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QUALITY OF LEAF PROTEIN CONCENTRATES IN *MISCANTHUS FLORIDULUS*[1,2]

CHANG-HUNG CHOU, CHIU-CHUNG YOUNG
and CHIA HUANG[3]

Institute of Botany, Academia Sinica, Nankang, Taipei
Taiwan, Republic of China

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Abstract

Three fractions of leaf protein concentrate (LPC) from *Miscanthus floridulus* were investigated. They are called the residue 100-mesh LPC, heat precipitate LPC, and acid precipitate LPC. These leaf protein concentrates were determined for their fiber content, crude protein yield, and amino acid composition. Among these concentrates, the acid precipitate LPC gave the highest protein yield (about 47.30 kg per hectare per harvest in fresh weight basis) and the lowest fiber content (8%, dry weight basis). Comparing the acid precipitate LPC with other foodstuffs, it was shown that the quality of protein in the former was as good as that in other foodstuffs. It is possible to use this grass as a new source for leaf protein concentrate.

Introduction

Protein demand for human nutrition has tremendously increased particularly in many developing countries. Faced with a limited amount of land for agriculture and a rapid population growth, scientists have made a great effort to find some new nutrition sources to prevent millions of people dying from starvation and illness from protein deficiency. An extensive study based on leaf protein concentrate (LPC) has been centered at the University of Wisconsin, Madison, where a group of scientists including biochemists, agronomists, agricultural engineers and nutrition scientists are involved. Their experimental findings were published in a variety of journals (Koegel and Bruhn, 1972; Russell, Jorgensen and Barrington, 1974; Smith, 1974; Stahmann, 1968). Stahmann and his associated reported the results of qualitative comparison of protein in leaf protein concentrates of many green plants (Akeson and Stah-

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mann, 1965; 1966; Gerloff, Lima and Stahmann, 1965; Oelshlegel, Schroeder and Stahmann, 1969; Lima, Richardson and Stahmann, 1965; Stahmann, 1968).

Although many herbaceous plants were used as the source for leaf protein concentrate, *Miscanthus floridulus*, a native dominant grass in Taiwan has never been used as a source. This grass has a great potentiality for making leaf protein concentrate because of its ubiquitous distribution in most of mountain hill in Taiwan (Chou and Chung, 1974). The grass has rarely been used as a forage due to its high fiber content. However, the protein constituent of *Miscanthus* was thought to be good comparing to other forages in Taiwan. It is possible to obtain leaf protein concentrate from it when the extraction techniques are established. Therefore, it is the aim of this study to evaluate the quality of protein and amino acids in the leaf protein concentrates of *Miscanthus* obtained from different extraction techniques.

Material and Methods

Preparation of leaf protein concentrates

Fresh leaves of *Miscanthus floridulus*, harvested at about one meter high, were chopped into piece about 2.5 cm long. One kilogram of the chopped leaves was added with 3 liters of distilled water, then the mixture was homogenized with a commercial waring blendor at middle speed for 5 min. This homogenized mixture was then filtered with two layers of cheese cloth. The filtrate collected was stored in a freezer before using, and the residue was allowed to dry in a dryer. The dried residue was further sieved with a 60-mesh screen and with 100-mesh later. The stuff passed through the 100-mesh screen was collected as a fraction of leaf protein concentrate called "residue 100-mesh LPC".

The filtrate stored was divided into two parts. One part was used for heat treatment, another part for acid treatment. To one liter of filtrate, 10 ml of glacial acetic acid were gradually added and adjusted the suspension to pH 5. Most of protein was precipitated, and the supernantant was carefully decanted. The precipitate collected was further centrifuged at 6,000 rpm, then the pellet was dried overnight with a Virtis lyophilizer. The fine green powder thus obtained was designated as "acid precipitate LPC".

The other part of filtrate of miscanthus extract was heated at temperature below 80 C. Most of protein was precipitated. Using the same techniques as described above, the fine powder of precipitate was obtained. This fraction of protein concentrate was called "heat precipitate LPC". The procedure of preparation of leaf protein concentrates is outlined in Fig. 1.

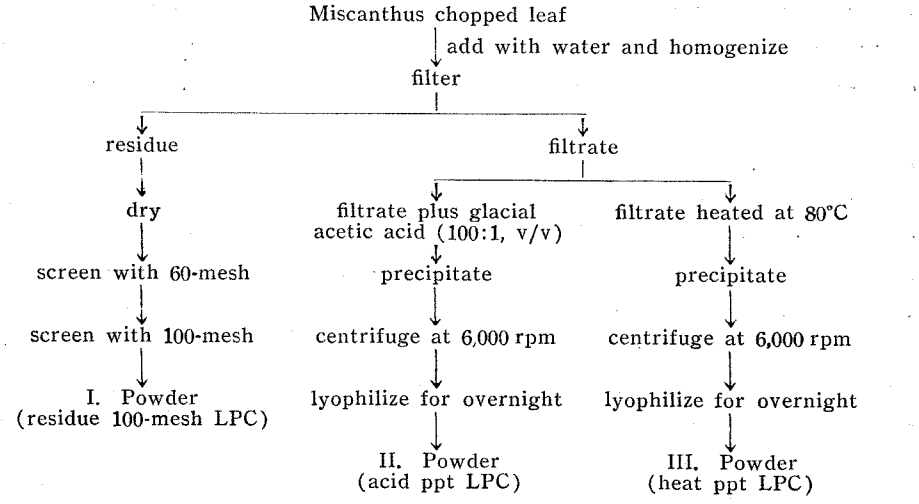

Fig. 1. A general scheme for preparation of miscanthus leaf protein concentrates.

Determination of fiber and crude protein content of miscanthus LPC

The dry samples of the residue 100-mesh LPC, heat precipitate LPC, and acid precipitate LPC were determined their fiber content by using the techniques described by van Soest (1963). The crude protein contents of these samples were analyzed by means of Kjedahl digestion (Horwitz, 1960).

Amino acids analysis of leaf protein concentrates

To 60 mg of each dry sample of miscanthus LPC, 25 ml of 6 N HCl was added for acid hydrolysis. The mixture was set in vacuo, then was heated at 108 C for 24 hr. After hydrolysis the solution was filtered with filter paper where the residue on the paper was washed with a small volume of 0.5 N HCl four times. The final volume of filtrate was built up to 25 ml with 0.5 N HCl. One milliliter of the filtrate was dried completely and then dissolved in 1 ml of 0.2 sodium citrate buffer with pH at 3.25, or 4.25, or 5.28 subsequently, depending on the character of amino acids analyzed. The solution was then analyzed for amino acid composition by using an amino acid analyzer (Amino acid analyzer model LC-5A, Yamagimoto Mfg., Co. Ltd.) The data of amino acid content in three miscanthus LPC was further analyzed statistically by the analysis of variance.

Results

Fiber and crude protein content in miscanthus LPC

The amount of fiber and protein content was determined by using the dry samples. The fiber content in these leaf protein concentrates is different; the residue 100-mesh LPC has 32.4% fiber, the heat precipitate LPC 22.97%,

and the acid precipitate 8.0%. On the other hand, the protein content in the residue 100-mesh LPC, heat precipitate LPC, and acid precipitate LPC is 8.74%, 21.69%, and 37.84%, respectively. It is clear that the acid precipitate LPC contains a great amount of protein, while the other two are much low in protein content. From 10,000 kg of fresh miscanthus leaves, about the production of a hectare per harvest, there is about 100 kg of fresh concentrate from the heat precipitate method and 125 kg from the acid precipitate method. Thus, the crude protein yield on fresh weight basis was about 384 kg (10,000 kg ×3.84%), 21.69 kg (100 kg×21.69%), and 47.30 kg (125 kg×37.84%) in fresh leaves, the heat precipitate LPC, and the acid precipitate LPC, respectively (Fig. 2). Only a small amount of protein goes to the fraction of the residue 100-mesh LPC (about 16.43 kg) comparing to the total crude protein yield in the fresh leaves. The rest of protein, out of these three protein concentrates, must be remained in the unharvestable portion of leaves extract. However, it is clear that the acid precipitate method provides a relatively high amount of protein from miscanthus leaves and the fiber content is low.

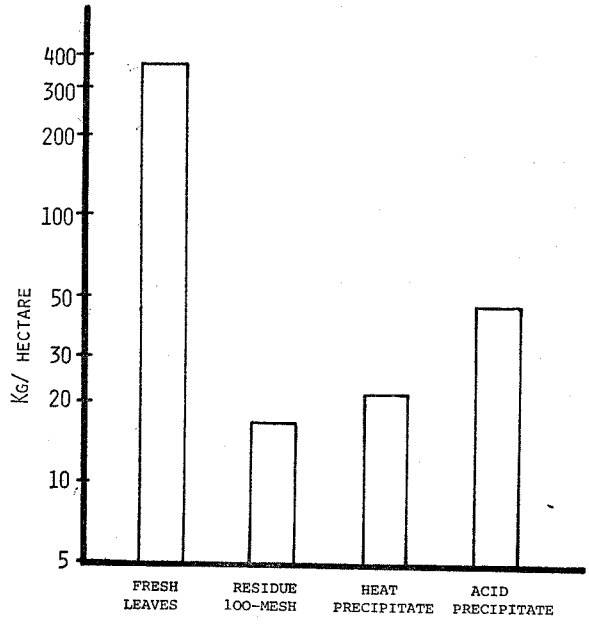

Fig. 2. The amount of protein yield in the fresh leaves, residue 100-mesh LPC, heat precipitate LPC, and acid precipitate LPC of *Miscanthus floridulus* after a harvest.

Amino acid composition of miscanthus protein concentrates

By using an amino acid analyzer, the amounts of various amino acids in these three fractions of miscanthus leaf protein concentrates were determined.

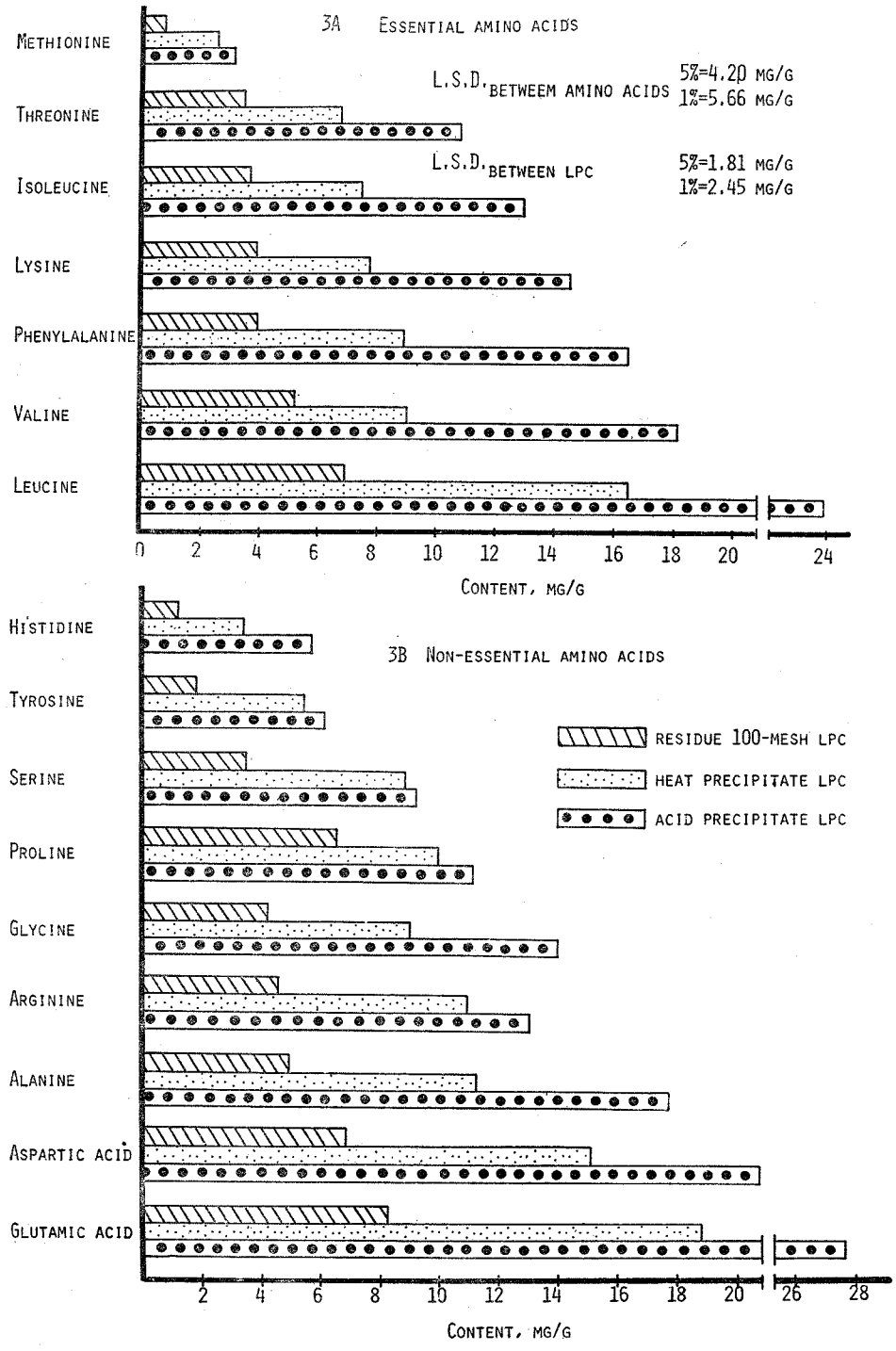

Fig. 3. Quantitative comparison of amino acid content in three fractions of miscanthus leaf protein concentrates of residue 100-mesh LPC, acid precipitate LPC, and heat precipitate LPC.

Results expressed as milligram per gram of leaf protein concentrate are shown in Fig. 3A and 3B. It was found that the amount of amino acids in the acid precipitate LPC was significantly higher than those in the residue 100-mesh LPC and the heat precipitate LPC. Among these essential amino acids, the content of leucine is generally higher than other amino acids, while methionine is the lowest one (Fig. 3A). On the other hand, glutamic acid exhibits the highest content and histidine is the lowest one among these non-essential amino acids (Fig. 3B).

Qualitative comparison of miscanthus LPC and other foodstuffs

To compare the quality of miscanthus leaf protein concentrates with those of other foodstuffs, such as IR corn, IR alfalfa, milk, cheese, and egg, the per cent of each amino acid was calculated from the total amount of amino acids

Table 1. *Comparison of amino acid composition in three miscanthus leaf protein concentrates and in other foodstuffs.*

Data are expressed as percentage of amino acid calculated from total amino acid recovered. Data between each protein concentrate are not significantly different by using the analysis of variance.

Amino acids	Miscanthus LPC			Other foodstuffs				
	Residue 100-mesh	Acid precipitate	Heat precipitate	IR(1) corn	IR(1) alfalfa	Milk(2)	Cheese(2)	Egg(2)
Essential amino acid								
Leucine	9.94	10.72	10.86	10.0	9.6	11.3	8.9	4.2
Valine	7.43	8.07	5.92	6.5	6.3	8.5	7.7	7.3
Phenylalanine	5.92	7.30	5.75	6.0	6.4	5.7	6.5	6.3
Lysine	5.64	6.45	5.14	4.6	6.3	8.2	8.5	7.2
Isoleucine	5.41	5.79	4.96	4.9	6.6	8.5	7.3	8.0
Threonine	5.10	4.76	4.52	5.1	5.1	4.5	3.6	4.3
Methionine	1.03	1.44	1.15	2.1	1.9	3.4	3.6	4.1
Non-essential amino acid								
Glutamic acid	11.77	12.18	12.37	13.2	11.4	—	—	—
Aspartic acid	9.73	9.18	9.92	10.0	10.2	—	—	—
Alanine	7.13	7.84	7.45	7.4	6.4	—	—	—
Arginine	6.48	5.77	7.21	5.8	5.8	4.3	4.2	6.4
Proline	9.36	4.92	7.02	5.1	4.8	—	—	—
Glycine	6.10	6.21	5.92	5.8	5.7	—	—	—
Serine	4.93	4.06	5.80	5.1	4.3	—	—	—
Tyrosine	2.50	2.74	3.52	3.9	4.5	5.3	6.9	4.5
Histidine	1.71	2.56	2.31	1.9	2.1	2.6	3.0	2.1

(1) Data from Gerloff, Lima and Stahmann (1965).
(2) Data from Block and Weiss (1956).

recovered (Table 1). Besides the data of milk, cheese, and egg, the rest of data shown in Table 1 was statistically analyzed by using the analysis of variance. As far as a single amino acid is concerned, the quality of amino acids in the miscanthus LPC is not significantly different from those of other foodstuffs. However, there was significant difference among the amino acids (Table 1). It is obvious that the quality of amino acids among the miscanthus LPC, IR corn, and IR alfalfa is about the same, sometimes the quality of the former is even better than other stuffs mentioned. For example, the per cent of leucine, valine, phenylalanine, lysine, glutamic acid, alanine, proline, glycine, and histidine is higher in the miscanthus acid precipitate LPC than in IR alfalfa, although many essential amino acids present in animal protein of cheese and egg are in high content. Thus it is concluded that the quality of miscanthus LPC, specially the acid precipitate LPC, is as good as other leaf protein concentrates. In fact, corn and alfalfa are the most important nutrition sources for animal. It is possible that we may use the miscanthus as a new nutrition source for animal ration.

Discussion

The above experimental results clearly showed that *Miscanthus floridulus* has a high potentiality for providing leaf protein concentrate. Among most of green plants, the amount of leaf protein in *Miscanthus* is about in the average. Thus, it is possible to use the miscanthus leaf as a new protein source. However, the difficulty is based on the extraction techniques. At the present, the acid precipitate method, provided high protein and low fiber, is a good one since the quality of amino acid composition is as good or even better than other foodstuffs (Fig. 3 and Table 1). The total amount of amino acids in the acid precipitate LPC (226.35 mg/g) is significantly higher than that in the heat precipitate LPC (152.08 mg/g), and the total crude protein harvested from the fresh leaves is also much higher in the former (47.3 kg/hectare) than the later (21.69 kg/hectare). Furthermore, the fiber content in the acid precipitate LPC (8%) is much lower than that in the heat precipitate LPC. Therefore, it is concluded that the acid precipitate LPC has better quality than the heat precipitate LPC and the residue 100-mesh LPC.

A series of experiments has been performed by using the residue 100-mesh LPC mixed with regular ration as material to feed mice. The results of this animal test showed that there was no detrimental sympton occurred (Wan, Chou and Young, 1975 unpublished data). Experiments of animal test by using the acid precipitate LPC as ration source are in progress.

Nevertheless, research on the removal of chlorophyll, undesirable favor, and reducing fiber content has to be carried out by improving extraction

techniques. Our present findings would be a good start for developing new techniques and also would be able to suggest to industry worker to make the mass production of miscanthus protein concentrate. As far as the necessary experiments, such as animal test and purity of the concentrates, have done, the miscanthus leaf protein concentrates would eventually be used for soup mate or any kind of nutrition source to meet the great demand of protein in many developing countries.

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五節芒中葉蛋白濃縮物的品質研究

周昌弘 楊秋忠 黃 嘉

中央研究院植物研究所

新鮮的五節芒葉子經打碎過濾後得草汁，此草汁經加酸，或加熱處理後得綠色沉澱物，再將此沉澱物離心及冷凍乾燥後得粉末，此謂之葉蛋白濃縮物（Leaf Protein Concentrate）。分析它們的纖維、粗蛋白、及十六種氨基酸的含量得知，由酸處理後所得的葉蛋白濃縮物的品質最好。此濃縮物與其他植物如苜蓿或玉米等的葉蛋白濃縮物比較其品質知五節芒的葉蛋白濃縮物品質很好。它很可能用來提高飼料蛋白的好材料來源之一。