SOME TRIALS FOR THE EXTRACTION OF PROTEIN FROM CASSAVA (*Manihot esculenta* L.) LEAVES

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ABSTRACT

Cassava is widely planted in tropical and subtropical areas. The leaves are mostly waste after the harvest of tubers. In the hope of effective utilization of this leaves, some trials for extraction of leaf protein were conducted.

The crude protein content of cassava matured leaves were varied according to their varieties, ranging from 2.77 to 11.88% (wet basis) or 8.11 to 35.75% (dry basis). The top shoot young leaves contain higher crude protein than the matured ones.

Extractability of leaf protein was tested by several trials. Results indicate that cassava leaf protein is difficult to extract by simple mechanical treatment. The highest extractability was attained by using "lumpang" as macerater and 0.2% NaOH solution containing 0.1% Mercaptoethanol and 0.5% Sodium Lauryl Sulfate (SDS) as extractant, at 80% recovery. Acetone Dried Powder of leaves resulted in lower extractability than the raw material.

Cyanide content in juice were varied from 11 to 37 mg/100 ml, which is higher than the safety level for food or feed products. Cyanide was not easily eliminated by simple heating of the juice.

INTRODUCTION

In the developing tropical countries, protein deficiency is widespread because diets generally are deficient in quality and in quantity of protein, especially in areas where cassava is a staple food. Green leaves usually also have a large amount of fiber. Then, if it is possible to isolate protein from the green leaves, it will become a potential source of edible protein.

Researches on protein isolation from leaves have been carried out for many years since 1965, especially using alfafa and the other leaves, as by Devi[1], Archcoll[2], Buchanan[3], Pirie[4], Sentheskan and Durand[5], Bestchart and Kinsella[6], Yasui and Yoshizawa[7], and many others. The report on the isolation of protein from cassava leaf, however, is very few.

Cassava is widely planted in tropical and subtropical areas as well, and as a C_3 plant containing more leaf protein fraction compared with a C_4 plant. Martin *et al.*[8] identified cassava leaves as one of the potential candidates for LPC.

In Indonesia, cassava is also widely planted mainly for the production of starch from tubers, called "tapioka". However the leaves are mostly wasted after the harvest of the tubers, although young leaves are consumed partly as vegetables in local food customs. Therefore, in order to use this leaves effec-

tively as a source of leaf protein, in this experiment, the protein extractability was tested in detail.

MATERIALS AND METHODS

Materials

Cassava (*Manihot esculenta* L.) leaves were obtained from plots in Muara and Cimanggu (Bogor Research Institute for Food Crops), Bogor, West Java, Indonesia.

The harvested leaves were immediately removed from the stem, then were treated according to the experimental procedures. Some samples were kept at -70°C until use. The cassava plants were 5—7 months old. The young top shoot leaves and well-matured leaves were separated from each other. For the extraction trials, well-matured leaves were used.

Methods

(1) Method for extraction of juice by mechanical pressing: Extraction of juice from cassava leaves was performed according to the method shown in Fig. 1.
(2) Several trials for extraction of protein from cassava leaves: Extractability of protein from cassava leaves was tested by the methods shown in Fig. 2.
(3) Extraction of protein from Acetone Dried Powder of Leaves: Cassava leaves were homogenized in cooled aceton at -20°C After filtration, the residue was repeatedly washed by adding aceton 4 times. Aceton remaining in the residue was removed under reduced pressure. Extraction of protein from Acetone Dried Powder (ADP) is shown in Fig. 3.
(4) Methods for Chemical Analysis.

Analysis of nitrogen content: Total nitrogen content was determined by the standard semi-micro Kjeldahl method. Samples were hydrolyzed by heating with H_2SO_4 containing a catalyzer (K_2SO_4 + HgO). In destilation steps; the destilate was collected in 4% boric acid solution, then titrated with 0.01 N HCl using BCG and MG mixture as an indicator. Crude protein content was calculated by multiplying 6.25 to the nitrogen content.

Determination of cyanide: The cyanogenic glycoside present in cassava leaf is hydrolyzed to free cyanide by enzyme reaction, then followed by spectro-photometric determination of cyanide content according to Cooke *et al.*[9] method.

Juice from cassava leaves was obtained by pressing well crushed leaves without any addition of reagents. Linamarase was prepared from cassava roots as follows: Cassava roots was homogenized in 0.1 M acetate,

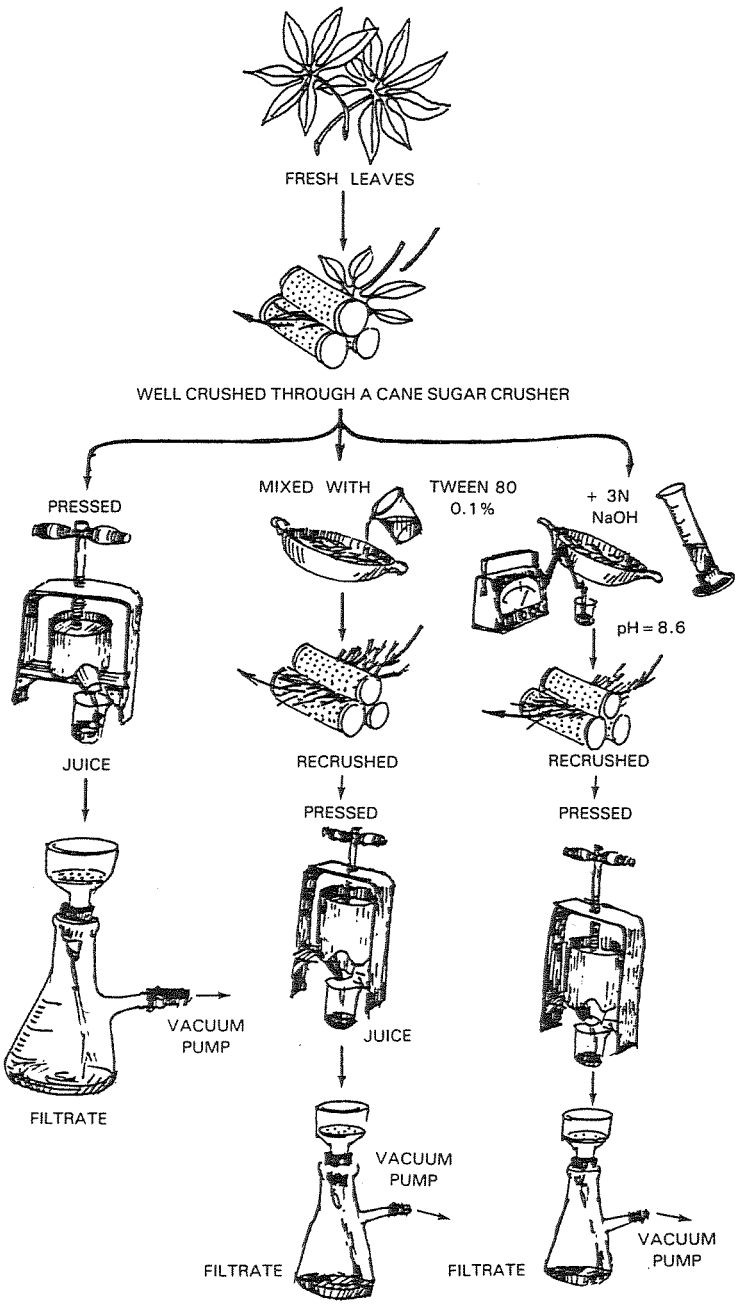

Fig. 1. Method for Extraction of Juice by Mechanical Pressures.

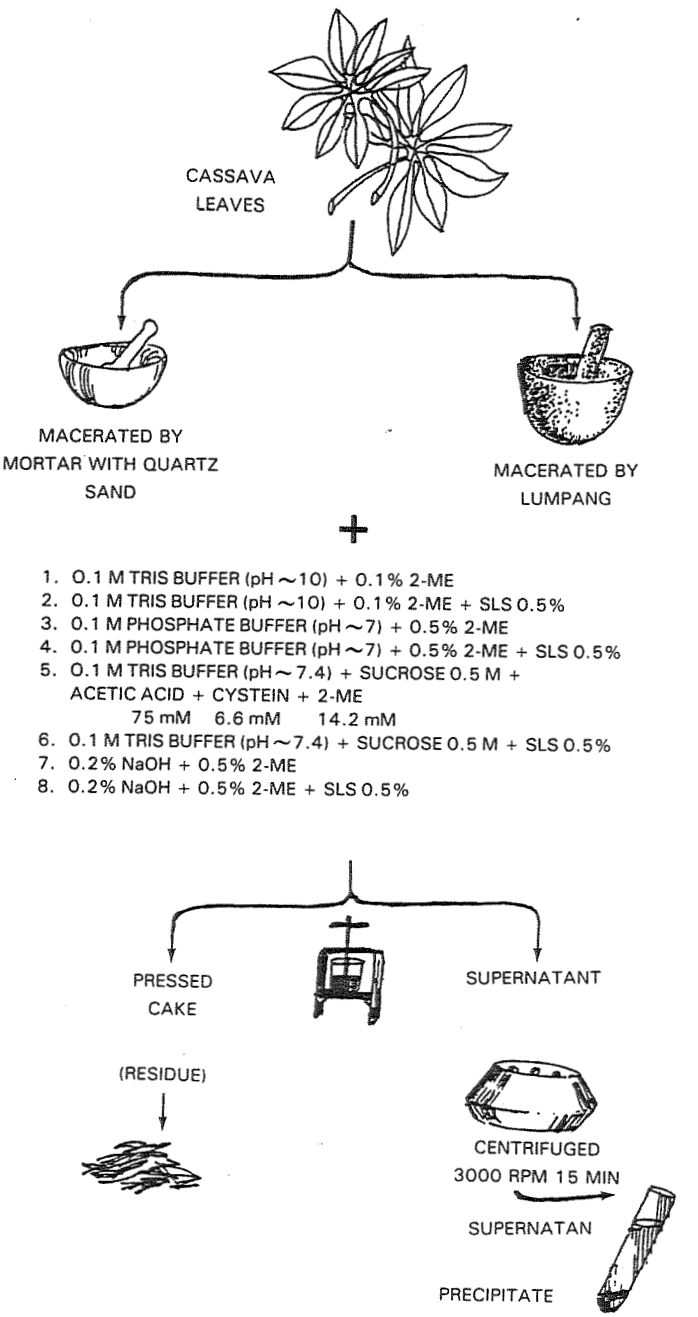

Fig. 2. Several Trials for Extraction of Protein from Cassava Leaves.

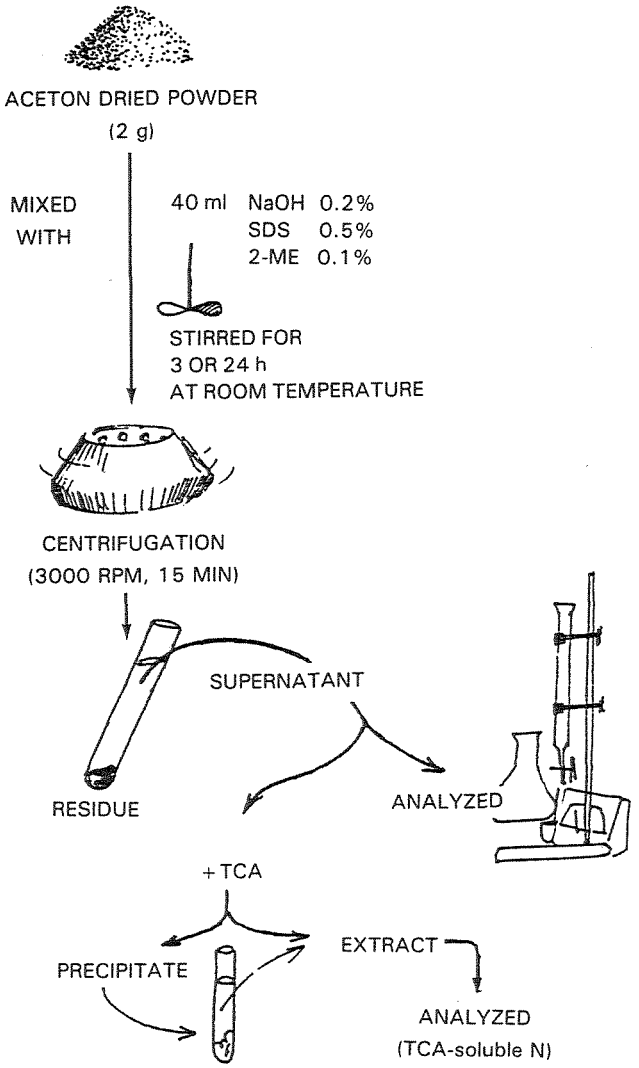

Fig. 3. Extraction of Protein from Acetone Dried Powder.

then filtered. The filtrate was brought to 70% saturation of $(NH_4)_2SO_4$. The precipitate containing enzyme was collected by centrifugation, then dialyzed, lyophilized and freeze dried.

The CN in cassava leaf juice was freed by incubating it with 0.1 ml of enzyme solution (30 mg/ml) and 0.5 ml of the juice at 30°C for 30 minutes. The reaction was stopped by adding 0.6 ml of 0.2 N NaOH. The cyanide

released was estimated as follows: 6.8 ml of 50 mM NaPi pH 6 was added to the tubes followed by 0.4 ml of 0.5% (w/v) chlroramine T. The tubes were mixed and placed in iced water for 5 min. 1.6 ml of a soln containing 0.2 g bispyrazolone and 1 g of 3-methyl-1-phenyl-5-pyrazolone in Py (200 ml) was then added and mixed. The blue colour developed after storage for 90 min at room temp. was measured at 620 nm.

Standard curve was prepared by using NaCN.

Determination of moisture content. The moisture content of cassava leaves was determined by drying samples (Ca 3 g) for 3 hrs at 110°C in an air oven.

RESULTS AND DISCUSSION

Protein Content of Cassava Leaves

The moisture and crude protein content of cassava leaves, are shown in Table 1. The crude protein of cassava leaves varied according to their varieties, ranging from 2.77 to 11.88% (wet basis), or about 8.11 to 35.75 by percentage of dry matter (dry basis). These contents are quite similar with the formerly reports which are 31.6% (Hall *et al.*[10]), 26.8% (Pirie[4]), 24.1% (Scientific Research Council of Jamaica[11]), (all in dry basis), 7.34 to 8.34% (Somaatmadja D. *et al.*[12]) 6.8% (Directorat of Nutrition of Public Health Indonesia[13]) (in wet basis), etc. There were no significant differences in protein content between the bitter varieties (e.g. WL_{54}, W_{20}) and the sweet varieties (e.g. A_{20}, M_{31}, A_5).

The content of protein is higher in the young top shoot leaves than the matured ones. It indicates that if the leaves can be harvested at an early age, it is suitable as the low cost material for the extraction of protein.

Table 1. Moisture and total crude protein content of cassava leaves.

Varieties	MOISTURE CONTENT (%)		TOTAL CRUDE PROTEIN (%)	
	Young	Old	Young	Old
WL_{54}	–	69.73	–	7.46
G_{168}	70.64	65.67	8.43	8.39
A_{20}	74.49	69.52	10.27	5.95
A_5	–	68.94	–	7.86
$Aldira_1$	–	66.77	–	11.88
M_{30}	–	65.85	–	2.77
M_{31}	–	67.72	–	8.60
$W_{236\text{-}20}$	73.91	64.54	10.30	7.25
$W_{236\text{-}19}$	–	73.78	–	4.42

Trials for The Extraction of Cassava Leaf Protein

First, cassava leaves were crushed and pressed, then the juices were analyzed for the protein content. The results obtained are given in Table 2.

Table 2. Total crude protein content of juices and the extraction by mechanical pressure.

Samples	Volume of juice (mg/kg)	Total Crude Protein (g/100 ml)	Total Crude Protein (g/100 g leaves)	% Extractability
Raw Leaves	—	—	7.274	(100)
Juice (No additives)	333	0.534	0.178	2.45
+ NaOH 3N to pH 8.6	400	0.345	0.138	1.90
+ Tween 80 (0.1%)	400	0.415	0.166	2.2

The extraction by this simple method, using pilot plant scale mechanical crusher and presser, without any addition, didn't give any satisfactory protein extractability. Addition of 3 N NaOH until pH 8.6 or 0.1% tween 80 into the raw material after the first crushing showed no improvement on the rate of protein extraction. A slight difference of extractability might be caused by the differences in the crushing and pressing rate in each treatment. Data indicates that cassava leaf protein is difficult to extract with a simple mechanical treatment.

Extraction was conducted by crushing the cassava leaves in mortar with addition of several additives which are expected to promote the protein release.

Table 3 shows the effect of several combinations of additives on the extractability of cassava leaf protein. The additives used are chemical compounds which worked as reducing agents, disulfide splitting agent, and detergent; e.g. 2-Mercaptoethanol, ascorbic acid, SDS, etc. The pH arrangement by NaOH or Tris or Phosphat buffers were also tested.

In several combination of additional agents, the highest extractability was achieved by using 0.2% NaOH solution containing 0.5% 2-ME and 0.5% SDS, especially macerated by "lumpang". In the case of pH arrangement, only at very high pH level gave good results on the extraction of the cassava leaf protein, although Pirie[4] reported that leaf protein was easily separated from fibrous fraction, if the pulp is made slighty alkaline (Ca. pH 8.2).

The high effect of 0.2—0.4% NaOH solution containing 0.1% 2-ME and 0.5% sorbitan monolaurate (surface active agent like SDS) to extract the protein materials has been reported by Yasui *et al.*[14], who can extract the protein as follows: spinach 87%, cabbage 86%. Japannese reddish 83%, soybean leaf 60% and so on.

Table 3. Several trials on extraction of cassava leaf protein.

No. of Samples	Total Crude Protein (g/100 g leaf)				% Extractability (extracted crude protein/crude protein in frozen leaf)			
	Mortar		Lumpang		Mortar		Lumpang	
	A_5	A_{20}	A_5	A_{20}	A_5	A_{20}	A_5	A_{20}
1	0.32	–	–	–	4.84	–	–	–
2	2.50	–	–	–	38.39	–	–	–
3	0.85	0.23	1.86	0.56	13.06	4.08	28.60	10.13
4	2.09	1.04	4.47	3.48	32.18	18.77	68.68	65.51
5	0.39	–	–	–	5.98	–	–	–
6	1.17	–	–	–	17.96	–	–	–
7	1.46	1.05	1.86	1.46	24.44	18.81	28.58	26.15
8	3.75	2.49	5.24	4.21	57.58	44.68	80.49	75.74

Tris buffer solution either at pH 10 containing 2-ME only, or pH 7.4 containing sucrose, reducing and disulfide agents didn't increase the extractability significantly, in spite of Betschart and Kinsella[6] could extract 60.8% total nitrogent from alfafa leaves with this extractant.

In almost all of the combinations, the presence of SDS gave very good effects on the extractability. The higher extractability in this case may be due to the disruptive activity of SDS as surface agent to lysis the cell wall.

The method for maceration affected the results. The conventional Indonesian mortar, "lumpang", gave better extractability than the laboratory mortar with sand quarzt; probably it has enough rough surface, that cell walls will be ruptured well.

Data in Table 3 also show that the extractability varies due to the variety of cassava, but have still a similar tendency.

To avoid the chemical changes while extraction, cassava leaves were also treated first by dehydration with cold aceton into Acetone Dried Powder (ADP). The extraction rate of leaf protein from acetone powder is shown in Table 4.

Table 4. Protein content in aceton dried powder of cassava leaves and their extracts.

Samples	Crude Protein (g/100 g ADP)	Pure Protein (g/100 g ADP)	% Extractability (crude prot./crude prot. in frozen leaf)
ADP	20.77 (100%)	18.39 (100%)	(67.08)
Juice of 3 hrs stirred	11.43 (55.03%)	9.89 (53.78%)	36.91
Juice of 24 hrs stirred	12.07 (58.11%)	10.36 (56.33%)	38.98

As the extractant of the protein from ADP, 0.2% NaOH solution containing 0.1% 2-ME and 0.5% SDS (the best extractant in former trials) was used. The ratios of extracted protein were lower than those directly extracted from raw materials.

The decrease in extractability is supposed to be caused by the effect of aceton, which may changes the molecul structure of several protein in leaves. The other reason of this decrease is the lower protein content in ADP itself compared with the raw one.

Three and twenty four hours stirring in reagent solutions didn't give a significant difference in extractability of protein.

Practically, the acetone treatment is effective for long term preservation of raw material, but it is impractical because of its high cost.

Free-Cyanide Content in Cassava Leaf Juices

Some varieties of cassava plants have been known to contain cyanide compound both in their tuber or in their leaves. According to Daryanto and Murjati[15] report, the cyanide content is higher in the leaf than in the tubers. The presence of this component in high level is harmful for health.

The free-cyanide in juice extracted from cassava leaves by simple pressure are given in Table 5. The content were varied from 11 to 36 mg/100 ml, which are higher than the safety level of free-cyanide content in food or feed products, shown in the work of Koch, Bolhuis and Coursey. According to their guideline, moderate toxic of cyanide is 50—100 ppm (5—100 mg/100 ml) and more than 100 ppm is dangerous toxic. This extract is therefore dangerous to be used directly without any treatment.

Table 5. Free cyanide content in juices from several cassava leaves.

No. of Samples	CN Content (mg/100 ml)
1	25.73
2	35.55
3	36.66
4	33.16
5	14.59
6	11.41
7	15.22

Unfortunately this cyanide wasn't easily eliminated by a simple heating of juice at 80°C and 100°C for 10 minutes (Table 6). By heating at higher temperature, however, a little more amount of cyanide was eliminated.

Table 6. Effect of heating on CN content in juice.

TREATMENTS	CN Content (mg/100 mg)	CN Remaining (%)
no heating	35.55	100.00
80°; 10 min	35.23	99.25
100°; 10 min	31.84	89.55

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