J. Agric. Sci. Mansoura Univ., 27 (7): 4763 - 4769, 2002

NON CONVENTIONAL METHODS FOR EXTRACTING PROTEIN FROM COWPEA LEAVES :
1- OPTIMUM CONDITIONS FOR PROTEIN ISOLATION

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ABSTRACT

Chemical composition and some antinutrational factors of cowpea leaves *(Vigna sinensis, Savi)* , as well as setting up the optimum conditions for maximum protein extraction were studied. The electrophoretic properties of protein extraction were also investigated . Our results led to the following conclusions: - Cowpea leaves had a good source of leaf protein because it's content was 30.40% as crude protein . The highest extractable protein yeild at 50° C, after 90 minuets and pH 9 was found to be the optimum value for maximum recovery using distilled water in a ratio 1:25 (w/w) (cowpea leaves pwoder : solvent) .

Most of protein fractions of cowpea leaves were focused in about 6 bands in the pH range of 3.5 – 4.7. Protein extract was precipated according to it's iso electric point which found at pH 4.

Keywords: Cowpea leaves, leaf protein, protein isolate , protein electrophoresis .

INTRODUCTION

Since it is highly unlikely that enough protein can be obtained from animal sources to meet increasing demand due to the growth in population, increasing the value of vegetable protein becomes of great importance (Fiorentini and Galloppini , 1983) . A rich source of protein is attracting the attention of scientists as a supplement to protein deficient diets . The extraction and utilization of protein from legumes have been reported as a good source of leaf protein , since the legumes fix nitrogen and have a high nitrogen content (Byers, 1961) . Abo Baker *et al.* (1982) studied the chemical composition of four species of leafy Egyptian crops namely; bean *(Phaseolus vulgaris)* , cabbage *(Brassica oleracea),* tomato*(Lycopersicon esculentum)* and suger cane (Saccharum *officin-arum)* . They pointed out that : their crude protein contents were 6.04-29.70% , total sugar 31.7 – 40.4 % , crude fiber 9.53 – 22.4 % .ether extract 6.50-9.33% and ash 16.3 – 19.57 % Metwalli *et al.* (1988) found that sweet potato leaves had percentage of crude protein(29.3%) highly enough to be considered a good source of leaf protein .

The protein was precipated according to it's isoelectric point which was delected at pH 4 . where, the values of chemical composition of suger beet leaves were ether extract (5.5%) , crude protein (27.9%) , non protein nitrogen (1.4%), crude fibers (13.6%) , ash (20.7%) and nitrogen free extract (32.3%) Shaleby (1990) .Also, he found that the isoelectric point of extracted protein was at pH 4

MATERIALS AND METHODS

Materials

Green leaves of cowpea *(Viga sinenesis, Savi)* were collected from Sabahia Fam of Horticulture Research Station, Agriculture Research Center, Alexandria.Leaves were removed from their stem and transported to the laboratory of Food Technology at Sabahia Hort. Res. Station, Alex. Leaves were cleaned with tap water to remove dust and undesirable materials, then dried in air oven at 50 ° C and milled to pass through 100 mesh seives . Dried material was kept in polyethylene bags and stored at room temperature.

Methods

Analytical methods: The procedures as described in AOAC (1984) were used to determine moisture,ash , ether extract, crude fibers,crude protein , non protein nitrogen and oxalate . Nitrate was estimated as mentioned by Bremner (1965), while nitrite as reported by Chapman and Pratt (1961) . Tannine was detected according to Ranganna (1977) .

Extraction of protein from cowpea leaves : The optimum temperature (50-100 ° C) and pH (1-12) for maximum protein extraction was determined.Effect of extract time (15,30,45,60,90 and 105 min) and sample to solevent ratios (1:10, 1:15,1:20,1:25 and 1:30) were estimated . Extraction from each treatment was centrifuged according to Dale (1981) . Total nitrogen was estimated in the supernatant as mentioned in AOAC method (1984) .

The optimum conditions for maximum protein extraction were carried out . The extractable protein was used for each electrophoretic studies, as described by Radola (1980) , where staining and destaining were conducted using Serva Viollt 49 dye, and protein preciptation based on it's isoelectric point . The preciptated protein was isolated by centrifugation and the yielded paste washed twice with acidic distilled water (pH 4) and recentrifuged . The washed paste was dried in an air oven at 50 ° C and milled to pass through 100 mesh screen sieve and stored in polyethylene packages.

RESULTS AND DISCUSSIONS

1- Chemical composition and antinutritional Factors: -

The major chemical constituent of the dried cow pea leaves were given in table (1) . It can be noted that the percentage of crude protein in cowpea leaves was hight enough to be considered a good source pf leaf protein . Similar results were found alsewhere (Abo- Bakr *et al.*, 1982 , 1983 and Pisulewska, 1991) .On other hand, moisture , ash,ether extract, crude fiber and nitrogen free extract contents of the dried cow pea leaves were in the range as reported by Metwalli, *et al.* (1988), who estimated these components in sweet potato leaves . Apparent also from table (1) that the percentage of antinutrition factors of cowpea leaves, oxalate, tannine , nitrate and nitrite contents , were in the normal range and similar to those found by Pirie(1971) who estimated these components in cassava . From the

nutritional view point nitrates are rlearly nontoxic,but consider a potential hazard precursor of nitrites , the acute toxic substance . Intestial bacteria can convert nitrate to nitrite . Also nitrate affects the utilization and conversion of caroten to vitamin A . whereas, some species of leaves are richer in tannins then others, e.g. lucerne, but common to ruptures tissues of all species are the Θ-quinines produced by enzymic oxidation the prepared leave protein contained only small amounts of extractable phenolic compounds when polyvinylpyrrolidone was present during the extraction procedure(Fafunso and Byers, 1977).

Table (1) : Chemical composition and antinutritional factors of cowpea leaves (on dry weight basis) .

Costitutentse	%
Moisture	82.0
Ash	14.75
Ether extract	2.34
Crude fibers	4.86
Crude protein	30.40
Non protein nitrogen	6.45
Nitrogen free extract *	41.20
Antinutritional Factors :	
Tannins	0.36
Oxalate	0.77
Nitrate	0.78×10^{-2}
Nitrite	0.12×10^{-4}

* Nitrogenfree extract was calculated by difference: -

2- Extraction of protein from cowpea leaves: -

A- Effect of extraction temperatures: -

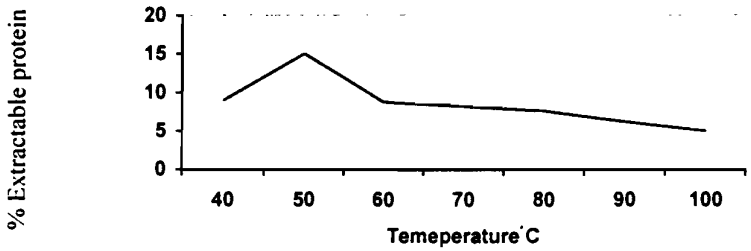


Fig (1) : Effect of temperature on extractability of protein from cowpea leaves .

It can be observed from figure (1) that the extractability of protein increased at 50 C and then decreased . Protein was estimated in the supernatant of extracted protein samples. The maximum solubility of protein was at 50 C, but by increasing temperature from 60 to 100 C, protein

coagulated. The used extraction conditions were 1:10 sample to water ratio for 90 minutes.

B: Effect of extraction time: -

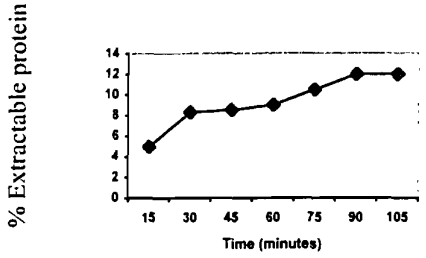


Fig (2): Effect of time on extractability of protein from cowpea leaves.

The results as shown in figure (2) indicted that extractable protein was increased with increasing time. The highest extractable protein yeilded after 90 minutes at pH 9 with 1:10 (sample: water) ratio, therafter, no increase in the recovered protein was observed even after 105 min. Obtained results in agreement with sekul *et al* . (1978). The protein extractability increased as a function of prolonged time of extraction was due to enough time for water to penetrate the cells and extract more of its protein.

C-Effect of pH of extraction solvent:

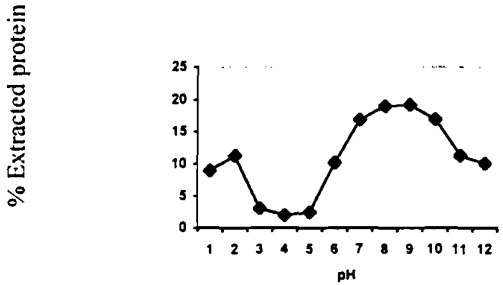


Fig (3) : Effect of pH on from cowpea extractability of protein from cowpea leaves .

As illustrated in figure (3) it can be seen that the extractability of protein increased at pH above 5 and the hightest yield of the protein was obtained at pH 9 (1:10 sample to water ratio after 90 minutes for extraction) . The lowest

extraction of protein from cowpea leaves was occurred at pH 4 . However it is clear from the same figure that the highest percentage of protein extractability was relatively low (only 19.2 % of the total protein present in cowpea leaves) . These results may be due to the effect of drying treatment. Similar results were found by Metwalli, *et al* . (1988) .

D- Effect of sample to distilled water ratio: -

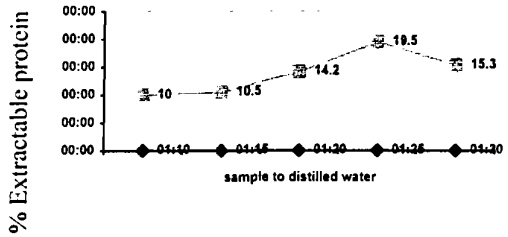


Fig (4) : Effect of sample to distilled water ratio on extractability of protein from cowpea leaves .

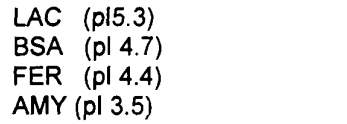
Results shown in figure (4) outlined that the sample to distilled water 1: 25 was the optimum ratio for maximum protein extraction from cowpea leaves (leaves were extracted for90 minutes at pH 9) .These results were in agreement with those obtained by Dale (1981) .

From the previous results it can be concluded that the optimum conditions for extracting protein from cowpea leaves were at 50 C for 90 minutes at pH 9 with sample to water ratio 1:25 .

3- Electroporetic studies: -

Figure (5) shows that most of protein fractions in dried cowpea leaves were focused in about six bands in the pH range of 3.5 - 4.7. All fractions were focused between amyloglucosidase (PI 3.5) and bovine serum albumin (PI 4.7) Extracted protein was precipitated according to it's isoelectric point which was found at pH 4

fig5



Fig(5) : Protein patterns of cowpea leaves , separated by isoelectic focusing in 5 % t ,3 % C polyacrylamide gel of 3-6 gradients .

Where :
S : Dried cowpea leaves
M : Marker proteins from serva
AMY : Amyloglucosidase
FER : Ferritin
BSA : Bovine serum albumin
LAC: β. Lactoglobulin

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إمكانية استخلاص بعض بروتينيات ورق اللوبيا بطريقة غير تقليدية
١- الظروف المثلى لعزل البر وتينات

فاطمة الزهراء على عبد العال – سلوى دانيال روفائيل

قسم الحاصلات البستانية– معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية

تم دراسة التركيب الكيماوى و بعض المتطلبات الغذائية لأوراق اللوبيا و تم تحديد أنسب الظروف لعزل البروتينات مع دراسة صفاتها عن طريق التفريد الكهربى باستخدام تكنيك isoelectric focusing و قد أوضحت الدراسة النتائج التالية :-

- وجد أن أوراق اللوبيا قد احتوت على كمية مناسبة من البروتينات بدرجة تجعلها من أغنى المصادر لها حيث كانت ٣٠,٤٠ % كبروتين خام .
- تبين أن درجة حرارة ٥٠م بعد ٩٠ دقيقة تعطى أعلى استخلاص للبروتين و ان رقم الحموضه ٩ كان أفضل رقم حموضة لاستخلاص البروتينات باستخدام ماء مقطر بنسبة واحد جرام عينة : ٢٥ مل ماء مقطر .
- قد أظهرت نتائج التفريد الكهربى أن بروتينات أوراق اللوبيا قد فصلت و تركزت فى ٦ مناطق فى مدى رقم حموضه ٣,٥ – ٤,٧ أى أنها من النوع الحامضى و أمكن ترسيب البروتين المستخلص باستخدام صفة نقطة التعادل الكهربى لها و التى وجدت عند رقم حموضه ٤ .