PRELIMINARY STUDIES ON WINGED BEAN-LEAF PROTEIN CONCENTRATES

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SUMMARY

Leaf protein concentrates (LPC) were prepared using immature, leaves and stems of an indigenous Sri Lankan cultivar of winged bean (SLS-40). Decolourization of the leaves was attempted by various solvent extractions to obtain a white protein concentrate, and ethanol (95%, V/V) gave the best results. The off-white LPC of winged bean contained 59.73% protein, 0.71% fat, 5.13% fibre and 10.41% minerals on a dry weight basis. The protein content of winged bean LPC was found to be greater than that of LPC from alfalfa, Sesbania grandiflora and Spinacea oleracea. However, the LPC of winged bean contained greater quantities of fibre compared to other leaf protein concentrates. Mineral content of LPC of winged bean was estimated using atomic absorption spectrophotometry and it contained 209 mg calcium, 321 mg potassium, 37 mg magnesium, 60 mg iron, 5 mg sodium and 229 mg phosphorus per 100 g dried LPC. However, leaching of most of the minerals, except iron, occured during the processing of LPC. The trypsin inhibitor in LPC was determined to be 1.65±0.73 units per mg dry LPC. Due to the heat treatment during the processing of LPC there was an appreciable reduction in the activity of trypsin inhibitors. The LPC showed higher in-vitro digestibility (87.36±1.51%) compared to that of the leaves (80.05±1.27). The greater protein dispersibility index for LPC (10±0.8%) compared to that of the leaves (2±0.5%)indicated greater concentration of soluble proteins in the LPC and lesser protein denaturation during processing.

The LPC of winged bean contained most of the essential amino acids in greater quantities (g/16 g N) compared to those in the alfalfa LPC. The methionine content in the winged bean LPC, was found to be almost double that of the soybean meal.

INTRODUCTION

Malnutrition is a mojor international problem. Food and Agriculture Organization (FAO) estimated that the number of severely undernourished people in the developing nations (excluding China and Japan) increased from about 360 million in 1969-1971 to about 500 million (22% of the population in these countries) in 1974-1977 (Economic Review, 1982). It has been estimated that some 100 million children aged 1 - 4 years are undernourished in Latin America, Africa, and the Asian countries (WHO Chronicle, 1974). A nutritional survey (1980-1982) carried out by the Food and Nutrition Policy Planning Division (FNPPD), Ministry of Plan Implementation, Sri Lanka has identified serious acute undernutrition, especially in the estate population (FNPPD, 1982).

As the world population increases at an accelerated rate with more mouths to feed, less grain will be available for animal feeds. Since conventional sources of protein are increasingly in short supply, paying attention to the development of unconventional protein sources such as leaf protein concentrates (LPC) becomes vital. Plant leaves are apparently available in vast quantities and offer the highest yield of protein of all crops. The protein production is said to be actually doubled in a given area by leaf protein fractionation (Houseman et al., 1975).

Several studies have been made in the past to produce and utilize LPC mostly from alfalfa, in human as well as in animal nutrition (Hartman et al., 1967; Kohler et al.,

1968; Stahmann, 1968; Oelshlegel et al., 1969; Pirie, 1978; Edwards et al., 1975). Waterlow (1962) reported that infants recovering from protein malnutrition tolerated LPC added to a formula in which milk protein provided 50 to 75% of the protein.

The tropical legume winged bean (Psophocarpus tetragonolobus L. DC.), with a promising future consisting of protein rich foliage (Masefield, 1961) is a potential source for leaf protein. The leaves of winged bean plants are a popular food item in many south east Asian countries, where they are eaten raw or cooked and added to salads and soups (Claydon, 1975). Okezie and Martin (1980) reported that the winged bean leaves of seventeen varieties analyzed contained 28-36% protein (dry weight basis). Senanayake and Sumanasinghe (1976) observed that the local Sri Lankan winged bean leaves contained 38.7% protein. The protein content of the leaves of winged bean is greater when compared to cassava and many other dark green tropical leaves (Platt, 1962).

The purpose of this study was to extract LPC from the leaves of a Sri Lankan cultivar (SLS-40) and to determine its proximate and mineral composition. The trypsin inhibitor levels, in-vitro digestibility, protein dispersibility index and essential amino acids profile of the LPC were also investigated.

MATERIALS & METHODS

Materials

SLS-40 was selected to be used in this study since it was observed to have profuse leafy growth. Fresh young and immature leaves with tender twining stems were utilized for the extraction of protein.

Methods

Extraction of leaf protein

The procedure adopted for the extraction is given in Fig. 1. Decolourization of the leaves was carried out with various organic solvents and ethanol gave the best results. Salt was added to preserve the precipitated protein and the final product was an off-white coagulum of leaf protein.

Proximate analysis

Analysis of moisture, protein, fat, fibre and ash was performed on the leaf protein concentrate and the leaves of winged bean (SLS-40). Methods provided in the Official Methods of Analysis of the AOAC (1975) were followed.

Mineral composition

Fat-free samples of leaves and LPC of winged bean were analyzed for the minerals by atomic absorption spectrophotometry as outlined in AACC (1976). Samples were prepared by the "wet ashing" method (AOAC, 1975). A Varian Techtron Spectrophotometer (model 635) was used for the study.

Determination of trypsin inhibitor activity

Crude winged bean extracts from LPC and leaves were prepared for trypsin-inhibitor assay, by homogenising 1.0 g sample in a blender with 50 ml phosphate buffer (0.1 M, pH 7.6. This stock suspension was centrifuged at 1,500 x 1 g for 10 minutes and the supernatant was diluted (1:30) with phosphate buffer, and 1.0 ml aliquots were used for assay. These 1.0 ml aliquots contained 670µg of winged bean sample. The method developed by Kakade et al (1969) and modified by Hettiarachchy et al. (1979) was used for estimating the trypsin inhibitor activity in the winged bean extracts.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the leaves and the LPC of winged bean cultivars SLS-40 are shown in Table 1. For comparison the proximate compositions of the leaves and LPC of alfalfa and two green leafy vegetables were included.

The leaves of winged bean and the LPC of winged bean contained greater quantities of protein compared to the corresponding values reported for alfalfa (Hartman et al., 1967, Bickoff and Kohler, 1974), Sesbania grandiflora and Spinacea oleracea (Kandiah, 1975). Okezie and Martin analyzed fresh leaves of seventeen strains of winged bean and their reported values for proximate composition agrees with the present study.

The crude fibre content of LPC of winged bean was found to be 5.13 percent (dry basis), which was greater than LPC from other sources (Table 1). The crude fat content of all the LPC including winged bean was found to be very low. The reason for the low fat content is due to the removal of the fat by the organic solvent used for the decolourization of the chopped up leaves. However, the low fat content would facilitate increased shelf-life of the LPC. The ash content of the winged bean LPC was also observed to be lower when compared to that of other LPC (Table 1).

Moisture content of the winged bean LPC prepared in this study was found to be high (55%) compared to that of alfalfa LPC which contained only 2.6% moisture (Hartman et al., 1967). The alfalfa LPC was prepared using 95% ethanol as a solvent and the pressed alfalfa juice was spray dried (Hartman et al., 1967). However, the moisture contents of Sesbania grandiflora and Spinacea oleracea were 78.6 and 93.0% respectively (Kandiah, 1975). Addition of 10% common salt to the green LPC of winged bean was found to increase its shelf-life.

One trypsin unit is defined as an increase of 0.01 absorbance units at 280 nm per 10 ml reaction mixture.

Determination of in-vitro digestibility

Ten ml of aqueous suspension of LPC and leaves of winged bean in distilled water were adjusted to pH 8.0 with 0.1 N HCL and/or 0.1 N NaOH. The slurry was then incubated for 10 min. at 37°C. Two to three ml of a multienzyme solution (consisting of 2 mg trypsin, 3 mg chymotrypsin and 1.5 mg peptidase per ml, previously adjusted to pH 8.0 and kept in a refrigerator) were added to the sample suspension with constant shaking at 37°C. The pH of the suspension after incubation for 10 min. at 37°C was recorded and the *in vitro* digestibility was calculated using the regression equation of Hsu *et al.*, (1977).

$$Y = 210.464 - 18.103X$$

where Y = *In vitro* digestibility (%), and

X = pH of the sample suspension after 10 min digestion with multienzyme solution.

Determination of protein dispersibility index (PDI)

PDI of LPC and leaves of winged bean were determined using the procedure given in AACC (1976). A sample of 10 g was used. Crude protein was determined by microkjeldal method (AOAC, 1975).

Amino acid analysis

Lipid-free samples of the winged bean were analyzed for amino acid content as outlined in the Technicon Manual (1978). The hydrolysis of protein was carried out using 6N HCL according to the method of Spitz (1973). Quantitation was based on the ninhydrin reaction. Calculations were based on the height - width (triangulation) method of determining the area under the curve.

Mineral composition

Mineral contents of the LPC and leaves of winged bean are given in Table 2. The amounts of calcium, potassium, magnesium, sodium and phosphorus were approximately four times greater in the leaves than in those present in the LPC. This indicates possible leaching out of minerals during the processing of leaves into LPC and is undesirable, hence improved methods have to be developed in order to minimize these losses. However, it was observed that leaching of iron was minimal (Table 2), and this may assume importance in the nutritional problem of iron deficiency, which is the most common cause of anemia in Sri Lanka and has been well documented for the population in estates (FNPPD, 1982). However, more work is needed to evaluate the bioavailability of minerals in LPC of winged beans especially calcium, phosphorus and iron, to identify any binding factors such as phytic acid.

Trypsin inhibitor, in-vitro digestibility and protein dispersibility index

The trypsin inhibitor activity, in-vitro digestibility and the protein dispersibility index of the leaves and LPC of winged bean are given in Table 3.

The leaves of winged bean showed 7.95±0.62 trypsin inhibitor units per mg of dry sample, but LPC of winged bean contained only 1.65±0.73 trypsin inhibitor units per mg of dry sample (Table 3). During the extraction of LPC, the pooled filtrate from the extraction of the leaves was heated at 82°C for one hour, and the heat treatment reduced the concentration of trypsin inhibitors appreciably. Several published data reported the presence of trypsin inhibitors in the winged bean plant and their destruction by heat treatment (Hafez and Mohamed, 1983; De Lumen and Salamat, 1980; De Lumen and Belo, 1981; Sathe and Salunkhe, 1981; SriKantha and Hettiarachchy, 1981). The reduction in the activity of trypsin inhibitors would improve the digestibility of protein.

The LPC of winged bean showed a high in-vitro digestibility and it was observed to be higher than that of the leaves (Table 3). This difference may be due to the higher fibre content in the leaves. Vap et al. (1981) reported that the in-vitro digestibility of young winged bean leaves was 74.91% and this value is lower than the present study (80.5 percent). The difference may be due to the different assay used to estimate the in-vitro digestibility. Badri (1983) reported that the in-vitro digestibility of LPC could vary according to the method of preparation.

The higher value of protein dispersibility index for LPC indicates greater solubility of proteins compared to that of leaves (Table 3). This also indicates that the heat treatment given to the leaf coagulum during processing was not in excess, and very low protein denaturation has occurred. According to Fan and Sosuki (1974), there is a high possibility of protein denaturation, and possible darkening of protein isolates at high alkaline pH. In the method of extraction of LPC (present study) the pH of the leaf coagulum was adjusted to 4.5 before it was heated, thus preventing excess denaturation of protein.

Essential amino acid contents

The LPC of winged bean contained more lysine and methionine than reported for alfalfa LPC. The LPC of winged bean also contained other essential amino acids in greater quantities compared to those of LPC of alfalfa (Table 4). Gerloff et al. (1965) reported that methionine was the limiting amino acid in most of the LPC and that the other essential amino acids were present in adequate amounts for a high quality protein. However, the methionine content of LPC of winged bean was determined to be nearly double that of soybean meal. These findings agree with the reported results of Telek (1981).

Further research is being planned to conduct feeding trials with rats to evaluate the protein efficiency ratio of the LPC of winged bean and to detect any toxic factors that may be present.

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Table 1. Proximate composition of winged bean leaves and leaf protein concentrate (LPC), compared to other plants (g/100 g dry matter)[a]

Composition	Winged bean (SLS-40)[b]		Alfalfa[c]		Sesbania grandiflors[d]		Spinaces oleracea[d]	
	Leaves	LPC	Leaves	LPC	Leaves	LPC	Leaves	LPC
Protein[e]	30.32±0.22	59.73±0.41	22.96±0.51	43.90±0.42	19.30±0.41	50.42±0.53	20.22±0.50	42.30±0.43
Fat (ether extract)	4.50±0.10	0.71±0.11	4.80±0.12	0.62±0.22	3.50±0.23	1.50±0.21	4.21±0.31	0.80±0.24
Fibre	10.00±0.21	5.13±0.13	24.33±0.22	0.83±0.21	12.00±0.14	3.80±0.06	10.81±0.07	2.71±0.08
Ash	6.00±0.12	10.41±0.14	8.25±0.15	15.00±0.31	7.00±0.23	12.00±0.14	9.02±0.08	13.22±0.07
Carbohydrate[f]	49.18±0.50	24.02±0.52	39.68±0.43	39.70±0.51	58.20±0.21	32.30±0.34	55.80±0.61	41.00±0.55

[a] Mean of 3 determinations ± standard deviation.

[b] Present study.

[c] Spray-dried juice extracted with 95% ethanol, Hartman et al., 1967.

[d] Kandiah, 1975.

[e] N x 6.25

[f] Calculated by difference.

Table 2. Mineral content of leaves and leaf protein concentrate (LPC) of winged bean (SLS-44), mg/100 g dry sample.

Minerals	Leaves	LPC
Calcium	897.00*	209.40*
Potassium	1415.00	320.80
Magnesium	155.00	36.60
Iron	67.40	60.00
Sodium	18.00	4.93
Phosphorus	937.20	228.60

*Values were mean of three determinations.

Table 3. Trypsin inhibitor, in-vitro digestibility and protein dispersibility index of leaves and leaf protein concentrates (LPC) of winged bean (SLS-44).

Parameters	Units	Winged bean (SLS-44)	
		Leaves	LPC
Trypsin inhibitor	Trypsin inhibitor units/mg dry sample.	7.95±0.62	1.65±0.73
In vitro digestibility	g/100 g sample	80.05±1.27	87.36±1.51
Protein dispersibility index	%	2±0.5	10±0.8

Values were mean ± S.D., 5 determinations.

Table 4. Essential amino acid content (g/16 g N) of leaf protein concentrate (LPC) from winged bean compared to those from other tropical species.

Amino acid (g/16 g N)	Source of LPC		
	Winged bean (SLS-44)	Alfalfa[a]	Soybean[b] meal
Threonine	6.3	5.2	3.9
Valine	5.9	5.7	5.0
Methionine	3.4	1.4	1.6
Isoleucine	5.2	4.8	6.4
Leucine	9.7	8.5	8.2
Phenylalanine	7.5	6.5	4.8
Lysine	6.3	6.0	6.2
Histidine	3.4	2.5	2.5
Arginine	6.1	6.3	6.0

[a]Hartman et al., 1967.

[b]Gerloff et al., 1965.

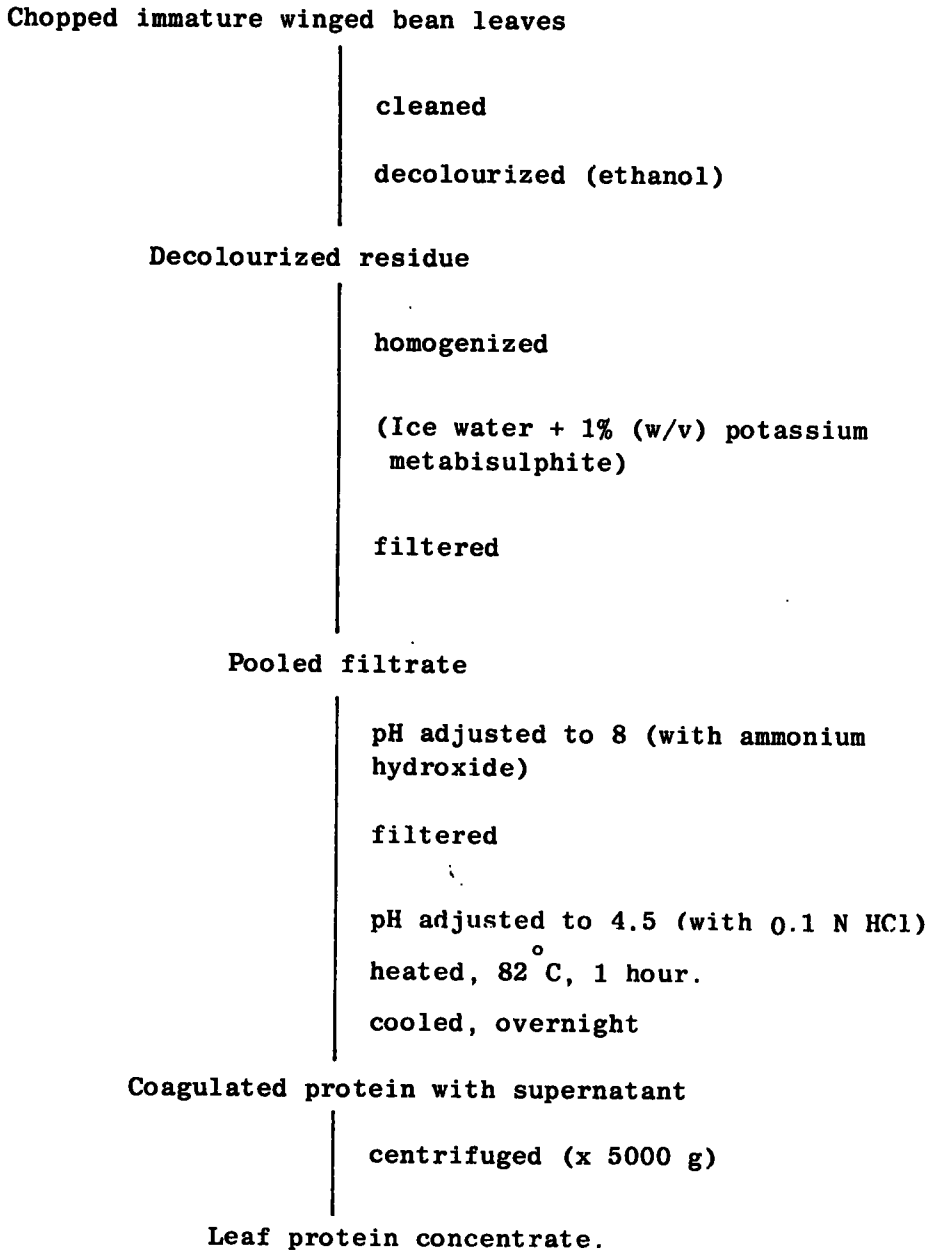

Fig 1. Flow chart for the preparation of leaf protein concentrate.