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QUANTITY AND COMPOSITION OF AMINO ACIDS OF LEAF PROTEIN CONCENTRATES IN FIVE TROPICAL LEGUMINOUS PLANTS[1,2]

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Abstract

Five tropical leguminous plants, namely *Centrosema pubescens*, *Desmodium intortum*, *Pueraria phaseoloides*, *Pueraria thunbergiana*, and *Stylosanthes guyanensis* were extracted for leaf protein which was then concentrated to dry powder called leaf protein concentrate (LPC). The homogenizer apparatus used to extract leaf protein was very successful, and the amounts of leaf protein from these plants were compared. The quantity of leaf protein concentrates in these plants was determined by an amino acid analyzer. *Centrosema pubescens* exhibited the highest protein yield and high amino acid content as compared to these of other food stuffs. These tropical leguminou plants revealed high potentiality for leaf protein source.

Introduction

Inasmuch as rapid population growth and limited land for agriculture, protein shortage for human nutrition has become a serious problem particularly in many developing countries. Although there are many protein sources, plant protein is one of the easiest obtained sources and is very economic comparing to other sources. In 1940, Pirie initiated the study of leaf protein. Since that time many papers concerning this study have been published by a variety of journals (Akeson and Stahmann, 1966; Byers *et al.*, 1965; Chou *et al.*, 1975; Gerloff *et al.*, 1965; Pirie, 1966, 1971, 1975). This study was strongly emphasized during the meeting of the 19th General Assembly of International Union of Biological Sciences. In Taiwan, the study has been started by this laboratory and other two laboratories. Because of rich flora in Taiwan the potentiality

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for extracting leaf protein is very high. Chou *et al.* (1975) reported that the quality of leaf protein concentrates (LPC) in *Miscanthus floridulus* was as good as that in alfalfa. Liu *et al.* (1976) isolated LPC from *Astragalus sincns* (Chinese Milk Vetch), and Peng (1976, personal communication) obtained LPC from napier grass and sugar cane leaf tip. We continue the study and report our findings of the amino acid composition and quantity of leaf protein concentrates in five tropical leguminous plants in Taiwan.

Materials and Methods

Materials

Fresh leaves of five tropical leguminous plants, namely *Centrosema pubescens*, *Desmodium intortum*, *Pueraria phaseoloides*, *Pueraria thunbergiana*, and *Stylosanthes guyanensis*, were collected in summer 1976 from the experimental farm of Taiwan Liverstock Research Institute, Hsin Hua, Tainan, Taiwan.

Preparation of leaf protein concentrates

About 10 kg of each aforementioned leaves was cut into small pieces of about 10 cm long, and was immediately placed in a LPC homogenizer (Fig. 1) designed by Professor C. K. Chen (National Cheng Kung University, Tainan, Taiwan), simultaneously enough tap water was added during homogenation process. The plant juice and residue was run out and collected in an 100-liter container, then the mixture was filtered by 2 layers of cheesecloth. The filtrate was adjusted to pH 4.5 with about 300 ml to 350 ml of glacial acetic acid, which was allowed to stand for 3 hr till the precipitate was found. The upper-layer supernantant fluid was decanted, and the precipitate was stored in a freezer below 5°C. The precipitate was then centrifuged at 5000 rpm and dry out by using a Virtis lyophilizer. Thus, the green dry powder of leaf protein concentrate was obtained.

Determination of protein content in leaves and LPC

Both dry powder of leaves and LPC from the aforementioned forage legumes were analyzed by means of Kjedal digestion (Hortwitz, 1960) for determination of total nitrogen content, and the value was calculated into protein content by timing 6.25.

Amino acid analysis of leaf protein concentrates

The content of amino acids in leaf protein concentrates of tropical leguminous plants was determined by a method described by Chou, Young and Huang (1975) and analyzed with an amino acid analyzer (Yamagimoto Mfg, Model LC-5A). The data then were analyzed by the analsis of variance.

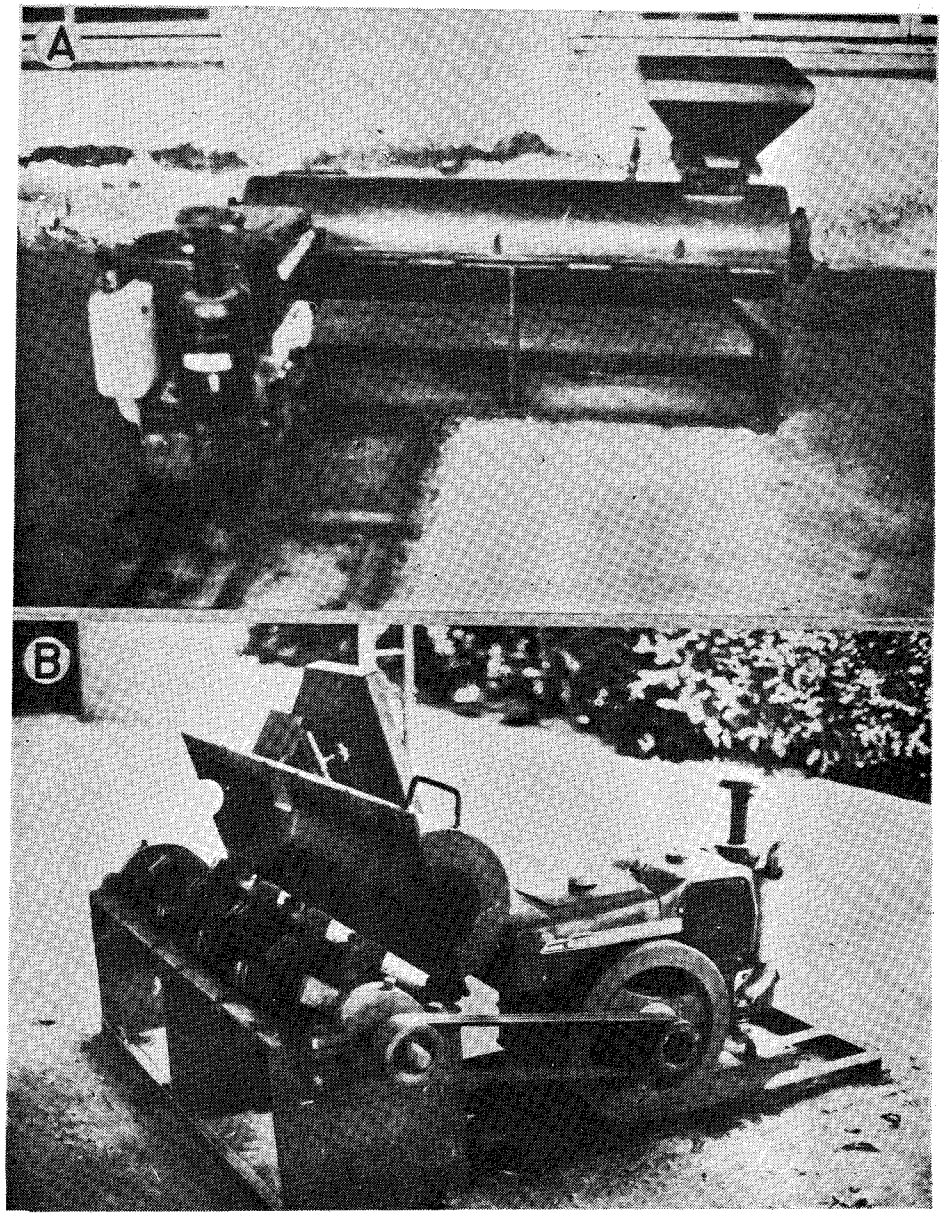

Fig. 1. Homogenizer apparatus for extracting leaf protein (A), and the cross section of the homogenizer (B).

Results

Production of leaf protein concentrates

By using the acid precipitation method described earlier, the leaf protein concentrates of the five tropical legumes were obtained. Table 1 shows that *Centrosema pubescens* and *Stylosanthes guyanensis* exhibite higher protein content on the basis of fresh weight, while *Desmodium intortum* reveals the lowest one. It was estimated that the average production of the forage legumes was at least 10,000 kg per hectare per cutting. Thus the aforementioned legumes would produce LPC ranged from 112.9 kg to 169.1 kg per hectare (Table 1). The percentage of leaf dry weight ranged from 18% to 26.2%, thus the amount of LPC by dry weight basis differed with plants. *Centrosema pubescens* still exhibited the highest protein content, while *Pueraria thunbergiana* the lowest one.

Comparing the leaf powder with LPC on dry weight basis, the protein content in the former ranged from 17.31% to 20.56%, while the latter ranged from 28.35% to 41.13% (Table 2). Among them, *C. pubescens* exhibited the highest protein content and *S. guyanensis* the lowest one.

Table 1. *Amount of leaf protein concentrates obtained from fresh leaves of five tropical leguminous plants*

Species	Fresh weight		Dry weight
	g/kg	kg/ha(1)	g/kg
Centrosema pubescens	16.91 (20.6%)(2)	169.1	82.09
Desmodium intortum	11.29 (18.0%)	112.9	62.72
Pueraria phaseoloides	15.71 (26.2%)	157.1	59.96
Pueraria thunbergiana	12.69 (25.6%)	126.9	49.57
Stylosantes guyanensis	16.43 (25.9%)	164.3	64.18

(1) About 10,000 kg of fresh leaves were produced from a hectare of experiment farm.
(2) Data in parenthesis indicated the percentage of leaf dry weight.

Table 2. *Protein content of leaf powder and LPC in dry weight basis in five tropical leguminous plants*

Species	% protein content	
	Leaf	LPC
Centrosema pubescens	20.34	41.20
Desmodium intortum	20.56	41.13
Pueraria phaseoloides	19.25	38.64
Pueraria thunbergiana	19.03	38.68
Stylosanthes guyanensis	17.31	28.35

Amino acid composition of LPC in 5 leguminous plants

By using an amino acid analyzer, the quantity of various amino acids in the five forage legumes was compared (Table 3). The amount of total amino acids is very high in *C. pubescens* (525.29 mg/g), and the following order is *P. phaseoloides*, *P. thunbergiana*, *D. intortum*, and *S. guyanensis*. Among 16 amino acids analyzed, glutamic acid, aspartic acid, glycine, valine, leucine, and phenylalanine revealed high amount, while histidine and methionine showed low amount. As an individual amino acid was concerned, the content was differed (Table 3).

To compare the quality of LPC of the aforementioned plants, the percent of each amino acid was obtained from the total amount of amino acid recovered (Table 4), the data of which were further analyzed by analysis of variance. As a single amino acid was concerned, the quantity of amino acid varied with plant species but was insignificantly different. However, the content was

Table 3. *The amino acid contents of LPC in 5 tropical leguminous plants*

Amino acid	Content, mg/g of LPC				
	CP(1)	PP	PT	DI	SG
Essential amino acid					
Methionine	10.32	8.95	8.35	10.29	5.37
Threonine	2.91	24.60	21.67	15.48	11.19
Isoleucine	30.29	26.22	23.34	30.29	13.90
Lysine	29.81	19.58	34.19	17.24	13.15
Phenylalanine	37.28	30.81	28.73	37.28	15.85
Valine	38.43	29.25	26.23	38.45	13.35
Leucine	59.17	48.64	42.54	59.17	23.34
Non-essential amino acid					
Histidine	11.60	7.44	12.41	6.20	6.20
Tyrosine	28.83	21.86	19.20	12.68	9.05
Serine	28.95	23.93	20.59	14.50	10.51
Proline	21.03	21.89	23.94	2.35	11.59
Glycine	40.71	27.62	24.77	40.71	12.91
Arginine	28.73	19.05	29.95	15.32	15.67
Alanine	37.69	30.39	27.07	37.69	13.36
Aspartic acid	63.71	52.16	44.18	31.93	22.35
Glutamic acid	60.63	58.16	50.30	33.53	27.65
Total	525.09	450.55	437.46	403.65	225.86

(1) The abbreviation of following plants are: CP=*Centrosema pubescens*, DI=*Desmodium intortum*, PP=*Pueraria phaseoloides*, PT=*Pueraria thunbergiana*, and SG=*Stylosanthes guyanensis*.

Table 4. *Comparison of amino acid composition of LPC in 5 tropical leguminous plants*

Data were expressed as % of amino acid calculated from total amino acid recovered.

Amino acid	Tropical legminous plants(1)					Other stuff		
	CP	DI	PP	PT	SG	IR-corn(2)	IR-alflafa(2)	Miscanthus(3)
Essential amino acid								
Methionine	1.97	2.55	1.99	1.91	2.38	2.10	1.90	1.44
Threonine	0.55	3.84	5.46	4.95	4.95	5.10	5.10	4.76
Isoleucine	5.77	7.50	5.82	5.34	6.15	4.90	6.60	5.79
Lysine	5.68	4.27	4.35	7.82	5.82	4.60	6.30	6.45
Phenylalanine	7.10	9.24	6.84	6.57	6.13	6.00	6.40	7.30
Valine	7.32	9.53	6.49	6.00	5.91	6.00	3.30	8.07
Leucine	11.27	14.79	10.80	9.72	10.53	10.00	9.60	10.72
Non-essential amino acid								
Histidine	2.21	1.54	1.65	2.84	2.75	1.90	2.10	2.56
Tyrosine	4.54	3.14	4.85	4.39	4.01	3.90	4.50	2.74
Serine	5.51	3.59	5.31	4.71	4.65	5.10	4.30	4.06
Proline	4.01	0.58	4.86	5.47	5.10	5.80	5.70	6.21
Glycine	7.75	10.09	6.13	5.66	5.72	5.10	4.80	4.92
Arginine	5.47	3.80	4.23	6.85	6.94	5.80	5.80	5.77
Alanine	7.18	9.33	5.62	6.19	5.92	7.40	6.40	7.84
Aspartic acid	12.13	7.91	11.58	10.10	9.90	10.00	10.20	9.18
Glutamic acid	11.55	8.91	12.91	11.50	12.24	13.20	11.40	12.18

(1) The abbreviations of following plants are: CP=*Centrosema pubescens*, DI=*Desmodium intortum*, PP=*Pueraria phaseoloides*, PT=*Pueraria thunbergianan*, SG=*Stylosanthes guyanensis*.
(2) Data after Gerloff, Lima and Stahman (1965)
(3) Data after Chou, Young and Huang (1975)

significantly different among amino acids. In the essential amino acids (Table 4), leucine revealed the highest content ranged from 9.72% to 14.79%, while methionine is the lowest one (less than 2.54). As lysine was concerned, the content was the highest in *P. thunbergiana*, *C. pubescens* the next, and *D. intortum* the lowest. In the non-essential amino acids, the content of glutamic acid and aspartic acid are mostly higher in the leguminous LPC than *Miscanthus* LPC. It is concluded that the quality of LPC in the five leguminous plants examined is better than *Miscanthus* LPC, and is as good as LPC in alfalfa and corn as far as most amino acids are concerned.

Discussion

In Taiwan, leguminous plants are highly productive due to favorable climatic condition. However, many leguminous plants are not highly utilized. The

above experiment results indicated that the forage legumes produced high protein yield and good quality of amino acids present in the aforementioned LPC. Among these five plants, *Centrosema pubescens* revealed the highest amount of protein content and the amino acid composition of leaf protein concentrate. The animal tests of these LPCs have been performed in Taiwan Liverstock Research Institute, and the results showed no detrimental effect on poultry. Nevertheless, many leguminous plants contain toxic amino acids (Bell, 1972), further animal test should be conducted. In addition, more research on removal of toxic amino acid, if any, chlorophyll, and undersirable favor, has to be done before the LPC becomes commercially available. Our present findings suggested food scientists to take this advantage to modify the aforementioned LPCs, and eventually these LPC can be edible as regular food for human or used as animal ration. This is one of ways to increase protein resource for the sake of great protein demand.

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五種熱帶豆科植物的葉蛋白濃縮物中的氨基酸組成份及量的研究

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五種熱帶豆科植物如山珠豆 (Centrosema pubescens)，營多藤 (Desmdium intortum)，臺灣葛藤 (Pueraria phaseoloides)，熱帶葛藤 (Pueraria thunbergiana)，及泰樂豆 (Stylosanthes guyanesis) 被用來萃取其植物葉蛋白。其草汁蛋白經離心冷凍乾燥後，得綠色粉末謂葉蛋白濃縮物簡稱 LPC。上述五種 LPC 經氨基酸分析儀分析後得氨基酸成份，並比較其品質。實驗結果指出山珠豆產葉蛋白量最高，且其氨基酸的量及品質均較其他四種豆科植物者爲高，又其品質亦不亞於前人用苜蓿所得的葉蛋白濃縮物者。