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EXTRACTING PROTEIN FROM COWPEA LEAVES AND EVALUATING THE ISOLATED LEAF PROTEIN .

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ABSTRACT

Chemical composition, nutritional evaluation, and functional properties of cowpea leaf protein isolate (LPI) were studied. The results revealed that cowpea leaf contained high percentage of protein (81.15%) and very low percentage of ether extract (0.62%). It had moderate amounts of total carbohydrate (5.51%), ash(8.24%), and crude fibers (4.12%). LPI was free of tannins, nitrate, nitrite, and small amount of oxalate (0.36%). The investigated LPI was rich in all essential and non-essential amino acids except sulfur containing amino acids comparing with casein. In Vitro protein digestibility and computation protein efficiency ratio (C-PER) of LPI were high enough to compete with the standard protein (casein). As for functional properties, protein solubility was high value when LPI was dissolved in sodium hydroxide followed by potassium hydroxide, sodium chloride and potassium chloride, with concentrations of 0.1 M for each solution , then by distilled water. Fat absorption capacity (FAC) of LPI was higher than water absorption capacity (WAC) of the same protein; also, it was higher than FAC of albumin. The foaming capacity was high (16 ml) whereas, foam stability decreased gradually up to 45 min. It can be concluded that cowpea leaf protein isolate had nutritional value and good functional properties therefore, it can be used in manufacture some foods.

Keywords: Cowpea leaf protein, extraction, isolation, evaluation .

INTRODUCTION

The plant foodstuffs such as cassava, yam, and millets constitute an important dietary source of protein for many segments of the world's population. Particularly where animal protein is in short supply or is forbidden by cultural or religious practices (Kakade, 1974). The usage of concentrated protein in human and pet foods has increased markedly during the past fifteen years because of greater knowledge of functional properties, processing technique and nutritive value (Sosulski and Fleming, 1977). Several investigators (Buchanan, 1969 and Betschart and Kinsella, 1974) evaluated LPI from wheat and soybean leaves and found that these proteins were rich in essential amino acids except sulfur containing acids, also these protein were easy to digest and accepted by humans when used in manufacturing some foods (Soup and Sausage). Protein solubility, water, and fat absorbing capacities are some of the major functional properties of protein that decidedly affect their utilization (Hung and Zayas, 1992). The functionality of protein influences the physical characteristics quality of food and the sensory properties of the food, in which they are incorporated. Therefore, studying the functional properties of protein is essential in order to monitor its suitability in any food product.

This work was carried out to study the chemical composition, and to evaluate nutritional and functional properties of cowpea LPI.

MATERIALS AND METHODS

A- Materials

Cowpea legume was planted at Sabahia Experiment Station in Alexandria, during summer season of 2002.The leaves were collected before the harvesting of the dried pods. Leaf protein was isolated from dried cowpea leaves using distilled water in ratio of 1:25 (w/v) sample to water. The optimum conditions for extracting protein, according to Fatma El-Zahraa and Salwa (2002), were at 50ºC for 90 minutes at pH 9 then the extract was centrifuged at 3000 rpm. The extractable protein supernatant was precipitated according to its isoelectric point at pH 4, then centrifuged at 5000 rpm and the obtained paste washed twice using acidic distilled water (pH 4) then recentrifuged at 5000 rpm. The yielded paste was dried in an air oven at 50ºC, then milled in a mill and sifted in 100-mesh metal screen sieve. The fine powder of leaf protein isolate was kept in polyethylene packages for analysis and evaluated (Atta *et al.*,1988).

B- Methods

1- **Analytical methods:** Moisture, ash, ether extract, crude protein, crude fiber and oxalate were determined according to the methods given in the AOAC (1995). Nitrate was estimated as described by Bremner (1965), while nitrite as reported by chapman and Pratt (1961). Tannine was detected according to Ranganna (1977). Amino acids were performed by the method of Mabbott (1990), where tryptophan was determined colorimetrically in the alkalin hydrolyzate following the method of Miller (1967). Computation protein efficiency ratio (C-PER) was calculated as described by Siam (1999). In Vitro pepsin, pancreatin and pepsin followed by pancreatin digestibilities were conducted according to the method of Akeson and Stahman (1964).

2- **Functional properties:** Protein solubility was carried out using different solvents, such as distilled water, 0.1 M of each sodium chloride, potassium chloride, sodium hydroxide, and potassium hydroxide (El-Adawy, 1986). Water absorption capacity (WAC) and fat absorption capacity (FAC) were determined using the method of Knuckle and Kohler (1982). Foaming capacity and foaming stability were measured by mixing 2 grams of protein sample with 50 ml distilled water in blender at room temperature. The suspension was blended for 5 minute at 1600 rpm. The contents along with the foam were poured into a 100 ml graduated measuring cylinder and the total volume was recorded after 30 sec. The percentage increase in volume was recorded after 30 sec and expressed as foam capacity. The volume of foam only (Total volume – Liquid volume) after 30 min of standing at room temperature is taken as foam stability (Lawhon and Cater, 1971).

RESULTS AND DISCUSSIONS

1- Chemical composition of leaf protein isolate (LPI).

Table (1) showed that protein content of leaf protein isolate from dried cowpea leaves was very high (81.15%). It had a very low percentage of ether extract (0.62), and moderate amounts of total carbohydrate (5.51%), ash (8.24%), and crude fiber (4.12%). Similar results were found by Atta *et al.*, (1988) and Shalaby (1990) who isolated proteins from sweet potato and sugar beet leaves, respectively. Also, the same Table revealed that LPI was free of tannins, nitrate, and nitrite. It had small amount of oxalate (0%). So, the nutritional problems caused by these anti-nutritional factors can be avoided. Carrera *et al.*, (1974) reported that tannins bind vitamin B_1 in the human body. LPI contained less than 50% of the initial oxalate value that found in dry cowpea leaves (0.77%) (Fatma El-Zahraa and Salwa, 2002). It is well known that the highest utilization of food could be obtained when the oxalate content was very low, since oxalate has the ability to bind calcium in the human body and cause trouble in the urinary track (Oke, 1969).

Table (1): Chemical composition of cowpea leaf protein isolate (LPI) (calculated as percent on dry weight basis).

Component	%
Moisture	7.31
Protein	81.15
Total carbohydrate #	5.51
Ether extract	0.62
Ash	8.24
Crude fiber	4.12
Antinutrational factors	
Oxalate	0.36
Tannins	N.D
Nitrate	N.D
Nitrite	N.D

Total carbohydrate was calculated by differences.
N.D : Not detected

B- Nutritional evaluation of leaf protein isolate (LPI)

1- Amino acid composition and their chemical scores.

Table (2) showed that the investigated LPI was rich in all essential and non-essential amino acids comparing with casein. Leucine and isoleucine were the predominant between essential amino acids followed by valine, where glutamic acid, alanine and aspartic acid were the highest among the non-essential amino acids. The remainder of the non-essential amino acids were in a adequate balance. It was clear from the same Table that the cowpea LPI had a shortage of sulfur containing amino acids (methionine and cystine). Essential amino acids (phenylalanine and thereonine) were present at higher level compared with those found in casein. Whereas, tyrosine and tryptophan in LPI were in favorable balance comparing with their levels in casein or FAO protein references. The obtained results were in agreement with those of Atta et al., (1988) and Shalaby (1990) who found that LPI were rich in essential and non-essential amino acids

.Leucine was the predominant between the essential amino acids, where aspartic acid and glutamic acid were the highest among the non-essential amino acids. All essential amino acids except sulfur containing amino acids were more abundant than needed for protein balance according to the (FAO,1973) reference protein.

Table (2) : Amino acid composition of cowpea leaves protein isolate (LPI), casein and essential amino acids content in the FAO provisional pattern (1985)

Amino acid (A.A.)	LPI	Casein	FAO
	(g A.A. / 100 g protein)		
Essential amino acids			
Lysine	381	7.51	5.50
S-containing A.A.			
Methionine	1.83	2.96	3.50
Cystine	1.24		
Threonine	4.20	3.43	4.00
Leucine + Isoleucine	10.82	14.21	11.00
Valine	7.49	5.42	5.00
Aromatic A.A.			
Phenylalanine	5.71	9.81	6.00
Tyrosine	3.50		
Tryptophan	1.62	1.21	1.00
Non-essential amino acids			
Aspartic acid	6.35	5.97	
Serine	5.63	5.59	
Glutamic acid	15.50	17.53	
Glysine	5.31	1.72	
Alanine	6.43	2.65	
Proline	5.38	2.92	
Arginie	5.78	4.22	

The nutritive value of a protein depends primarily on its capacity to satisfy the needs for essential amino acids. Thus, the amino acids requirements are the logical yard-sticks by which protein quality can be measured. Chemical scores of essential amino acids found in LPI and casein as a reference are given in Table (3). Cowpea LPI showed high amino acids score as high as casein with exception of lysine and sulfur containing amino acids. The results coincide with Pisulewsk (1991) who determined the nutritive value of leaf protein extracted from vetch and cereal mixture.

Table (3) : Chemical scoring # of essential amino acids of cowpea leaf protein isolate (LPI)

Amino acid	Chemical score	
	LPI	casein
Lysine	69.27	136.54
Methionine + Cystine	87.71	84.57
Threonine	105.00	85.75
Leucine + Isoleucine	98.36	129.18
Valine	149.80	108.40
Phenylalanine + Tyrosine	153.50	163.50
Tryptophan	162.00	121.00

Chemical scores were calculated as a percentage of the FAO (1985) essential amino acids.

1- In Vitro enzymatic digestibility of cowpea leaf protein isolate and their computed protein efficiency ratio (C-PER).

The results in Table (4) indicated that casein was easily digested compared with leaf protein isolate. This could be explained as reported by Free and Satterlee (1975) who reported that protein bound chlorogenic acid inhibit the action of proteolytic enzymes, hence, lower protein digestibility could be expected in plant protein concentrates. Moreover, digestibility of LPI with pancreatin was higher than that with pepsin. This may be attributed to the modes of action and specificities of pepsin and pancreatic enzymes, as well as to the amino acids make up and sequence in the peptides of the LPI. In addition, the results showed that digestibility of LPI using pepsin followed by pancreatin was higher than those of pepsin or pancreatin alone. This may be related to the natural sequence prevailing in gastro-intestinal tract, whereby proteins were initially exposed to the action of pepsin, which first splits the large protein molecules into smaller units, which becomes more easily to be attack by the pancreatic proteolytic enzymes.

The computed protein efficiency ratio (C-PER) of cowpea LPI was similar to casein, so, it is suppose that it has a high biological values, there it would be easily to digest. These results were agreement with those observed by Buchanan (1969) who isolated proteins from wheat leaves and Shalaby (1990) who isolated proteins from sugar beet leaves. They found that C-PER of LIP was lower than that of standard casein protein, but it was high enough to be closed to that of casein.

Table (4): In Vitro enzymatic digestibility # and computed protein efficiency ratio (C-PER) of cowpea leaf protein isolate (LPI) and casein.

	Digestibility%			
Sample	Pepsin	Pancreatin	Pepsin followed by Pancreatin	C-PER
LPI	48.5	60.00	78.80	1.92
casein	54.00	87.00	90.03	2.50

Digestibility was calculated as a percentage of the non-protein nitrogen to the total nitrogen in the sample.

C-Functional properties of leaf protein isolate .

The functional properties of LPI were studied to determine its suitability as an additives in some foodstuff.

1-Protein solubility

Protein solubility of cowpea LPI was determined in 0.1 M sodium hydroxide, 0.1 M potassium hydroxide, 0.1 M sodium chloride, 0.1 M potassium chloride and distilled water as shown in Table (5). The highest value of protein solubility was obtained with sodium hydroxide solution followed by potassium hydroxide, both sodium and potassium chloride solutions, then by distilled water. This may be due to that water extracts only albumins, globulins, and other protein fractions, such as prolamin and glutelins. Similar results were obtained by El-Hadidy (1995) who found that the highest value of protein solubility index was obtained when sodium hydroxide was used ,followed by both potassium and sodium chloride solutions, then by distilled water.

Table (5): Protein solubility of cowpea leaf protein isolate (LPI) in different solvents (%).

Solvents	Solubility (%)
Sodium hydroxide(0.1M)	67.40
Potassium hydroxide (0.1M)	64.06
Sodium chloride (0.1M)	45.26
Potassium chloride(0.1M)	40.38
Distilled water	30.00

2-Water and fat absorption capacity (WAC and FAC)

It is clear from the results present in Table (6) that WAC of the cowpea LPI was lower than that of albumin. This may be due to the differences in protein purity and conformation characteristics of these proteins. Sathe and Salunkhe (1981) reported that water binding by proteins is a function of several parameters including size, shape, conformational characteristics, hydrophilic-hydrophobic balance of amino acids in the protein molecule, lipids and carbohydrates associated with proteins and solubility of the protein molecules. WAC of cowpea LPI bind 2.24 folds its weight of water (Table 6). Polar amino acids residues of protein have an affinity for water molecule. An equal important consideration is whether the conformation of the protein permits, its binding sites to be available for interaction with water (Lin *et al.*, 1974).

Table (6): Water absorption capacity (WAC) and fat absorption capacity (FAC) of cowpea leaf protein isolate (LPI) and albumin

Tested protein	WAC g Water / 100 g protein	FAC g Oil / 100 g protein
Cowpea LPI	224.00	246.35
Albumin	327.00	230.00

The fat absorption capacity of cowpea LPI was higher than that of albumin as standard protein (Table 6). Cowpea LPI absorbed 2.46 fold as its weight of fat. LPI's oil absorption may be due to the lipophilic nature of this protein, besides the presence of several non-polar side chains which may bind the hydrocarbon chains of fat, thereby resulting in higher absorption of oil as reported by Sathe et al., (1982).These results were in accordance with those obtained by El-Hadidy (1995) who found that FAC of LPIs from potato, sweet potato and cabbage were 294.80, 266.18, and 264.44 g oil / 100 g protein, respectively.

3-Foaming properties

The foaming capacity and foaming stability of protein are important in food such as, cake, ice cream, soufflés and whipped topping. A strong viscoelastic protein film or lamellae around air bubbles is necessary for foam formation and stabilization. This requires protein to be rapidly diffused to and be adsorbed at the air / water interface (Barbeau, 1990). Results given in Table (7) showed that foaming capacity was 16 ml whereas, foam stability decreased gradually up to 45 min. The foam stability value did not change beyond 30 min, indicating good foam stability (Nath and Narasinga Rao, 1981). Similar results were found by El-Hadidy (1995)

Finally, it can be concluded that leaf protein isolated from cowpea leaves had a high nutritional value and good functional properties which can be used in fortification of some foots.

Table (7): Foaming properties of cowpea LPI

Time (min)	End volume (ml)	Foam volume (ml)
0.0	66	16
15	61	11
30	60	10
45	55	5
60	54	4
75	54	4
90	54	4

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استخلاص البروتين من أوراق اللوبيا وتقيم البروتين المعزول

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تم تقييم البر وتينات المعزولة من أوراق نباتات اللوبيا المنزرعة فى مزرعة الصبحية التابعة لمعهد بحوث البساتين بالإسكندرية ؛ عن طريق تقدير تركيبها الكيماوي وقيمتها التغذويه ، وكذلك صفاتها الطبيعية والوظيفية .

وقد أظهرت النتائج أن هذه البر وتينات تحتوى على نسبة عالية من البروتين (٨١,١٥ %) ، ونسبة منخفضة جدا من الدهن . كما احتوت على نسبة متوسطة من الكربوهيدرات الكلية (٥,٥١ %) ، والرماد (٨,٢٤ %)، والألياف الخام (٤,١٢ %). وكانت هذه البر وتينات المعزولة خالية من التانينات والنيتريت والنترات ، غير أنها احتوت على نسبة بسيطة من الأكسالات (٠,٣٦ %). كما كانت هذه البر وتينات غنية فى محتواها من الأحماض الأمينية الأساسية وغير الأساسية ؛ باستثناء الأحماض الأمينية الكبريتية . هذا إلى جانب أن معامل الهضم وكفاءة الاستفادة من هذه البروتينات كانت مرتفعة مقارنة بالكازين كبروتين قياسى .

أما بالنسبة للصفات الطبيعية والوظيفية لهذه البر وتينات فقد وجد أن لها ذائبية عالية عند الذوبان في محاليل (٠,١ ع) من كل من هيدروكسيد الصوديوم يليه هيدروكسيد البوتاسيوم ثم كلوريد الصوديوم وكلوريد البوتاسيوم وأخيرا الماء المقطر. ووجد أن ارتباطه بالدهن أعلى من ارتباطه بالماء ، وأيضا أعلى من قيمة ارتباط الألبيومين بالدهن . كما وجد أن قدرة هذه البر وتينات المعزولة على تكوين الرغوة عال (١٦ مل) فوق المحلول الأساسي (٥٠ مل). بينما ثبات هذه الرغوة انخفض تدريجيا لأكثر من ٤٥ دقيقة.

يتضح مما سبق أن البر وتينات المعزولة من أوراق اللوبيا تتميز بقيمة تغذويه عالية، وتصلح للاستخدام فى الأغراض الغذائية.