proved that dried tropical leaves, even those of cassava which are unfit for LPC production, supported the growth of rabbits and gave good results.

In a proper leaf protein preparation process, all products must be effectively used and not wasted. Since deproteinized juice cannot be used in small village units for yeast production, it should be used to irrigate fields. The fractionation process to white proteins would be a prolonged procedure with small yield for feeding trials. Similarly, the use of complicated purifications by ultrafiltration and gel filtration would be impractical.

The quality of life in rural areas of less developed countries has to be raised; however, a single, small industry like the bicycle-driven LPC production system used in India and involving a $3,000 investment will not substantially help alleviate the problems (fig. 16),

More moral and material support should be given to the plan of Joshi (41), which is a healthy transition to the on-farm use system. He suggested that in India LPC production could be incorporated into a small, cooperative dairy farm to improve animal husbandry for selected cows and to increase milk production, This would be practical, Dairy cooperatives are being used successfully in Europe, where the milk is collected for central processing and distribution.

Figure 16.—Screw Press Operated by Human Power in India

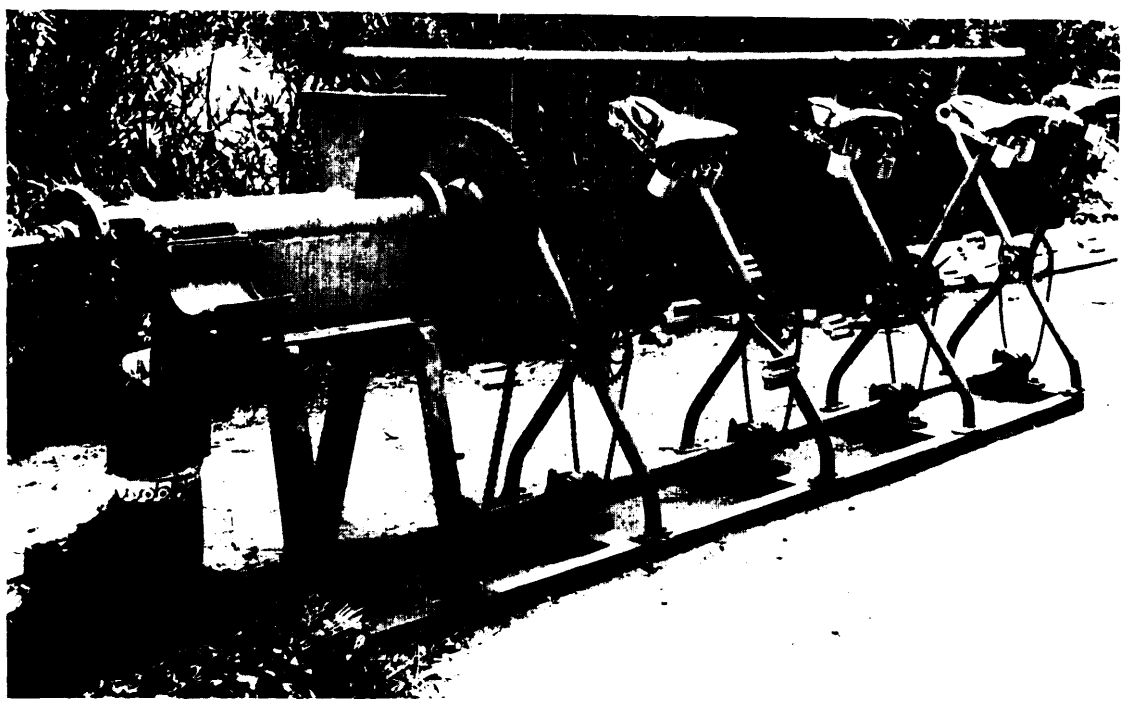


Photo *credit: Courtesy of R. J. Joshi*

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The on-farm use system has been studied only with alfalfa and ryegrass. Using legumes, these model experiments could be duplicated in tropical countries, especially in Latin America where agricultural practices are well developed, land is available, and relatively simple technology could be successfully adapted to individual needs. The introduction of this type of system into areas of sugarcane production would reduce the acreage given to that crop, an economically sound decision, There is the possibility of substituting leaf protein for high protein feeds, such as soybean meal, in poultry and hog protein rations which are now imported into tropical areas.

As discussed in the on-farm use section, a production system of medium capacity is probably the level that will have a tangible effect on the protein resources within an area without disturbing its environment. There is no waste; the animal manure can be returned to the fields to improve the soil, and some of the pressed juice can be used for irrigation. The production facilities could process plant material contracted from neighboring farmers. A higher income level would result from more intense agriculture, which easily could be adapted to the existing conventional system. This size of agro-industry will not put a strain on electricity. The processing machinery could use small tractors as a source of power, It will not require a large amount of water, and only simple tools are needed for maintenance.

Research Needed

Leaf protein fractionation would increase the protein production of a given area universally. The basic farm equipment has been designed and properly evaluated, and existing machinery can process any fresh green crop with proper N level and extraction. The production level should be selected according to the needs of an ecosystem and modified according to the traditions and religious bias of the local population. Suitable plants should be studied in more detail, possibly using a medium-scale experiment at the production site. However, before expansion to the Pro-Xan type of operation can be contemplated, it is imperative that long-range studies be made of potential plant material at the on-farm use level.

USDA research centers and U.S. land-grant universities and tropical research centers in developed countries should have sufficient funds for the remaining basic and applied research needed for LPC extraction. An international cooperation between U.S. institutions and host institutions funded by AID should be developed for applying the research results in countries in the Western Hemisphere that would benefit from agricultural development based on LP extraction. A parallel program should enable scientists from host countries to learn the chemical and physical methods of production and quality control of raw material and finished products.

The host country scientists would offer the necessary data required for successful implementation. The following salient points must be carefully investigated by local scientists before a system and site are chosen:

1. Market research to determine need and acceptance of products
2. Availability of suitable land
3. Likelihood of undisturbed flow of plant material for processing.

The next phase of investigation should be nutritional evaluation of LPC products with large and small animals: cattle, milk cows, goats, rabbits, pigs, and chickens, The preparation of proper feed mixes should not be neglected. It is not sufficient to prepare a nutritionally balanced feed mixture; it should be readily acceptable by the animal and should have good keeping qualities,

Farm-level processing machinery has been well designed for disintegration and pressing. Only that used for the separation of green protein concentrate needs more investigation to obtain simpler and more effective equipment. An inexpensive basket centrifuge would probably be useful in this step of the process,

Well organized on-farm use of LPC could provide a thorough evaluation of tropical plants using medium-size technology and pave the way for the development of large-scale production of LPC—especially in tropical countries where advanced technology and sufficient amount of capital are available, such as Brazil, Puerto Rico, and Venezuela. After evaluation of plant sources on a farm scale, a commercial-size production facility is more likely to succeed.

While the capital requirements for large-scale production are high, the return on investment is also likely to be high because of the low labor costs and year-round operating season. The effect of the length of the operating season on return on investment is illustrated in table 13. The use of government subsidies to set up cooperative, commercial-scale plants might help initiate the new industry (26). Additional research funds should also be allocated to study low cost, more efficient extraction, dewatering, separation, and evaporation tech-

niques that are suitable for large-scale LPC production from tropical plants studied previously and selected from intermediate-size studies.

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