Potential Food Uses for Protein from Tropical and Subtropical Plant Leaves

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Protein deficiency is one of the most widespread nutritional disorders in underdeveloped tropical countries. The potential usefulness of tropical and subtropical leaves as a source of supplemental protein is discussed. Protein contents of 60 leaves and potential protein values of grasses and aquatic plants are compared. Preparation, composition, nutritional value, and flavor acceptability of several leaf protein fractions are discussed.

Prevailing rates of population growth and current patterns of population dispersion threaten the health and welfare of many peoples of the world. It took 33 years, from 1927 to 1960, for the world's population to increase from 2 to 3 billion, and from 1960 to 1976 to increase to 4 billion. By the year 2000, with a projected population of 7 billion, some 80% of the world's population will live in Asia, Africa, and Latin America. The average population growth rate is 2.5% for developing countries and 1.0% for developed countries (Prager and Duncan, 1976). Those dissimilar growth rates forebode problems related to nutrition and food supply in the countries that can least accommodate population growth.

The United Nations World Food Conference (1974) estimated that 460 million of the world's people are malnourished and cited protein deficiency as the commonest form of malnutrition. Cereal grains, which are a dominant food in the malnourished and undernourished two-thirds of the world, contain proteins that generally are deficient in the essential amino acids, threonine, tryptophan, lysine, and methionine (Kaul, 1975). In Latin America, legume seeds (specifically, beans) are the most important protein sources. Economically disadvantaged people, living on subsistence agriculture, cannot afford high-quality protein derived from fish, animals, and animal products (milk, eggs).

In the developing tropical countries, protein deficiency is widespread because diets generally are deficient in quality and quantity of protein, especially in areas where cassava is the staple food. Importation of high-grade animal products, cereal grains, and processed legume seed proteins has partially alleviated malnutrition in those regions. Continued importation of protein foods, however, imposes considerable economic strains on these countries' financial resources. In tropical countries capable of limited agricultural investigations, efforts have largely focused on improving the use of arable lands, development of high-yielding crop varieties, improving indigenous cultigens (corn, beans, rice, cassava), eliminating crop monocultures, evaluating uncommon edible tropical leaves (Terra, 1964; Martin and Ruberte', 1975; Oliveira and De Carvalho, 1975; Martin et al., 1977), and studies on extractable leaf proteins from tropical leaves (Scientific Research Council of Jamaica, 1965; Valli Devi et al., 1965; Telek et al., 1978). This symposium paper is addressed to the general subject of proteins from leaves of tropical plants.

HISTORICAL

The detection of leaf proteins dates back to 1773 when Rouelle demonstrated the formation of a coagulum when leaf extracts were heated (translation of Rouelle's article from Pirie, 1971). Although the value of leaf protein was recognized in animal nutrition during the intervening years, research on the extractability of proteins from leaves and on the preparation of a leaf protein concentrate (LPC) remained virtually dormant until the 20th century. Investigations by Osborne and Wakeman (1920) and Chibnall and Schryver (1921) on laboratory-scale extractions of leaf proteins were soon followed by patentable processes for separating large quantities of proteins from green plants (Ereky, 1926; Goodall, 1936; Slade and Birkinshaw, 1939). Leaf protein research was given its greatest impetus by Norman Pirie of Rothamsted Experimental Station, England. In the early war years of the 1940's, Pirie recognized the potential of leaf proteins for human consumption to help mitigate a protein shortage if there were a blockade of England and to ease a general wartime food shortage (Pirie, 1942, 1975a). After the war, research efforts devoted to development of human foods from leaf proteins waned considerably. Interest into this potential food source, however, was rekindled in 1964 with the establishment of the International Biological Programme (IBP) under the auspices of the International Council of Scientific Unions (Pirie, 1975b). One of the seven sections of IBP was concerned with the use and management of biological resources. That section was entrusted with the responsibility for the development and evaluation of novel protein sources. Under the guidance of IBP, investigations into tropical leaf proteins became international in scope and were conducted in Jamaica, Nigeria, Ceylon, and India. Progress was made on the determination of compositional and nutritional values of tropical leaf proteins, but the research was not complete when IBP was terminated in 1974. Fortunately, research on this novel and valuable tropical protein source is still conducted in India, Pakistan, Nigeria, Mozambique, and Venezuela and by the USDA (Mayaguez Institute of Tropical Agriculture, Puerto Rico; U.S. Citrus and Subtropical Products Laboratory, Winter Haven, Fla.; Western Regional Research Center, Berkeley, Calif.).

COMPARATIVE EFFICIENCIES OF PROTEIN PRODUCTION

Green leaves of plants are the original source of man's protein; however, the direct consumption of leaves to satisfy man's protein requirements is not practical. Although leafy crops contain carbohydrates, salts, proteins, lipids, and vitamins beneficial to man, they also contain large amounts of fiber, pigments, and other indigestible components which adversely affect palatability and digestibility. Confronted with this dilemma, man has satisfied most of his protein needs by consuming plant storage proteins (tubers, seeds) and animal proteins. The translocation of protein from leaves into plant storage tubers is often coupled with the production of a storage

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Table I. Yearly Crude Protein Production for Some Selected Leaves and Seeds and Potential Yields of Extractable Protein from Leaves

protein source	kg of protein/ha	yields of protein (kg/ha) based on extractability of		
		30%	40%	50%
leaves				
alfalfa (*Medicago sativa*)	2690,[a] 3000,[b] 4483[c]	807-1345	1076-1793	1345-2242
sorghum × sudan grass hybrid (*Sorghum sudanesis*)	2320[a]	696	928	1160
Transvala digitgrass (*Digitaria decumbens*)	2179[d]	654	872	1090
Tetrakalai (*Phaseolus aureus*)	1760[e]	528	704	880
seeds (oil, cereal, legume)				
soybean (oil)	510,[f] 785[a]			
wheat (cereal)	392[a]			
cowpea (legume)	330,[g] 204-485[h]			
urd bean (legume)	171-338[h]			

[a] Stahmann, 1968. [b] Singh, 1967. [c] Kohler et al., 1976. [d] Sotomayor-Rios et al., 1976. [e] Bagchi and Matai, 1976. [f] *Food Ind. S. Afr.*, 1975. [g] FAO, 1961. [h] Jeswani, 1975.

protein that is so diluted with carbohydrates that diets based on those storage tubers must be supplemented with high-protein foods. The biological process by which leaf protein is converted into animal protein for human consumption is expensive and inefficient. It is estimated that approximately 90% of leaf protein and/or plant storage protein fed to domestic animals is used in maintenance and only 10 to 15% of the original protein is recovered as animal products suitable for human consumption (Stahmann, 1968). Even though many tropical leaves are digestible, palatable, and relatively free of toxic substances (Martin and Ruberte', 1975), humans would have to eat an inordinate amount to satisfy their protein requirements.

More efficient utilization of leaf protein is achieved by mechanical processes (Morrison and Pirie, 1961; Pirie, 1971; Koch, 1973; Kohler, 1974; Kohler et al., 1976) in which the protein is extracted from the leaf and used for food, and the remaining fibrous residue used as silage for cattle, sheep, and other ruminant animals. This process for silage production has an enormous potential in areas where part of the year seasonal rains provide lush grazing but during the remainder of the year drought-like conditions exist. In many tropical regions animals produce well and gain weight during the rainy season but loose much of this weight during the following dry season when feed is scarce. Grazing animals also show slow and disproportionate development during these dry months (Oyenuga, 1959). Hence, it often takes twice as long to raise cattle to market size in the tropics as in temperate regions where preserved forages can be fed as hay or silage. The feed value of palatable silage (Oelschlegel et al., 1969; Stahmann and Whitaker, 1977) produced by the LPC process could markedly increase animal production in tropical countries where it is difficult to dry and store forage for use during the dry season. An additional advantage of the LPC process is that leaf protein coagulated from the juice can be used as a human food supplement.

Comparative examination of protein production by leaves and seeds (Table I) shows that leaves produce more crude protein than seeds. The percentage of protein expressed from leaves varies considerably depending upon species, horticultural practices, and on the mechanical process employed. Pirie (1975c) estimated that approximately 40 to 60% of protein in a forage crop, e.g., alfalfa, can be extracted. However, protein extraction values as high as 60 to 80% were reported in species of *Amaranthus* and *Chenopodium* (Crewther, 1976). Table I shows the potential yields of crude protein for four kinds of leaves at theoretical extraction efficiencies of 30, 40, and 50%. Crude protein is a measure of a combination of soluble protein, insoluble protein, and nonprotein nitrogen components (e.g., peptides and free amino acids). It is the soluble protein fraction which will yield edible protein fractions. An interpretation of data from Kohler and Knuckles (1976) suggests that about 575 kg of edible protein could be extracted from alfalfa containing 4000 kg of crude protein.

PROTEIN CONTENTS OF TROPICAL AND SUBTROPICAL LEAVES AND LEAFY VEGETABLES

In the tropics, green plants grow wherever soil and water conditions are favorable. The year-round availability of high amounts of sunlight and the agronomic potential for multiple cropping portend abundant protein yields from tropical leaves. As a protein source, leaves may originate from (a) leafy vegetables, (b) by-products of crops grown for commerical purposes, (c) cover crops and fodders, and (d) plants especially cultivated for their high protein contents and/or high percentage of protein extractability.

The crude protein contents of 60 tropical and subtropical leaves are reported in Table II. We selected leaves that have crude protein contents (as a percentage of dry matter) greater than 20%. Many tropical leaves not shown in Table II because of the 20% limitation also have been evaluated for their protein contents (Byers, 1961; Valli Devi et al., 1965; Scientific Research Council of Jamaica, 1965; Pirie, 1971; Hall et al., 1975). Leaves with protein values lower than 20% should not be unalterably rejected as potential sources of LPC because many show favorable properties of multiple cropping, protein extractability, and the relative absence of toxic components. Ultimate selection would depend on several factors, including the agronomic factor, i.e., protein produced/area of land or unit cost.

Table II shows that the protein contents of most of the selected tropical leaves were within the range of 20–30%. Nineteen leaves had protein contents in excess of 30% and the highest was Coriander (60.8%). Sixteen leaves, viz., cowpea, cassava, chaya, hyacinth bean, amaranth, horse bean, castor bean, okra, purslane, sweet potato, pigeon pea, scarlet bean, Ceylon spinach, Jack bean, cocoyam and cup panax, were identified as potential candidates for LPC studies by Martin et al. (1977), Telek et al. (1978), and the authors. The following is a brief description of five of the most promising leaves.

Vigna sinensis, the cowpea, a twining woody vine that climbs to tops of trees and shrubs, is normally grown as an annual. As a source of fodder and edible seeds, the cowpea probably originated in Central Africa but is now widely distributed throughout the tropics. The young leaves, which are widely consumed as cooked greens, are very nutritious. The cooked greens possess a strong odor

Table II. Crude Protein Contents of 60 Tropical and Subtropical Leaves (Protein Contents Expressed as Percentage of Dry Matter)

scientific name	common	% protein
30% and above		
Coriandrum sativum	coriander	60.8[a]
Amaranthus gangeticus	amaranth[i]	57.8[a]
Atriplex rosea	chakothra	57.3[a]
Ricinus communis	castor bean[g]	41.3,[c] 36.9[d]
Cucurbita maxima	pumpkin	34.7[d]
Trigonella faenumgraceum	methi	32.6[a]
Momordica charantia	balsam pear	32.5[c]
Physalis angolata	ground cherry	32.0[b]
Vigna sinensis	cowpea[g]	31.8,[c] 31.0,[d] 28.0[b]
Manihot esculenta	cassava[g]	31.6,[c] 26.8,[b] 24.1[d]
Crotalaria juncea	sun hemp	31.5[b]
Solanum nodiflorum	lumbush	31.4[f]
Corchorus olithorus	jute mallow	31.3[f]
Physalis peruvianum	peruvian cherry	31.2[b]
Cnidoscolus chayamansa	chaya[h]	30.7[e]
Talinum triangulare	surinam spinach	30.5[f]
Vernonia amygdalina		30.2[f]
Clitoria ternatea	butterfly pea	30.1[b]
Amaranthus viridis	calalu	30.0[d]
25 to 30%		
Leucaena leucocephala	lead tree	29.2[d]
Lablab niger	hyacinth bean[g]	29.0[b]
Colocasia esculenta	taro	28.8[b]
Hibiscus esculentus	okra[g]	27.6[b]
Desmodium uncinatum	Spanish clover	27.6[b]
Canavalia ensiformis	horsebean[g]	27.3[b]
Portulaca oleracea	purslane[g]	27.3[c]
Centrosema pubescens		27.2[b]
Stizolobium niveum	velvet bean	26.8[b]
Indigofera subuluta		26.8[d]
Ipomoea batatas	sweet potato[g]	26.7[b]
Aspilia latifolia	bush marigold	26.6[b]
Desmodium spp. ?		26.2[d]
Brassica chinensis	popchow	25.8[d]
Phaseolus lathyroides	scarlet bean[i]	25.4,[b] 23.4[d]
Sesbania grandiflora	sesbania	25.3[a]
Samanea saman	guango	25.2[d]
Xanthosoma spp. ?	Indian kale	25.0[d]
20 to 25%		
Sida acuta	broomweed	24.8[d]
Sechium edule	chayote	24.4[c]
Brassica oleracea	cauliflower	24.3[d]
Morus alba	mulberry	23.9[c]
Glycine max	soybean	23.8[a]
Solanum ficifolium	susumber	23.6[d]
Dolichos lablab	bonavist bean	23.4[d]
Cajanus cajan	pigeon pea[g]	23.3[c]
Basella rubra	Ceylon spinach[i]	23.3[c]
Canavalia ensiformis	jack bean[g]	23.1[c]
Xanthosoma sagittifolium	cocoyam[i]	22.6[d]
Nothopanax scutellarum	cup panax[g]	22.4[c]
Boehmeria nivea	ramie	22.1[d]
Peperomia pellucida	peperomia	21.6[c]
Melicocca bijuga	genip	21.4[d]
Brassica oleracea	cabbage	21.2[d]
Musa paradisiaca	banana	20.9,[c] 19.3[a]
Lactuca sativa	lettuce	20.9[d]
Erythrina berteroana	dwarf coral tree	20.4[c]
Fleminga strobilifera	wild hops	20.2[d]
Murraya koenigii	curry leaves	20.1[a]
Bambusa vulgaris	bamboo	20.1[d]
Alysicarpus vaginalis		20.0[d]

[a] Valli Devi et al., 1965. [b] Pirie, 1971. [c] Hall et al., 1975. [d] Scientific Research Council of Jamaica, 1965. [e] Telek et al., 1977. [f] Bassir and Fafunso, 1976. [g] Suggested as a possible source of LPC by Martin et al., 1977. [h] Suggested as a possible source of LPC by Telek et al., 1978. [i] Suggested as a possible source of LPC by authors.

but the finished dish has a mild flavor (Martin and Ruberte', 1975).

Manihot esculenta, cassava, which has been cultivated as a food plant since prehistoric times by the natives of Brazil, is a perennial, vigorous, soft-wood shrub in the tropics. Its principal food value (starch) resides in the large fleshy roots, which grow in a cluster at the base of the stem. The leaves of cassava may be used as greens. The horticultural advantages of cassava are its ready propagation from stem cuttings, rapid growth, leaf yield/hectare and drought resistance.

Cnidoscolus chayamansa, chaya, which was included in the diet of ancient civilizations in the Yucatan Peninsula of Mexico (Telek et al., 1978), is a perennial, vigorous

Table III. Crude Protein Content of Tropical Forage Grasses Harvested at Three Intervals Over a 2-Year Period[a]

species	PRPI[b]	30 days: crude protein as % of dry matter	30 days: crude protein yields (kg ha^{-1} yr^{-1})	45 days: crude protein as % of dry matter	45 days: crude protein yields (kg ha^{-1} yr^{-1})	60 days: crude protein as % of dry matter	60 days: crude protein yields (kg ha^{-1} yr^{-1})
Digitaria setivalva	6402	13.3	1946	11.1	2066	9.3	1790
Brachiaria sp.	9626	11.1	1870	9.0	1824	8.3	1617
Brachiaria mutica	6451	13.8	2051	10.3	1902	9.3	1790
Digitaria decumbens	6439	13.8	2179	10.3	1834	9.5	1859
Brachiaria decumbens	5365	11.6	1866	9.5	1961	7.9	1887
Brachiaria brizantha	5909	12.0	1958	9.3	1907	7.8	1696
Digitaria pentzii × *D. sumtsii*	9621	14.3	1929	11.5	1836	9.7	1764
Digitaria milanjiana	6416	13.8	1938	10.8	2009	9.5	1925
Brachiaria brizantha	5569	14.0	2062	11.4	2018	9.7	1711
Brachiaria decumbens	9625	13.3	1910	10.1	1800	8.5	1718
Digitaria smutsii	6434	13.8	1830	11.2	1864	9.7	1721
Brachiaria ruziziensis	5366	11.2	1437	9.7	1460	8.9	1483
Digitaria decumbens	5124	13.8	1757	11.6	1811	10.1	1567
Digitaria decumbens	0560	13.3	1711	10.5	1622	9.6	1546
Brachiaria brizantha	5567	12.8	1758	10.4	1754	7.7	1827
Brachiaria brizantha	1525	12.0	1618	9.8	1504	8.8	1401
Cynodon nlemfuensic var. *nlemfuensis*	2341	14.0	1883	10.3	2141	9.2	2030
Digitaria pentzii × *D. pentzii*	9620	13.8	1525	11.3	1482	11.1	1158
Digitaria pentzii × *D. milanjiana*	9619	14.0	1440	12.0	1642	10.3	1437
Average		13.1	1824	10.5	1814	9.2	1692

[a] Published with permission of Sotomayor-Rios et al. (1976). [b] Puerto Rico plant introduction number.

growing, soft-wood shrub and generally grows as a hedge or dooryard plant. The young shoots and tender leaves of chaya are cooked and eaten as greens. Chaya appears free of pests and diseases that plague green garden vegetables in tropical climates (National Academy of Science, 1975). Its horticultural advantages are propagation from stem cuttings, ease of pruning, tolerance to heavy rainfalls, drought resistance, and high productivity.

Lablab niger, hyacinth bean, is generally represented as a tall-twining, climbing vine, but bush forms are also common. The hyacinth bean is grown in many tropical areas where the pods and seeds are eaten. It is variable and some types (cultivars) offer the advantages of vigor, high leaf yields and a perennial nature. Agronomic studies in India indicated that the hyacinth bean has considerable potential as an LPC source (Joshi, 1971).

Amaranthus spp., amaranth, a coarse annual plant, grows best in hot, sunny places. When grown in rich soil, the leaves become very large but usually lack bright color. The leaves are edible and uses range from spinach or salads to pot herbs. The cooked leaves are somewhat soft, have a spinach-like flavor, and are not bitter. This edible plant is best appreciated in Southeastern Asia and West Africa (Oliveira and DeCarvalho, 1975) and was suggested as an LPC source by Joshi (1971).

GRASSES

The tropical forage grasses are unexploited leaf sources that could supply large quantities of protein. Tropical forage grasses photosynthesize more efficiently, grow during most of the year, show excellent regeneration after repetitive cuttings, and with increased nitrogen fertilization respond with increased green forage and protein yields. Extensive investigations on tropical grasses by Caro-Costas et al. (1960, 1961, 1972), Vicente-Chandler et al. (1959, 1961, 1964), and Sotomayor-Rios et al. (1973, 1974, 1976) have considerably broadened our understanding of the effects of fertilization and repetitive cuttings on green forage, dry matter, and protein yields.

As a rule, under constant fertilization conditions, yields of green forage and dry forage increase with length of cutting interval (usually 30 to 90 days; Vicente-Chandler et al., 1964; Sotomayor-Rios et al., 1976). As shown in Table III, crude protein contents show a reverse trend, i.e., as harvest intervals increase, crude protein decreases. At the 30-, 45-, and 60-day cutting intervals, the average crude protein yields (Table III) of 19 grasses were 1824, 1814 and 1692 kg ha^{-1} year^{-1}, respectively. As the length of cutting increases, the proportion of leaf blades decreases and of stems increases. Since leaves possess a higher proportion of protein than stems, the end result is lower protein yields. In addition, protein contents of both leaf blades and stems drop sharply with age (Vicente-Chandler et al., 1964). Therefore, if forage grasses are to be seriously considered as an LPC source, a compromise would be required between long harvesting intervals, which produce high forage yields, and short harvest intervals which produce high-protein contents.

AQUATIC PLANTS AS A PROTEIN SOURCE

Information is limited on the potential of aquatic plants as a possible LPC source. Aquatic and semiaquatic plants are of numerous forms, viz., floating, submerged, floating leafed, and emergent (Boyd, 1971). In most tropical countries, floating species abound. *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce) possess high water contents (92–97%) and 6 to 24% crude protein (as percent of dry matter). In the tropics these plants grow rapidly and, under a continuous cropping procedure, yields in excess of 100 000 kg/ha of green matter appear feasible (Boyd, 1971). A major economic obstacle to the full utilization of these plants could be the lack of suitable mechanical harvesting equipment.

Submerged and floating leafed plants contain 12 to 24% crude protein. These aquatic plants hold little promise as a leaf crop because of harvesting difficulties, poor protein extractabilities and because they produce small standing crops (Boyd, 1968), e.g., *Brasenia schreberi* (water shield), *Nymphaea odorata* (water lily), and *Nelumbo lutea* (American lotus).

Emergent plants have potential as a leaf protein crop because they would be accessible for mechanical har-

vesting. The crude protein levels (percent dry matter) of some representative emergent plants show the following ranges (Pirie, 1971): *Justicia americana* (water willow, 9.6–22.8) *Alternanthera philoxeroides* (alligator weed, 7.8–21.0), and *Typha latifolia* (cattail, 3.0–14.4). Protein extractability (25–54% total nitrogen) and protein yields were high for water willow (590 kg/ha), alligator weed (478 kg/ha), and arrowhead (362 kg/ha) (Boyd, 1968). Before these aquatic plants could be seriously considered as an LPC source, more experiments on crop yields, protein yields and extractabilities, harvesting parameters, and nutritive studies are required.

LEAF PROTEIN EXTRACTABILITY, PREPARATION, AND FRACTIONATION

Laboratory Scale. Over the past 50 years the development of extraction and precipitation techniques for the laboratory preparation of leaf protein concentrates have changed only slightly since the pioneering explorations of Osborne and Wakeman (1920) and Chibnall and Schryver (1921). Proteins are expressed from leaves by rupturing the cells by grinding or mincing. The addition of water (1 volume of H_2O:1 volume of leaves) greatly enhances expression and yield of the protein-containing leaf juice. The protein yield from ruptured leaf cells also depends upon succulence and fiber content of leaves and on the presence of the following: mucilage, acids, tanning agents, and proteolytic and lipoxidase enzymes (Crewther, 1976).

Protein extracts more readily from soft succulent leaves than from those which are fibrous and dry. Succulence, in turn, apparently is related to the growth rate of the plant (Lexander et al., 1970) and frequency of harvesting (Sotomayor-Rios et al., 1976). In general, leaves which produce glutinous or slimy juices are not expressed effectively and are difficult to handle (Pirie, 1971). Leaves of *Corchorus olitorius* (jute) and *Ipomoea batatas* (sweet potato) (Table II) are extractable, however, in spite of the slimy texture of their pulps.

Plant leaves containing high proportions of acid yield acid juices from which protein prematurely precipitates within the insoluble fibrous fraction (Crook, 1946). It was shown by Pirie (1971) that leaf protein separates readily from the fibrous fraction if the pulp is made slightly alkaline (ca. pH 8.2). Addition of alkali to acid leaves during pulping and expression should increase the yield of protein; however, more pectic substances are extracted and phenolic substances oxidize more rapidly at alkaline than at acid pHs (Pirie, 1975c).

Plant tissues contain the ubiquitous polyphenolases. These enzymes oxidize *o*-diphenols to *o*-quinones which, in turn, react with protein through polymerization to yield insoluble tannin–protein complexes. This tanning process not only decreases the extractability of leaf protein but also damages the protein's nutritive value through reaction with the ϵ-amino groups of lysine and the sulfhydryl group of cysteine. The addition of sodium metabisulfite enhances protection of the sulfhydryl group. In the Pro Xan II process, addition of sulfite during grinding and pressing of leaves results in higher levels of cystine and methionine in the white protein fraction (Bickoff et al., 1975).

Freshly extracted juice, which is allowed to remain at room temperature for extended periods before processing, produce reduced protein yields because of the actions of proteolytic and lipoxidase enzymes. The proteases cleave the protein molecule to amino acid units and subunits while the lipoxidases, through reaction with unsaturated fatty acids to generate highly reactive oxygenated derivatives (e.g., epoxides, dicarbonyls), cause the formation of oxidized lipoprotein fractions that have reduced water solubility and digestibility.

There are several basic methods for the extraction and preparation of LPC. In the commonest, and perhaps simplest, extraction procedure, the leaves are macerated in the presence of ice, and the protein-laden juice is filtered through some type of linen cloth (e.g., potato masher lined with a filter bag, muslin, other fine woven linen cloths). Protein extractability is enhanced by expression of leaves in an aqueous alkaline medium, by the presence of detergents (Festenstein, 1961), or by use of cellulolytic enzymes, but these techniques are seldom used and would increase the cost.

For the preparation of tropical LPC, we use either of the two laboratory procedures illustrated in Figure 1. Heat coagulation is generally accepted as the simplest and most satisfactory procedure for preparing a protein curd from expressed leaf juice. The first protein coagulum formed on heating juice between 50 and 64 °C is regarded as "chloroplastic" protein. This term stems from the fact that the first protein coagulum contains almost all the chlorophylls and a large quantity of lipids. The second protein coagulum separates between 64 and 82 °C and is termed "cytoplasmic" protein. It is a mixture of proteins from the cytoplasm and from subcellular bodies in the cytoplasm. Although the term, cytoplasmic, is widely used, some investigators regard this term as inappropriate because a large percentage of the proteins in this fraction originate in the chloroplasts (Bickoff et al., 1975). The enzyme, ribulose diphosphate carboxylase (also known as fraction I protein or 18 S protein), might constitute as much as 50% of the cytoplasmic or white protein fraction. This enzyme comes from the chloroplasts during maceration and pressing of the leaves (Siegel et al., 1972). Bickoff et al. (1975) suggest that the terms, chloroplastic and cytoplasmic, be abandoned as descriptions of fractionated protein products because some proteins found in the chloroplastic fraction originate in the cytoplasm and, likewise, some proteins found in the cytoplasmic fraction originate in the chloroplasts. Because of widely recognized usage and for convenience, we will employ the terms chloroplastic and cytoplasmic in this paper.

The percentage distribution of chloroplastic and cytoplasmic proteins in a leaf juice depends upon many factors, viz. plant species, physiological state of the leaf, juice pH, and method of heating and fractionating (Pirie, 1975c). Procedure 1 (Figure 1) is representative of the most common of the schemes based on differential temperatures. Employing this scheme Lexander et al. (1970) reported high chloroplastic to cytoplasmic percentage ratios for *Chenopodium quinoa* (quinoa) (75:25), *Atriplex hortensis* (mountain spinach) (67:33) and *Medicago sativa* (alfalfa) (65:35). We, unfortunately, have not obtained these propituous percentages with some tropical leaves and grasses. In fact, the percentage of cytoplasmic protein coagulating at 82 °C was rarely above 2% of the total precipitable protein.

Procedure 2 (Figure 2) produces a chloroplastic protein fraction from heat coagulation at 62–64 °C. The cytoplasmic fraction is, however, precipitated by the action of a relatively polar organic solvent (Telek et al., 1978). The use of organic solvents (e.g., ethanol, isopropyl alcohol, acetone) to precipitate total proteins from leaf juice has been established by the works of Osborne and Wakeman (1920), Chibnall and Schryver (1921), and more recently, by Huang et al. (1971) and Tragardh (1974). Precipitation of cytoplasmic proteins with acetone yields a protein which is light colored and of high purity (Telek et al., 1978). At

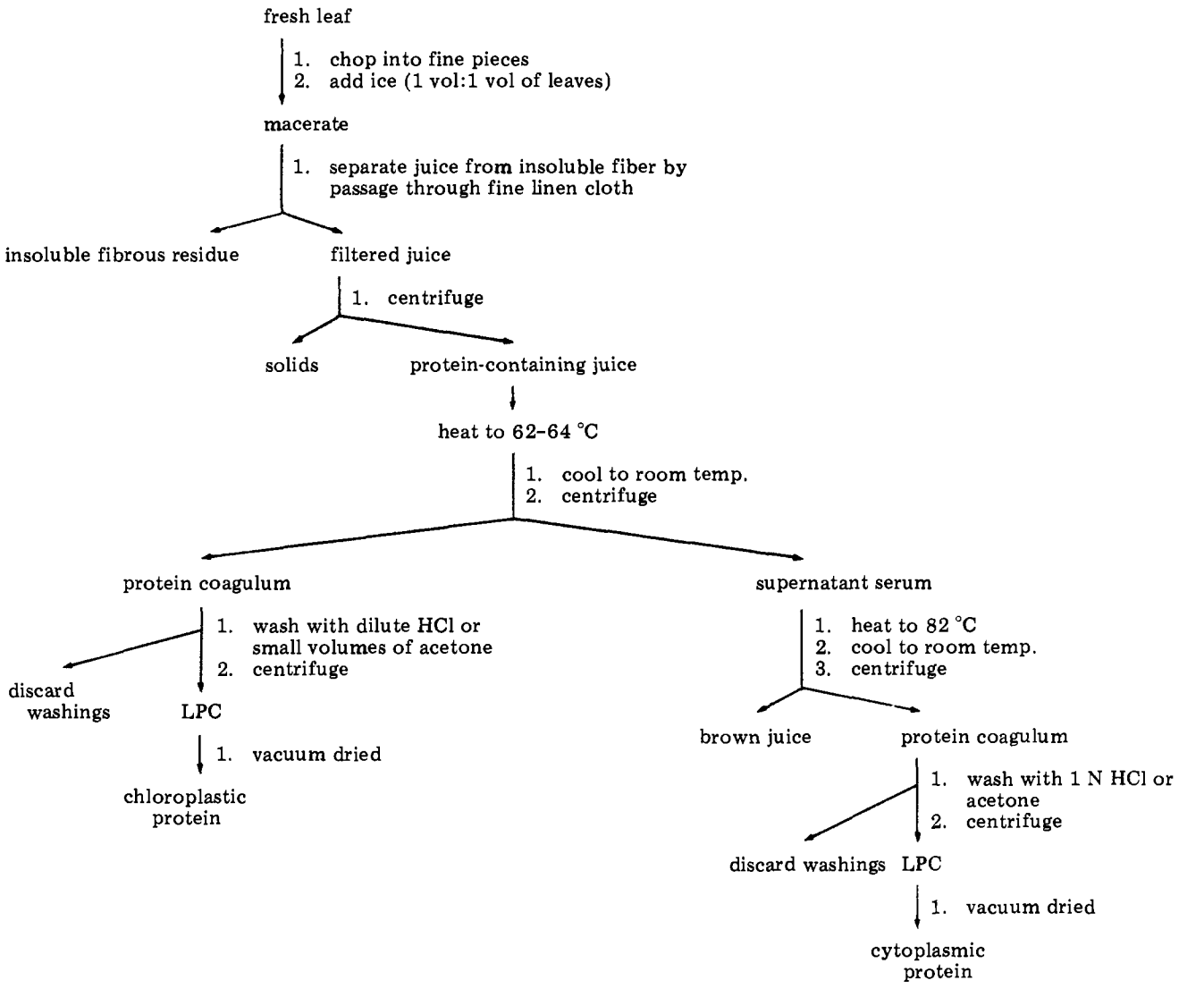

Figure 1. Procedure 1.

the Mayaguez Institute of Tropical Agriculture a new process has been developed by which a white protein can be separated from tropical leaves with considerable energy conservation (Telek, 1977). By this method green fraction protein is separated from a white protein, which is assumed to be a denatured fraction I protein, in about 3:1 ratio. Another protein, probably cytoplasmic derived, is also isolated but in lower proportion. Cytoplasmic fractions have a tendency to turn brown during drying.

The following plants yielded three protein fractions by the Mayaguez process: *Amaranthus* spp. (amaranth, several varieties), *Brassica alba* (mustard), *Brassica oleracea* var. *acephala* (collard), *Calotropis procera* (milkweed), *Glycine max* (soybean), and *Lablab niger* (hyacinth bean).

However, only two fractions were separated from the young and old leaves of the following plants: *Cnidoscolus chayamansa* (chaya), *Panicum maximum* Jacq. (Guinea grass), *Leucaena leucocephala* (lead tree), *Manihot esculenta* Crantz (cassava), *Sauropus androgynus* Merr. (Katuk), and *Sorghum sudanesis* hybrids.

Commercial Production. Commerical processes for dewatering fresh plant tissue to produce a press cake and usable products (LPC, molasses, growth medium for yeasts) from the expressed juice serum have been under constant development for many years in England, Hungary, and the United States. Most processes (Figure 3) begin by rupturing and pressing the plant material to produce a fibrous cake and green juice. The juice, containing soluble protein, carbohydrates, nucleic acids, lipids, vitamins, minerals, suspended chloroplasts, and other constituents, may be concentrated or spray dried to yield a heterogeneous whole juice LPC. As an alternative, the juice may be heated to 75–85 °C (process 3, Figure 3) causing the formation of a green LPC curd. The supernatant solution (brown juice), remaining after separation of the green curd, may be evaporated to yield a concentrated soluble fraction (nonprotein nitrogen compounds, carbohydrates, vitamins, minerals). The fourth process produces one soluble fraction and two protein fractions through differential temperature treatment. This last process is intended to yield a protein fraction (cytoplasmic LPC) that is suitable for human consumption.

In England, Pirie and co-workers (Pirie, 1953, 1959; Davys and Pirie, 1960, 1965; Davys et al., 1969; Pirie, 1971) were among the first to develop processing equipment for a moderate scale production of LPC. Pirie's basic objective was to develop equipment that was simple and compact so villagers in remote areas could use it to produce LPC from locally grown leaves. Pirie and co-workers have given highest priority to the preparation of LPC for human consumption. Fractionation of a leaf crop into fodder for ruminant and nonruminant animals has been of secondary importance. Their process has been the most extensively

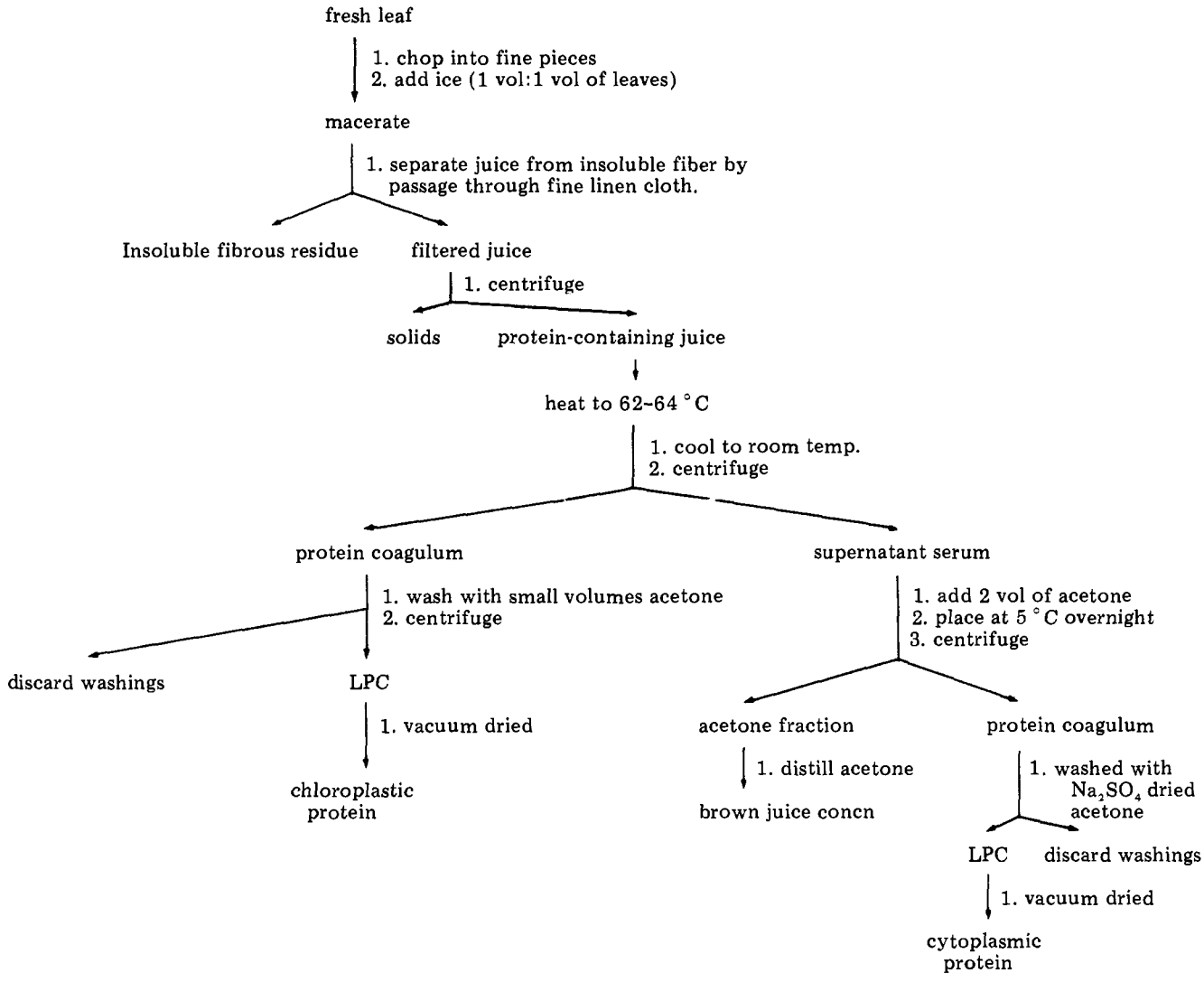

Figure 2. Procedure 2.

employed for preparation of tropical LPC in Nigeria, Jamaica, and India. Pirie's process, however, is not currently adaptable to large-scale commerical production of LPC.

There are currently two industrial processes for the commercial production of LPC, viz., the Hungarian process (VEPEX) and the American process (Pro-Xan) (Pirie, 1971). Each process was originally developed to produce a fibrous press cake for ruminant feed and a green press juice that could be used directly as a swine feed or heat treated to produce a green LPC suitable for other monogastric animals. The main product, both in weight and economic value, was the press cake while the juice was considered a by-product (Kohler, 1974). With increasing demands upon man's limited food supplies, the developers of VEPEX (Hollo and Koch, 1970; Koch, 1973) and of Pro-Xan (Kohler et al., 1968; Kohler and Bickoff, 1971) have since shifted their emphasis from a process solely devoted to the production of fodder for ruminant and nonruminant animals to a process for the production of protein concentrates for both the animal feed and human food industries.

Figure 4, a general schematic for the commercial production of LPC, is an abbreviated flow chart of the VEPEX and Pro-Xan II processes. More extensive schematics of the Pro-Xan and Pro-Xan II processes may be found in the works of Kohler and Bickoff (1971), Edwards et al. (1975), and Kohler et al. (1976). A VEPEX schematic may be found in a publication by Scole (1973). Both VEPEX and Pro-Xan II processes produce four basic products: (a) press cake (as a pelletized feed known as VEPEX IV), (b) green LPC (Pro-Xan II, VEPEX I), (c) white LPC (Welpro, VEPEX II), and (d) leaf solubles (remain after coagulation and removal of the LPC). Because of different processing conditions, the products from these two processes are not identical and differ in chemical composition and other physicochemical properties. As an example (Figure 4), white LPC by the Pro-Xan II process contains 90% crude protein (Welpro) while by the VEPEX process, this product contains about 60% crude protein (VEPEX II). LPC's may be utilized in various ways, e.g., in the production of single cell proteins by yeast culture (VEPEX III). Although these processes are still geared to produce important economical products (press cake, green LPC), there have been concerted efforts within the last few years to produce a white LPC for human consumption. There is at present no commerical production of LPC from tropical leaves; however, processing plants are at the stage of being constructed in southeast Asia which will employ the VEPEX process (Neal, 1975). It is possible that, within the near future, both the VEPEX and Pro-Xan II processes could be extensively employed to prepare LPC from tropical leaves.

A new energy-saving method which does not require heating of the pressed juice to yield a protein coagulum

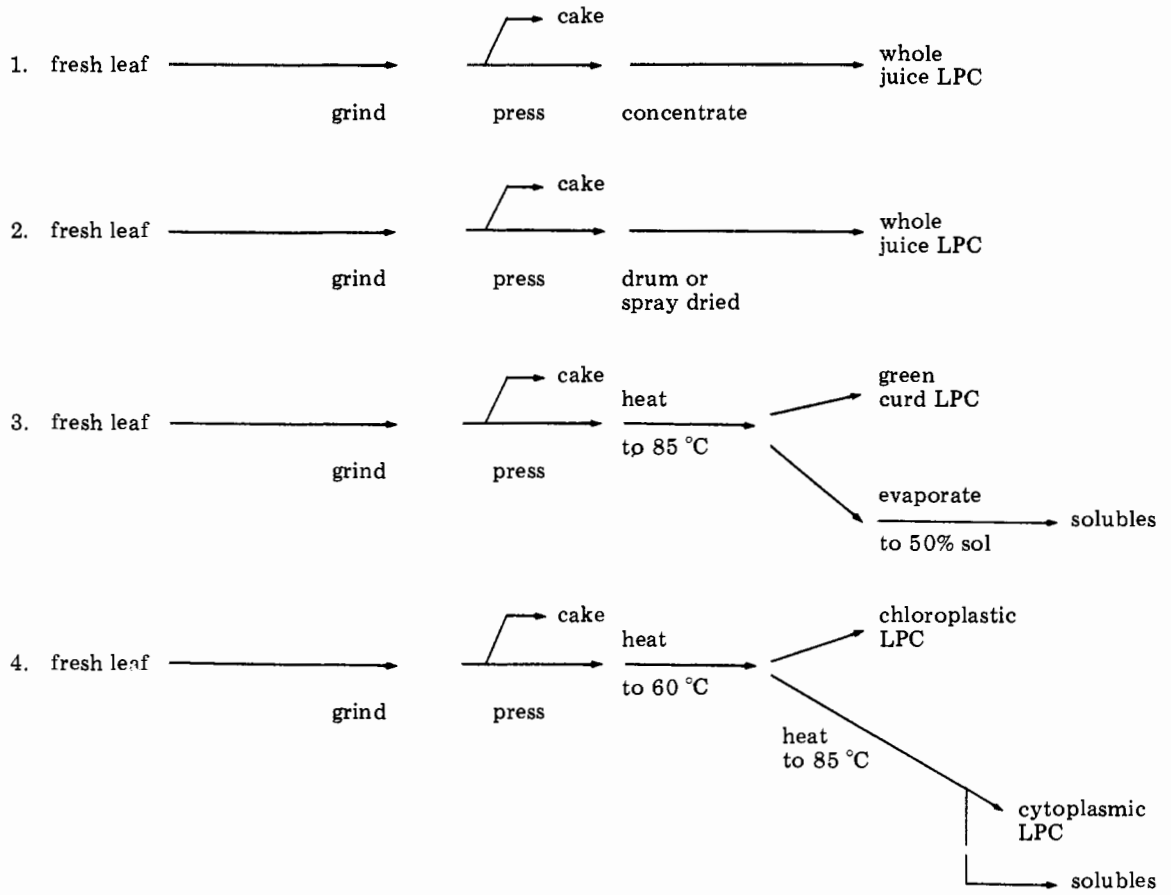

Figure 3. Several processes for preparing LPC and by-products. Published with permission from Kohler et al. (1976). Copyright 1976 University of California.

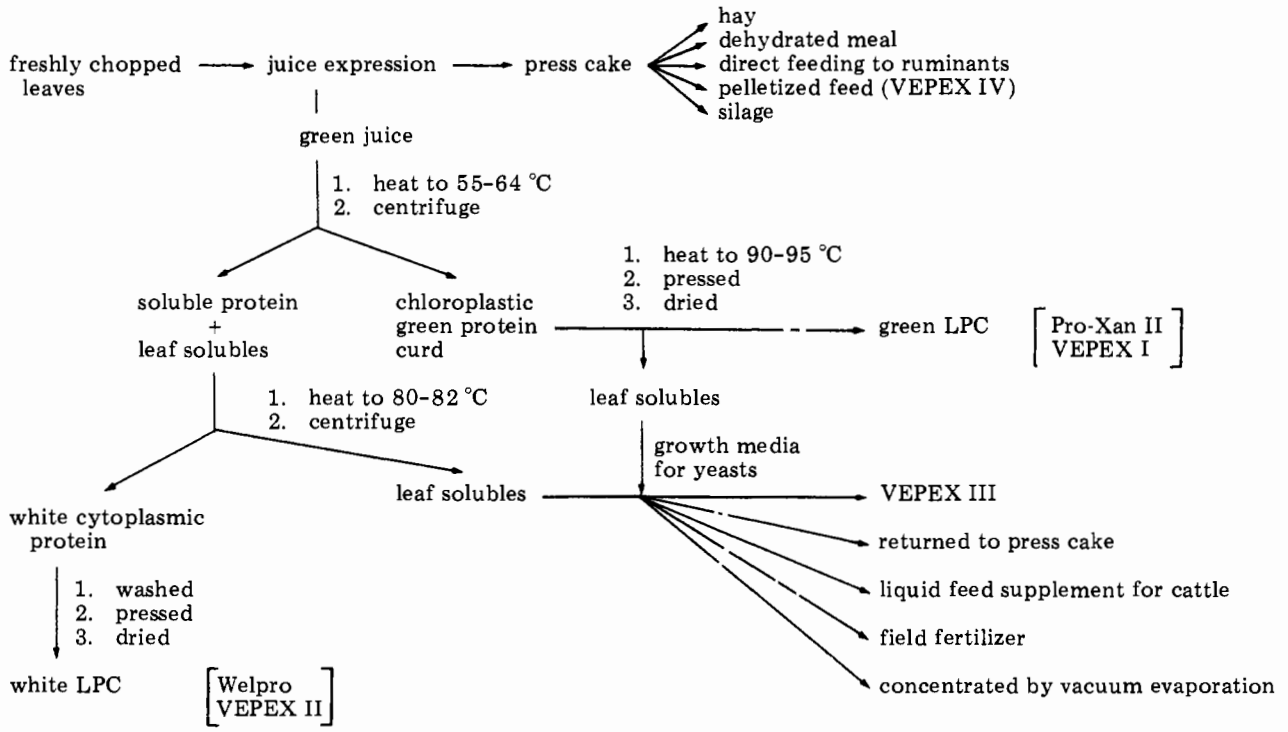

Figure 4. General schematic for the commercial production of LPC. Schematic indicates the general method for production of products by the VEPEX and Pro-Xan II processes. Because of different processing conditions, the products are not identical in chemical composition, nutritive value, functional properties, and other characteristics.

has recently been patented by Stahmann (1976). That method utilizes a simple anaerobic fermentation of the expressed leaf juices to produce organic acids which, in turn, lowers the pH and causes coagulation and preservation of the protein. Fermentable carbohydrates in the juice were also converted into protein that was collected with the protein coagulum. These proteins showed good nutritive value because less oxidative destruction occurred

Table IV. Comparative Amino Acid Compositions of Whole Leaf Proteins from Temperate and Tropical Leaves

	temperate				tropical		
amino acid	alfalfa[a,c]	chenopodium[b,d]	barley[b,e]	*Brassica chinensis* (Chinese cabbage)[b,e]	*Brassica carinata* (TAMU-Tex Sel)[b,f]	cassava[a,g]	*Miscanthus floridulus*[b,h]
essential							
lysine	6.3	7.2	6.6	7.1	6.0	6.7	5.2
phenylalanine	5.8	5.8	6.2	6.2	2.5	5.9	5.8
methionine	2.0	2.0	2.2	1.9	1.4	1.6	1.2
threonine	5.0	5.2	5.1	5.2	5.0	4.8	4.5
leucine	8.9	9.2	9.3	9.3	9.5	9.9	10.9
isoleucine	5.2	5.2	5.0	4.6	5.0	5.2	5.0
valine	6.4	6.4	6.4	6.1	6.3	6.6	5.9
tryptophan	1.7	1.6					
nonessential							
arginine	6.2	6.6	6.9	6.4	6.0	6.7	7.2
histidine	2.4	2.8	2.3	2.4	2.4	2.4	2.3
tyrosine	4.6	4.4	4.5	4.7	0.8	4.0	3.5
cystine	1.3	0.9	2.0	2.1	1.3		
aspartic acid	10.4	9.7	9.6	10.0	11.1	10.3	9.9
serine	4.4	4.9	4.4	4.5	4.7	4.2	5.8
glutamic acid	11.2	11.0	11.4	11.9	11.5	13.0	12.4
proline	4.7	5.3	4.7	4.7	4.8	5.3	7.0
glycine	5.2	5.6	5.6	5.4	5.3	5.8	5.9
alanine	6.1	6.3	6.7	6.1	5.8	7.5	7.4

[a] Expressed as g/16 g of N. [b] Expressed as g of amino acid/100 g of recovered amino acid. [c] Bickoff et al. (1975). [d] Gerloff et al. (1965). [e] Byers (1971b). [f] Brown et al. (1975). [g] Otoul (1974). [h] Chou et al. (1975).

with the sulfur amino acids and lysine. This fermentation process has considerable potential for use in the tropics.

LPC COMPOSITION

Prior to 1961 the amino acid compositions of leaf protein concentrates were unknown (Stahmann, 1974). With the advent of new and improved techniques (ion-exchange and gas–liquid chromatographies) for amino acid analysis, data increased substantially on the distributions of amino acids in whole or "unfractionated" leaf proteins, chloroplastic and cytoplasmic protein preparations, and specific leaf protein fractions (e.g., fraction I protein from tobacco leaf; Kawashima, 1969). On the basis of several studies on the amino acid compositions of whole leaf protein preparations (Chibnall et al., 1963; Gerloff et al., 1965; Wilson and Tilley, 1965; Oelschlegel et al., 1969; Byers, 1971a), Byers (1971b) concluded that the amino acid patterns of unfractionated proteins were essentially similar regardless of plant species. Reported amino acid compositions of some representative temperature and tropical leaves are compared in Table IV. Amino acid data are expressed either as g of amino acid/16 g of protein nitrogen or g of amino acid/100 g of recovered amino acid. Equating amino acids to 16 g of protein nitrogen is based on the principle that, on the average, 6.25% of the protein molecule is composed of nitrogen; thus, 6.25 × 16 g of protein nitrogen equals 100 g of protein. Those two methods for reporting amino acid concentrations are the most widely used and unquestionably, the most reliable (Byers, 1971b). In comparison with other leaves tested, the tropical leaf *Brassica carinata* (TAMU-TexSel) appears to contain lower levels of phenylalanine and tyrosine; the tropical grass *Miscanthus floridulus* contains less lysine and threonine but more serine and proline. Extensive investigations of whole leaf amino acid compositions would be required to determine whether or not the amino acid patterns differ between leaves of temperate and tropical plants.

The evidence is not clear as to whether or not the various unfractionated proteins of plants have similar amino acid compositions, but it is clear that within and among plant species the chloroplastic and cytoplasmic protein fractions do have different amino acid compositions. To illustrate this point, amino acid patterns are presented for chloroplastic and cytoplasmic protein fractions of two temperate leaves and one tropical leaf and for chloroplastic fractions of two tropical leaves (Table V). The cytoplasmic fraction, in contrast to the chloroplastic fraction, shows higher percentages for lysine, histidine, and glutamic acid. That difference has also been observed in *Lupinus albus* (lupin) and *Brassica chinensis* (Chinese cabbage) by Byers (1971a). The valine contents of chloroplastic fractions appear to be higher for the three tropical than for the two temperate leaves.

NUTRITIONAL VALUE

Amino acid composition and biological value, common criteria of the nutritive quality of proteins in foods, food preparations, and diets, are important in the evaluation of protein supplies in developing countries and as a basis for developing nutritionally sound food policies (FAO, 1970). There are eight amino acids (Table V) considered essential in human nutrition because of man's inability to synthesize them (National Academy of Sciences, 1974). The limiting essential amino acids in a food (expressed as g of amino acids/100 g of food protein) are determined by relating their concentrations in that food to their concentrations in the reference protein of egg (g of amino acid/100 g of egg protein) (FAO/WHO, 1965).

The essential amino acid compositions of chloroplastic and cytoplasmic protein fractions (Table V) show that methionine is the first limiting amino acid. Although methionine is the most important sulfur-containing amino acid, nutritional data should include the sum total of all sulfur-containing amino acids (methionine, cysteine, cystine). It is a well-established fact that cystine has a sparing action on methionine. Cystine can replace about one-sixth of the methionine requirement but has no growth effect in the absence of methionine (Rose et al., 1948). The tryptophan content of LPC is about equal to that of egg protein. The human requirement for tryptophan is low and most diets, except in cases of famine, supply ample amounts (Jacobs, 1951). Lysine, phenylalanine, threonine, leucine, isoleucine, and valine contents are from 1.1 to 2.6

Table V. Amino Acid Distributions of "Chloroplastic" and "Cytoplasmic" Protein Fractions in Temperate and Tropical Leaves

	temperate				tropical				
	barley[a,c]		alfalfa[b,d]		chaya[a,e]		bonavist bean,[a,f]	sorghum sudanesis,[a,f]	FAO[b]
amino acid	chloroplastic	cytoplasmic	chloroplastic	cytoplasmic	chloroplastic	cytoplasmic	chloroplastic	chloroplastic	reference protein
essential									
lysine	5.6	7.1	5.9	6.4	5.9	8.3	5.9	6.3	4.2
phenylalanine	7.0	5.8	6.6	6.4	6.3	4.8	7.8	7.2	2.8
methionine	2.3	2.4	2.2	2.7	1.8	1.8	1.7	2.1	2.2
threonine	4.8	5.4	4.7	5.7	5.0	4.4	5.1	4.9	2.8
leucine	10.4	8.4	10.3	9.4	10.1	8.4	10.8	10.7	4.8
isoleucine	5.3	4.7	6.2	5.3	5.6	4.5	5.6	5.7	4.2
valine	6.2	6.5	7.0	6.9	6.9	5.8	6.6	7.0	4.2
tryptophan			1.0	2.5	1.7	0.5	1.4	1.3	1.4
nonessential									
arginine	6.3	7.0	6.2	8.0	6.6	7.0	7.1	5.8	
histidine	1.8	2.7	2.4	3.2	1.1	4.3	1.5	2.2	
tyrosine	4.5	4.9	4.5	5.6	5.5	4.6	4.3	4.0	
cystine	1.0	1.7	1.0	1.7	1.3	1.9	0.2	0.2	
aspartic acid	9.8	9.6	10.3	10.0	9.0	10.7	10.5	10.1	
serine	4.9	4.1	5.1	3.7	4.9	4.6	4.1	4.3	
glutamic acid	11.0	11.9	11.0	12.0	10.3	12.5	10.8	11.7	
proline	4.9	4.6	4.5	4.4	5.3	4.5	5.4	5.0	
glycine	6.1	5.4	5.7	5.5	6.0	5.2	4.9	4.9	
alanine	7.1	6.5	6.3	6.4	6.5	5.6	6.3	6.9	

[a] Expressed as g of amino acid/100 g of recovered amino acid. [b] Expressed as g/16 g of N. [c] Byers (1971a). [d] Bickoff et al. (1975). [e] Telek et al. (1978). [f] Nagy et al. (1977).

times the contents of the reference protein. Phenylalanine and tyrosine should be grouped together and designated as total aromatic acids. While tyrosine is a nonessential amino acid, it has a sparing action on phenylalanine. Tyrosine can form phenylalanine but the reverse reaction, tyrosine → phenylalanine, does not occur in humans. Tyrosine can replace about one-half the phenylalanine requirement but has no growth effect in the absence of phenylalanine (Rose et al., 1948). The data in Tables IV and V indicate that LPC has considerable potential as a protein supplement to unbalanced diets such as the cereal based diets (low in lysine) common to Asia and diets based on cassava, beans, rice, and maize which are common foods in tropical areas of Asia, Africa, and Central and South America.

Before LPC could be added to foods for human consumption, preliminary feeding trials on animals were needed to determine (a) possible adverse toxic reactions, (b) biological values including protein utilization, and (c) amino acid availability. In terms of nutritive value, it is usually the availability of the essential amino acids, rather than their absolute amounts, which determine a protein's effectiveness. Ford (1964, 1970) showed with barley proteins that the availability of methionine in a cytoplasmic fraction was about 100% but in a sedimented chloroplastic fraction was only 40%. The unavailability of methionine in chloroplastic protein might be due to the presence of nonproteinaceous chloroplastic contaminants. The biological value of LPC has been studied extensively on rats (Davies et al., 1952; Larson and Halverson, 1962; Henry and Ford, 1965; Subba Rau and Singh, 1970; Eggum, 1970), chickens (Carpenter et al., 1952, 1954; Hughes and Eyles, 1953; Ellingher, 1954; Cowlishaw et al., 1956; Raymond and Tilley, 1956; Duckworth and Woodham, 1961, Aziz et al., 1971; Adegbola and Oke, 1973) and on pigs (Barber et al., 1959; Duckworth et al., 1961). In vitro enzymatic digestion (Akeson and Stahmann, 1965; Buchanan, 1969; Byers, 1971a; Oke and Umoh, 1974; Saunders et al., 1973; Fafunso et al., 1976) and microbiological assay (Henry and Ford, 1965) have also been employed to assess LPC's biological value. The enzymic and microbiological assays are excellent tools and give data that correlate closely with data from rat-feeding experiments, but they are less sensitive to availability of certain essential amino acids (Oke and Umoh, 1974).

Waterlow (1962) employed LPC of alfalfa in the first quantitative feeding experiments with humans. Infants, who were recovering from malnutrition, were fed either diets containing milk as sole source of nitrogen or diets with half the milk replaced by LPC. At ordinary levels of nitrogen intake, nitrogen retention was equal for the two diets. Waterlow's tests indicated that leaf protein was equal to milk in biological value.

With the foundation laid by many animal feeding trials and Waterlow's infant feeding success, Doraiswamy and co-workers (1969) began several quantitative feeding trials with children. Unfractionated alfalfa LPC was used as the protein supplement to a native diet (composed mainly of *Eleusine coracana*, i.e., ragi). The tests, involving 80 boys ages 6 to 12, showed that LPC could be used to improve lysine-deficient diets and add extra protein. After 6 months, increase in growth (weight and height) and improved blood properties (higher hemoglobin concentration and higher red cell counts) were greatest in boys that ate the LPC supplemented diet. In tests conducted by Singh (1971) with 10- to 12-year-old children over 12-day periods, leaf protein contributed 72–74% of the total protein content in test diets. Biological values based on nitrogen retention showed that when a low-quality protein diet was supplemented with leaf protein, nitrogen retention increased substantially.

Presently in the Coimbatore area of India a 2-year feeding trial, sponsored by the British relief organization "Find Your Feet Ltd.", involves 600 children in the 2- to 5-year age group (L.I.F.E., 1975). In one group, 15 g of alfalfa LPC are fed to each child each day, while in the other test groups a basic native diet is supplemented with (a) 500 calorie supplement (not named), (b) 1 horsegram of (*Dolichos biflorus*) supplement (cheapest local source of good protein), or (c) skimmed milk. Preliminary results

indicate that the leaf protein supplemented group had a 50% higher weight gain than the control group and more than double the height increase. The leaf protein was readily accepted by the children who ate it in the form of a sweet and was less expensive to produce than locally available legumes (*Food Eng.*, 1976). There are plans to organize a program for the inclusion of LPC supplements in the diets of 10 000 children now fed under an Indian government program and, later, for other official nutrition programs with 2 million children.

LPC ACCEPTABILITY AND PALATABILITY

The feeding trials with LPC generated considerable confidence in the nutritional value of this novel protein supplement. Long before the Jamaican (Waterlow, 1962), Indian (Doraiswamy et al., 1969), and Nigerian (Olatunbosum, et al., 1972) LPC feeding studies were published, Morrison and Pirie (1960) suggested trials with LPC as a protein supplement in local foods. A responsible quantity of LPC was suggested at 10% of the total protein requirement (equivalent to about one-third pint of milk or an egg).

In a second Jamaican study (Scientific Research Council of Jamaica, 1965), LPC products from wheat, maize, barley, rape, sugar cane, Guinea corn, and cowpea were incorporated into local recipes. Foods were prepared from local recipes and distributed to school children mostly as cookies, cakes, candies, breads, and spreads. Acceptability was low, apparently because the green color and the taste were too pervasive in all but the most highly colored and seasoned dishes (chocolate and ginger). Acceptability was increased by supplying less than 8 g of LPC child^{-1} day^{-1}. Sugar cane, maize, and barley LPC were the preferred supplements.

Since palatability is a key factor in the acceptability of a dietary supplement, LPC-supplemented foods must be evaluated by taste panels. Kamalanathan and Devadas (1971) showed that LPC acceptability increased as judges became accustomed to supplemented foods. When supplements with 0, 5, 10, and 15 g LPC were compared, only the 15-g supplement was unacceptable and was characterized by a leafy, bitter flavor and possessing a sawdust-like texture. For acceptance of a new protein food, the preparation, besides supplying adequate protein, must be ready to eat, convenient to distribute, palatable, and popular with the local populace (Kamalanathan et al., 1969, 1970).

In all of the human LPC feeding trials described in this paper, the leaf protein preparation was of the whole unfractionated type. This type preparation has the advantages of containing (a) good quality protein, (b) lipid materials including nutritionally important fatty acids, (c) vitamins, (d) minerals, and (e) β-carotene. Its disadvantages might result from the presence and/or characteristics of (a) toxic substances, (b) components, e.g., phenolics, that contribute to poor digestibility and/or unavailability of specific amino acids, (c) rancidity caused by oxidized fatty acids, (d) green color, (e) strong and hard-to-mask leafy taste, caused by chlorophyll and its breakdown products, and (f) poor texture. Solvent extraction might eliminate some of the objectionable features of unfractionated LPC but also would eliminate some nutritious components such as β-carotene, fat-soluble vitamins, and unsaturated fatty acids.

White cytoplasmic protein has been suggested as an alternative to chloroplastic and unfractionated leaf proteins in protein supplementation. Cytoplasmic protein has nutritional value and digestibility approaching those of casein and, in general, contains higher amounts of available histidine and lysine over that of chloroplastic protein (Byers, 1971b). Cytoplasmic protein, however, is considerably more difficult and complicated to produce and purify and, therefore, would probably be more costly than chloroplastic protein.

Functional properties, such as capacities for foaming, emulsifying, solubilizing, and texturizing, are important factors in the successful use of LPC in food applications (Wang and Kinsella, 1976). LPC's foaming properties make it useful in light products such as angel food cakes, whipped toppings, desserts, and souffle.

CONCLUSION

Leaf proteins are currently among the most underdeveloped protein supplementary sources (Pirie, 1975b). If developing countries are to prevent massive starvation, this abundant protein source should be seriously considered. The Malthusian axiom, that population growth will outstrip food supplies and thereby lead to biological catastrophe, has proven all too correct for too many countries in recent years.

The technical feasibility for production and use of leaf protein concentrates has been studied through two approaches: (1) on-farm or at-site (remote villages) production, followed by direct consumption (Pirie, 1971; Stahmann, 1974; Bray, 1975) and (2) large-scale commerical production by the Pro-Xan (Kohler and Bickoff, 1971) and VEPEX (Koch, 1973) processes. The small-scale on-farm production require a well-managed farm and sufficient capital to invest in equipment and operations (Koegel et al., 1974). Large-scale production by the Pro-Xan or VEPEX process would require a relatively large growing area in which the plant could be centrally located to minimize the cost of hauling raw materials.

Since many areas in the tropics have prolonged dry seasons, a crop rotation system must be designed to secure leaf material for year-round operation. Further studies should be directed toward the agronomic evaluation of novel tropical plant leaves as raw materials.

In processing leaves into leaf protein concentrates, a part of the leaf's protein is nonextractable. This nonextractable protein is associated with the fiber and has considerable economic value as a feed for ruminants. Leaf protein concentrates (green and white LPC) possess considerable potential as human food. Objections have been raised to the use of green LPC from some leaf sources for human food, but green LPC can be transformed into a stable, nutritious, bland off-white product by solvent extraction (Singh, 1975). Green LPC, which is the largest protein fraction derived from the fractionation process, has been used in many food preparations without any solvent treatment. More research should be directed toward improving leaf protein concentrate for human consumption.

LITERATURE CITED

Adegbola, A. A., Oke, O. L., *Nutr. Rep. Int.* 8, 313 (1973).
Akeson, W. R., Stahmann, M. A., *J. Agric. Food Chem.* **13**, 145 (1965).
Aziz, T. A., El Shazly, K., El-Zayat, S., Abou-Akkado, A. R., Naga, M. A., *Alexandria J. Agric. Res.* **19**, 193 (1971).
Bagchi, D., Matai, S., *J. Sci. Food Agric.* **27**, 1 (1976).
Barber, R. S., Braude, R., Michell, K. G., *Proc. Nutr. Soc.* **18**, iii (1959).
Bassir, O., Fafunso, M., *Plant Foods Man* **1**, 209 (1976).
Bickoff, E. M., Booth, A. N., de Fremery, D., Edwards, R. H., Knuckles, B. E., Miller, R. E., Saunders, R. M., Kohler, G. O., in "Protein Nutritional Quality of Foods and Feeds", Friedman, M., Ed., Marcel Dekker, New York, N.Y., 1975, p 319.
Boyd, C. E., *Econ. Bot.* **22**, 359 (1968).
Boyd, C. E., in "Leaf Protein: Its Agronomy, Preparation, Quality

and Use", Pirie, N. W., Ed., Blackwell Scientific Publications, Oxford, 1971, p 44.
Bray, W. J., 169th National Meeting of the American Chemical Society, Philadelphia, Pa., April 10, 1975.
Brown, H. E., Stein, E. R., Saldana, G., *J. Agric. Food Chem.* **23**, 545 (1975).
Buchanan, R. A., *Br. J. Nutr.* **23**, 533 (1969).
Byers, M., *J. Sci. Food Agric.* **12**, 20 (1961).
Byers, M., *J. Sci. Food Agric.* **22**, 242 (1971a).
Byers, M., in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", Pirie, N. W., Ed., Blackwell Scientific Publications, Oxford, 1971b, p 95.
Caro-Costas, R., Abruna, F., Vincente-Chandler, J., *J. Agric. Univ. P.R.* **56**, 267 (1972).
Caro-Costas, R., Vicente-Chandler, J., *J. Agric. Univ. P.R.* **45**, 46 (1961).
Caro-Costas, R., Vicente-Chandler, J., Figarella, J., *J. Agric. Univ. P.R.* **44**, 107 (1960).
Carpenter, K. J., Duckworth, J., Ellinger, G. M., *Br. J. Nutr.* **6**, xii (1952).
Carpenter, K. J., Duckworth, J., Ellinger, G. M., Proceedings of the European Grassland Conference, Paris, 1954.
Chibnall, A. C., Rees, M. W., Lugg, J. W. H., *J. Sci. Food Agric.* **14**, 234 (1963).
Chibnall, A. C., Schryver, S. B., *Biochem. J.* **15**, 60 (1921).
Chou, C. H., Young, C. C., Huang, C., *Bot. Bull. Acad. Sin.* **16**, 191 (1975).
Cowlishaw, S. J., Eyles, D. E., *Emp. J. Exp. Agric.* **24**, 223 (1956).
Crewther, W. G., *Search* 7(4), 140 (1976).
Crook, E. M., *Biochem. J.* **40**, 197 (1946).
Davies, M., Evans, W. C., Parr, W. H., *Biochem. J.* **52**, xxii (1952).
Davys, M. N. G., Pirie, N. W., *Engineering (London)* **190**, 274 (1960).
Davys, M. N. G., Pirie, N. W., *J. Agric. Eng. Res.* **10**, 142 (1965).
Davys, M. N. G., Pirie, N. W., Street, G., *Biotechnol. Bioeng.* **11**, 528 (1969).
Doraiswamy, T. R., Singh, N., Daniel, V. A., *Br. J. Nutr.* **23**, 737 (1969).
Duckworth, J., Hepburn, W. R., Woodham, A. A., *J. Sci. Food Agric.* **12**, 16 (1961).
Duckworth, J., Woodham, A. A., *J. Sci. Food Agric.* **12**, 5 (1961).
Edwards, R. H., Miller, R. E., deFremery, D., Knuckles, B. E., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **23**, 620 (1975).
Eggum, B. O., *Br. J. Nutr.* **24**, 761 (1970).
Ellinger, G. M., *Proc. World's Poult. Congr.* **2**, 128 (1954).
Ereky, K., British Patent 270629 (1926).
Fafunso, M. A., Bassir, O., Oke, O. L., *Nutr. Rep. Int.* **14**, 353 (1976).
FAO, FAO Agricultural Studies, No. 55, FAO, Rome, 1961.
FAO, Amino Acid Content of Foods and Biological Data on Protein, FAO, Rome, 1970.
FAO/WHO, FAO Nutr. Mtg. Rep. Ser. No. 70, FAO, Rome, 1965.
Festenstein, G. H., *J. Sci. Food Agric.* **12**, 305 (1961).
Food Eng. 29 (May 1976).
Food Ind. S. Afr. **9** (Oct. 1975).
Ford, J. E., *Br. J. Nutr.* **18**, 449 (1964).
Ford, J. E., National Institute for Research in Dairying, Reading, England, 1970, as cited by Byers, M., in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", Pirie, N. W., Ed., Blackwell Scientific Publications, Oxford, 1971b, p 95.
Gerloff, E. D., Lima, I. H., Stahmann, M. A., *J. Agric. Food Chem.* **13**, 139 (1965).
Goodall, C., British Patent 457789 (1936).
Hall, N. T., Nagy, S., Berry, R. E., *Proc. Fla. State Hortic. Soc.* **88**, 486 (1975).
Henry, K. M., Ford, J. E., *J. Sci. Food Agric.* **16**, 425 (1965).
Hollo, J., Koch, L., *Process Biochem.* **5**(10), 37 (1970).
Huang, K. H., Tao, M. C., Boulet, M., Riel, R. R., Julien, J. P., Brisson, G. J., *Can. Inst. Food Technol. J.* **4**, 85 (1971).
Hughes, G. P., Eyles, D. E., *J. Agric. Sci.* **43**, 136 (1953).
Jacobs, M. G., "The Chemistry and Technology of Food and Food Products", Interscience, New York, N.Y., 1951.
Jeswani, L. M., in "Food Protein Sources", Pirie, N. W., Ed., Cambridge University Press, Cambridge, 1975, p 13, 15.
Joshi, R. N. in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", Pirie, N. W., Ed., Blackwell Scientific Publications, Oxford, 1971, p 19.
Kamalanathan, G., Devadas, R. P., in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", Pirie, N. W., Ed., Blackwell Scientific Publications, Oxford, 1971, p 145.
Kamalanathan, G., Nalinakski, G. S., Devadas, R. P., *J. Nutr. Diet.* **7**, 288 (1970).
Kamalanathan, G., Usha, M. S., Devadas, R. P., *J. Nutr. Diet.* **6**, 12 (1969).
Kaul, A. K., in "Food Protein Sources", Pirie, N. W., Ed., Cambridge University Press, Cambridge, 1975, p 1.
Kawashima, N., *Plant Cell Physiol.* **10**, 31 (1969).
Koch, L., *Vaxtodling* **28**, 129 (1973).
Koegel, R. G., Barrington, G. P., Bruhn, H. D., Fourth Annual Alfalfa Symposium, Madison, Wis., April 9, 1974.
Kohler, G. O., Proceedings of the Twelfth Technical Alfalfa Conference, Overland Park, Kan., Nov. 6–7, 1974.
Kohler, G. O., Bickoff, E. M., in "Leaf Protein: Its Agronomy, Preparation, Quality, and Use", Pirie, N. W., Ed., Blackwell Scientific Publications, Oxford, 1971, p 69.
Kohler, G. O., Bickoff, E. M., deFremery, D., *Univ. Calif. Spec. Publ.* **3058**, 116 (1976).
Kohler, G. O., Bickoff, E. M., Spencer, R. R., Witt, S. C., Knuckles, B. E., *Tenth Tech. Alf. Conf. Proc.* **ARS 74-76**, 71 (1968).
Kohler, G. O., Knuckles, B. E., 36th Annual IFT Meeting, Anaheim, Calif., June 6–9, 1976.
Larson, R. L., Halverson, A. W., *J. Agric. Food Chem.* **10**, 422 (1962).
L.I.F.E., League for International Food Education Newsletter, March 1975, p 1–2.
Lexander, K., Carlsson, R., Schalen, V., Simonsson, A., Lundborg, T., *Ann. Appl. Biol.* **66**, 193 (1970).
Martin, F. W., Ruberte', R. M., "Edible Leaves of the Tropics", Antillian College Press, Mayaguez, Puerto Rico, 1975.
Martin, F. W., Telek, L., Ruberte', R., *J. Agric. Univ. P.R.* **51**, 32 (1977).
Morrison, J. E., Pirie, N. W., *Nutrition (London)* **14**(1), 3 (1960).
Morrison, J. E., Pirie, N. W., *J. Sci. Food Agric.* **12**, 1 (1961).
Nagy, S., Hall, N. T., Telek, L., Berry, R. E., amino acid compositions of chloroplastic and cytoplasmic tropical leaf proteins, ARS, USDA, Winter Haven, Florida, unpublished data (1977).
Neal, A. E., 68th Annual ASAE Meeting, University of California, Davis, 1975.
National Academy of Sciences, "Recommended Dietary Allowances", 8th ed, Washington, D.C., 1974.
National Academy of Sciences, "Underexploited Tropical Plants with Promising Economic Value", Washington, D.C., 1975.
Oelschlegel, F. J., Schroeder, J. R., Stahmann, M. A., *J. Agric. Food Chem.* **17**, 791 (1969).
Oke, O. L., Umoh, I. B., *Nutr. Rep. Int.* **10**, 397 (1974).
Olatunbosum, D. A., Adadevoh, B. K., Oke, O. L., *Nig. Med. J.* **2**, 195 (1972).
Oliveira, J. S., DeCarvalho, M. F., *Econ. Bot.* **29**, 255 (1975).
Osborne, T. B., Wakeman, A. J., *J. Biol. Chem.* **42**, 1 (1920),
Otoul, E., *Bull. Rech. Agron. Gembloux* **9**, 159 (1974).
Oyenuga, V. A., "Nigeria's Feeding-Stuffs: Their Composition and Nutritive Value", 2nd ed, Ibadan University Press, Ibadan, Nigeria, 1959, p 8.
Pirie, N. W., *Nature (London)* **149**, 251 (1942).
Pirie, N. W., Rep. Rothamsted Exp. Stn., 1953, p 173.
Pirie, N. W., *Microbiol. Technol. Eng.* **1**, 13 (1959).
Pirie, N. W., Ed., "Leaf Protein: Its Agronomy, Preparation, Quality and Use", Blackwell Scientific Publications, Oxford, 1971.
Pirie, N. W., *Nature (London)* **253**, 239 (1975a).
Pirie, N. W., Ed., "Food Protein Sources", Cambridge University Press, Cambridge, 1975b.
Pirie, N. W., *Nutr. Clin. Nutr.* **1**, 341 (1975c).
Prager, D. J., Duncan, G. W., *Battelle Today* **2** (Nov 1976).
Raymond, W. F., Tilley, J. M. A., *Colon. Plant Anim. Prod.* **6**, 3 (1956).
Rose, W. C., Oesterling, M. J., Womack, M., *J. Biol. Chem.* **176**, 753 (1948).
Scientific Research Council of Jamaica, S.R.C. Technical Report 1/65, 1965.

Saunders, R. M., Connor, M. A., Booth, A. N., Bickoff, E. M., Kohler, G. O., *J. Nutr.* **103**, 530 (1973).
Scole, R., *Chem. Eng.* **27**, 68B (1973).
Siegel, M. K., Wishnick, M., Lane, M. D., in "The Enzymes", Vol. VI, 3rd ed, Boyer, P. D., Ed., Academic Press, New York, N.Y., 1972, p 169.
Singh, N., Central Food Technological Research Institute Report, Mysore, India, 1967.
Singh, N., in "Leaf Protein: Its Agronomy, Preparation, Quality and Use", Blackwell Scientific Publications, Oxford, 1971, p 131.
Singh, N., *J. Sci. Ind. Res.* **34**, 538 (1975).
Slade, R. E., Birkinshaw, J. H., British Patent 511525 (1939).
Sotomayor-Rios, Acosta-Matienzo, A., Velez-Fortuno, J., *J. Agric. Univ. P.R.* **57**, 173 (1973).
Sotomayor-Rios, A., Julia, F., Arroyo-Aguilu, J. A., *J. Agric. Univ. P.R.* **58**, 448 (1974).
Sotomayor-Rios, A., Velez-Santiago, J., Torres-Riveria, S., Silva, S., *J. Agric. Univ. P.R.* **60**, 294 (1976).
Stahmann, M. A., *Econ. Bot.* **22**, 73 (1968).
Stahmann, M. A., U.S. Patent 3975546 (1976).
Stahmann, M. A., Proceedings of the Twelfth Technical Alfalfa Conference, Overland Park, Kan., Nov 6–7, 1974.
Stahmann, M. A., Whitaker, R. W., presented in part at the 1977 Summer Meeting of the American Society of Agricultural Engineers, Raleigh, N.C., June 1977.
Subba Rau, B. H., Singh, N., *Indian J. Exp. Biol.* **8**, 34 (1970).
Telek, L., Patent Application, 1977.
Telek, L., Telek, G., Hall, N. T., Smoot, J. M., *J. Agric. Univ. P.R.*, in press (1978).
Terra, G. J. A., *Trop. Geogr. Med.* **2**, 97 (1964).
Tragardh, C., *Lebensm.-Wiss. Technol.* **7**, 199 (1974).
United Nations World Food Conference, Items 8 and 9 of the Provisional Agenda E/Conference, Rome, 1974.
Valli Devi, A., Rao, N. A. N., Vijayaroghavan, P. K., *J. Sci. Food Agric.* **16**, 116 (1965).
Vicente-Chandler, J., Caro-Costas, R., Pearson, R. W., Abruna, F., Figarella, J., Silva, S., *Agric. Exp. Stn., Univ. P.R. Bull.* **187**, 152 (1964).
Vicente-Chandler, J., Figarella, J., Silva, S., *J. Agric. Univ. P.R.* **45**, 37 (1961).
Vicente-Chandler, J., Silva, S., Figarella, J., *Agron. J.* **51**, 202 (1959).
Wang, J. C., Kinsella, J. E., *J. Food Sci.* **41**, 498 (1976).
Waterlow, J. C., *Br. J. Nutr.* **16**, 531 (1962).
Wilson, R. F., Tilley, J. M. A., *J. Sci. Food Agric.* **16**, 173 (1965).

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Retention of Minerals in Protein Isolates Prepared from Peanut Flours

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Atomic absorption spectroscopy or X-ray fluorescence was used to determine concentrations of the essential minerals Ca, Fe, Cu, Mn, Mg, Na, K, and Zn in defatted peanut flours and their retention in protein isolates. To compare the effects of variety and environmental factors on mineral content, several cultivars of peanuts grown in different areas were acetone-extracted to produce defatted flours. Portions of the flours were extracted with 10% NaCl at pH 7.0, and the extracts were dialyzed vs. water and freeze-dried to obtain the protein isolates. In general, those elements that may be associated with phytic acid in the flours are present in lower, but still significant, concentrations in the protein isolates.

Oilseeds are expected to play an important role in filling world needs for edible protein. This need is intensifying because of increasing world population and heightened awareness of nutritional deficiencies in today's diets. Previous research on oil-free meals from peanuts has emphasized protein quality and/or functional properties. Little attention has been given to mineral content, despite a 1971 USDA survey that showed the three most important areas of consumer interest were planning balanced meals, weight control, and knowledge of vitamin and mineral needs (Dwyer and Alston, 1976).

Galvao et al. (1976) determined the levels of 13 essential elements in raw peanuts and peanut butter. Derise et al. (1974) reported the levels of nine elements in raw and roasted peanuts. Conkerton and Ory (1976) reported a limited comparison of mineral contents of peanut flours prepared from Virginia and Spanish peanuts. No attempt was made to determine the effects of growing area on mineral composition of the flours or on retention of minerals in protein isolates prepared from them. This report compares eight essential mineral contents in peanut flours and protein isolates prepared from three cultivars of peanuts from four different growing areas.

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MATERIALS AND METHODS

Different peanut cultivars grown in four geographic areas were obtained. The cultivars examined and their growing locations are Florunner, Ga.; Starr, Ga.; Florigiant, Ga.; Starr, Va.; Florunner, Va.; Florigiant, Va.; Starr, Okla.; and Starr, Tex.

Peanut flours (50–60% protein content) were prepared from raw, blanched peanuts by acetone extraction. The flours represent about 50% of the seed on a weight basis and have a protein content of 50–60% (N × 5.46). Isolates (90–100% protein) were prepared from the defatted flours by 10% NaCl extraction at pH 7.0. Details of the preparation are given by Conkerton and Ory (1976) and by Conkerton et al. (1973). Minerals were determined by atomic absorption (AA) or X-ray fluorescence. The concentration of the element to be determined and sensitivity of the method for that element influenced the choice of analytical method.

The atomic absorption instrument was a Perkin-Elmer Model 306, equipped with the HGA-2000 graphite furnace, a Model 56 recorder, and a deuterium background corrector. A Cahn Model G electrobalance was used to weigh milligram amounts of the samples. The instrumental