Chemical, Nutritional and Biochemical Studies of Garden Cress Protein Isolate

[1]Ahmed, M. Gaafar; [2]Azza, A. Morsi and [3]Heba, E. Elghamry

[1] Food Technology Research Institute, Agricultural Research Center, Giza, Egypt
[2]Faculty of Education, Hail University, Saudi
[3]Department of Nutrition and Food Science Faculties of Science and Home Economics, King Khalid University
ahmad_gafaar@yahoo.com

Abstract: Garden cress or "hab arachad" seeds are considered one of the popular medicinal herbs used in Arabian countries Garden cress meal (*Lepidium sativum*) is a by-product remaining after the extraction of the oil from seeds. Protein represents the most abundant nutrient in this product, which contains 34.15% protein, 1.86% crude oil, 9.85% crude fiber, 5.89% ash and 48.25% nitrogen free extract (NFE), on a dry weight basis. The garden cress meal protein isolate was prepared using the isoelectric point technique. The chemical composition of this isolate was 92.43% protein, 0.34% crude oil, 1.46% crude fiber, 1.39% ash and 5.56% NFE, on a dry weight basis. The minerals and amino acids of garden cress meal and the protein isolate were also determined. The results of the biological experiment indicated that the isolate had a high PER value, being 1.35, as compared to a value of 1.46 in the case of rats fed on a diet containing 15% casein. The biochemical parameters of kidney function of the rats fed on a diet containing 15% of garden meal and garden isolate were normal.
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Key word: garden cress, "hab arachad", protein isolate, mineral content, amino acid and PER.

1. Introduction

Lepidium sativum locally known as 'garden cress' or 'hab arachad' has been used widely in different parts of the world for its wide therapeutic application, plant and seeds are considered one of the popular medicinal herbs used in the community of Saudi Arabia, Sudan and some other Arabic countries as a good mediator for bone fracture healing in the human skeleton **(Gil & MacLeod, 1980)**. Garden cress is usually cultivated for its leaves, which are used in salad, sandwiches etc. The leaves and seedpods have a peppery taste (**Maghrani *et al*., 2005**). The seedlings are consumed in Europe as salad and spice. A number of recent studies pointed out the traditional uses of *Lepidium sativum* seeds extract in controlling many clinical problems. They were used as anti-asthmatic antiscorbutic, aperients, diuretic, galactogogue, poultice and stimulant. The leaves are antiscorbutic, diuretic and stimulant (**Eddouks *et al*., 2002**).

Lepidium sativum L. seeds increase weight gain as they are found to contain 18-24% of fat. Thirty four percent of the total fatty acids are alpha linolenic acid; and the oil has alpha linoleic acid which could give it nutritional advantages (**Diwakar *et al*., 2008**). The primary fatty acids in *Lepidium sativum* oil were oleic (30.6 wt %) and linolenic acids (29.3 wt%) and was found to contain high concentrations of tocopherols. It contains good amount of lignans and antioxidants, which can stabilize the n-3 polyunsaturated fatty acids in its seed oil. The primary phytosterols in *Lepidium sativum* were sitosterol and campesterol, with avenasterol (**Bryan *et al*., 2009**).

In different regions of Saudi Arabia, *Lepidium sativum* seeds are used commonly to treat abdominal discomfort, such as dysentery and diarrhea, in additional to its other beneficial effects, such as febrifuge, diuretic, antirheumatic, antiflatulent and antihiccup (**Ageel *et al.*, 1987; Duke *et al.*, 2002**). In various countries of Africa, *Lepidium sativum* seeds are thought to be a better medicinal remedy to cure respiratory disorders such as bronchitis and asthma (**Kloos, 1976**). The herb is also used to compensate vitamin C deficiency and to strengthen the immune system (**Fleming, 1998**).

The garden cress seed, *Lepidium sativum* L. is a fast growing annual herb belonging to the Brassicaceae family that is native to Egypt and west Asia. The seeds are wildly consumed as salad and spice (**Gokavi *et al.*, 2004**). *Lepidium sativum* is documented to possess alkaloids, riboflavin, a-tocopherol, b-carotenes, b-sitosterol, ascorbic, linoleic, oleic, palmitic and stearic acids. It is considered a good source of mono-unsaturated fatty acids and L-arabinose (**Duke, 1992**). Moreover, cucurbitacins and cardenolides have also been identified as plant constituents (**Fleming, 1998**). *Lepidium sativum* has been studied pharmacologically for its laxative (**Rehman *et al.*, 2011b**), antibacterial (**Darwish and Aburjai, 2010**), bronchodilatory (**Rehman *et al.*, 2011a**), contraceptive effects (**Sharief and Gani, 2004**) and in inflammatory bowel disease (**Rahimi *et al.*, 2010**).

So the present study was conducted to analyses of garden cress seeds meal and isolate for their chemical composition, mineral contents, protein, amino acid and PER as a preliminary work to explore the possibility of using this as a functional ingredient in product formulations.

2. Material and Methods

2.1. Materials

Garden cress seeds (*Lepidium sativum* L.) were obtained from the local market at Shibin El-Kom City, Egypt. The seeds were cleaned and rendered free of dust, then stored in polyethylene bags in the refrigerator until used.

2.2. Methods

2.2.1. Preparation of Garden cress seed flours

Garden cress seeds were crushed, using a household mill (Braun, Germany), and then defatted by soaking in n-hexane for 48 hrs with several changes of the solvent. The defatted flour was air-dried at room temperature (~25°C) and ground again to pass through a 60-mesh (British Standard Screen) sieve. The fine flour of each seed variety was then used for preparing protein isolates.

2.2.2. Preparation of protein isolates

One kg of flour was suspended in 10 l distilled water containing 0.25% Na_2SO_3, then adjust to pH 9.0 using 1 M NaOH. The suspension was stirred for 1 h at room temperature, then centrifuged at 3000 x g for 30 min. In order to obtain higher yields, the extraction and centrifugation were repeated on the residue. The extracts were combined and acidified to pH 4.5 for protein. The precipitate was recovered by centrifugation at 3000 x g for 30 min, then neutralized by 1.0 M NaOH to pH 7 and washed by distilled water, several times. The neutralized precipitate was freeze-dried (Lab Conco Freeze Dry 64312. Kansas, Missouri), then milled using a household mill (Braun, Germany) and finally sieved through 60-mesh.

2.2.3. Chemical composition

Moisture (14.004), fat (14.018), ash (14.006), crude fiber (14.020) and protein N_6.25 (14.026) were determined as described by AOAC (1990). Carbohydrates were calculated by differences. All determinations were performed in triplicate.

2.2.4. Minerals:

Minerals were determined after wet-ashing by concentrated nitric acid and perchloric acid (1:1, v/v). Na, K and Ca were determined by flame photometery (Corning 410, England), while Mg, Mn, Zn, Fe and Cu were determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA). Phosphorus was estimated photometrically via the phosphorus molybdate complex described by **Taussky and Shorr (1953).**

2.2.5. Amino acids

Amino acids were determined using a Mikrotechna AAA 881 automatic amino acid analyser according to the method of **Moore and Stein (1963).** Hydrolysis of the samples was performed in the presence of 6 M HCI at 110°C for 24 hrs under a nitrogen atmosphere. Sulfur containing amino acids were determined after performic acid oxidation. Tryptophan was chemically determined by the method of **Miller (1967)**. The amino acid score (AAS) was calculated for each essential amino acid using the **FAO/WHO/UNU (1989)** reference protein as follows:

2.5 Biological experiments

Twenty four adult male albino rats with an average weight of 100-120 g were housed in metal cages and fed on a standard diet as described by **A.O.A.C (2000).** Water and a meal composed of 15% corn oil, 15% protein, 1% vitamin mixture, 4% mineral mixture, 1% fiber and 64% starch were provided *ad libitum* for 10 days as an adaptation period. After this period, the rats were divided into 3 groups of 8 animals: the control group was fed on the standard diet containing 15% casein, and the study group on the same diet containing 15% Garden meal and garden protein isolate instead of casein. The experiment was carried out for 5 weeks. The protein efficiency ratio (PER) was calculated as the gain in body weight/protein consumed (**Bender and Doehl, 1957).**

2.6 Blood sampling

Blood samples of all the animals were taken from the orbital vein using capillary tubes at the beginning and after 2, 4, 6 and 8 weeks of experimentation. Sera were obtained by centrifugation at 1100 g for 20 min and kept frozen until analyzed.

2.7 Serum analysis

Urea, uric acid, creatinine and total bilirubin were estimated according to the methods of **Fawcetl and Soctt (1960), Barham and Trinder (1972), Larsen (1972) and Doumans *et al.* (1987),** respectively. Each determination was carried out in triplicate and the mean values presented in the text.

Statistical analysis:

Results are expressed as the mean value ± standard deviation (SD) of three separate determinations, except for the minerals and amino acid contents, which were determined in duplicate.

Data were statistically analyzed using analysis of variance and least significant difference using **SAS (1985)**. Significant differences were determined at the $p \leqslant 0.05$ level.

3. Results and Discussion

3.1 Chemical composition of garden cress seeds, meal and protein isolate

The data presented in Table 1 show the proximate composition of the garden cress seeds, meal and protein isolate. The moisture content of these products was 5.12, 6.96 and 4.82%, respectively. The highest protein content was found in the protein isolate, being about 3.8 and 2.7 times higher than those found in the whole seeds and meal, respectively. The seeds showed the highest level of crude oil, 27.85%, and the protein isolate the lowest, 0.34%. The garden cress meal had the highest level of crude fiber, being about 1.26 and 6.75 times as great as that in whole seeds and protein isolate, respectively. With regard to the ash content, the garden cress meal showed the highest level of 5.89%, and the whole seeds and protein isolate moderate amounts of 4.26 and 1.39%, respectively. **Mathews *et al.*, 1993** reported 24.3 ± 0.67% protein, 14.9 ± 0.79% fat, 55.4 ± 1.8% carbohydrate, 27.3 ± 0.43% acid detergent fiber and 35.7 ± 0.82% neutral detergent fiber in *L. sativum* seeds. **Andersson *et al.*, 1999** reported 19% protein, 20% crude fat and 40% dietary fiber in *L. campestre*. Also, **Sumangala *et al.* (2004)** reported that the Protein and fat were concentrated in endosperm whereas dietary fiber, minerals and carbohydrate in the bran fraction. The high protein, fat, dietary fiber, calcium, phosphorous and iron contents in this seed bring out its high nutritive value which may be making it useful in post pregnancy diets.

Table 1. Chemical composition of garden cress seed, garden cress meal and garden protein isolate (dry weight basis).

Component	Whole seed	Garden meal	Protein isolate
Moisture	5.12±0.96^{b}	6.96±0.66^{a}	4.82±0.25^{c}
Crude protein (Nx 6.25)	24.29±1.65^{c}	34.15±2.14^{b}	92.43±2.37^{a}
Crude lipids	27.85±1.73^{a}	1.86±0.14^{b}	0.34±0.08^{c}
Crude fiber	7.79±0.85^{b}	9.85±0.96^{a}	1.46±0.11^{c}
Ash	4.26±0.91^{b}	5.89±0.85^{a}	1.39±0.23^{c}
Total carbohydrates	35.81±2.37^{b}	48.25±3.14^{a}	4.38±0.54^{c}

3.2. Mineral content of garden cress seeds, meal and protein isolate.

The results showed that the garden cress meal had the highest levels of each mineral whilst the protein isolate had the lowest amounts (Table 2). Potassium is highest in all the products followed by phosphorous, magnesium and calcium. Iron content is considerably high which in whole seed are 8.43, 9.21 in meal and 5.46 mg/100 g in protein isolate. Calcium, potassium and sodium are concentrated in bran whereas phosphorous, iron, zinc and magnesium are in endosperm. **Gopalan *et al.*, 2000** reported 377 mg calcium, 723 mg phosphorous and 100 mg of iron in *L. sativum*. The difference between the reported values and the values obtained in the present study may be attributed to the varietal variations and also to the agronomical conditions. All the fractions have low sodium and high potassium content which makes it beneficial as an ingredient in health foods. High potassium diet is recommended for athletes who are involved in hard exercise and also for disorders related to high blood pressure **(Luft, 1987)**.

Table 2. Mineral contents of garden cress seed, garden cress meal and garden protein isolate.

Minerals	Mg/100g		
	Whole seed	Garden meal	Protein isolate
Calcium	243.12±6.51^{b}	317.26±7.16^{a}	198.24±6.53^{c}
Phosphorus	427.36±9.16^{b}	486.57±9.85^{a}	314.36±8.72^{c}
Magnesium	239.47±4.19^{b}	253.68±5.16^{a}	176.18±4.65^{c}
Potassium	975.16±10.66^{b}	1104.35±11.46^{a}	635.85±10.37^{c}
Iron	8.34±1.02^{b}	9.21±1.13^{a}	5.46±0.94^{b}
Sodium	19.65±1.52^{b}	21.74±1.25^{a}	13.39±0.77^{c}
Zinc	1.19±0.13^{b}	1.93±0.11^{a}	1.07±0.03^{b}
Cooper	1.25±0.18^{b}	2.16±0.23^{a}	1.08±0.12^{b}

3.3. Amino acid composition of the garden cress meal and protein isolate

The data shown in Table 3 show that the essential amino acids of garden cress meal and protein isolate corresponded to 45.23% and 45.68 of the total amino acid content, and that the garden cress meal and protein isolate showed good levels of most of the individual essential amino acids as compared with the amounts recommended by **FAO/WHO/UNU (1989).** Garden cress meal protein isolate were rich in essential amino acids such as histidine, valine and tryptophan compared with the **FAO/WHO/UNU (1989)** reference. Therefore, garden cress protein could very well complement those protein sources that are low in tryptophan. However, leucine, total sulfur amino acids, threonine and lysine were slightly deficient in garden cress protein compared with the reference pattern. The results indicated that aspartic

(9.53and 9.89 mg/100g protein) and glutamic acids (16.33 ± 0.19%) were the major abundant amino acids in this oil seed. This observation is in close agreement with **Olaofe [1994]** for melon seed, pumpkin seed and gourd seed. Glutamic acid is an important excitatory neurotransmitter and also plays a vital role in metabolism of sugars and fats.

Table (3). Amino acid composition of the garden cress meal and protein isolate

Amino acids	Mg/100g		FAO/WHO 1989
	Garden meal	Garden isolate	
Histidine	3.74	3.65	2.60
Isoleucine	4.07	4.26	4.60
Leucine	8.31	8.24	9.30
Lysine	5.18	5.39	6.60
Cysteine+Methionine	3.29	3.48	4.20
Tyrosine+phenylalanine	8.36	8.51	7.10
Threonine	3.62	3.55	4.30
Tryptophan	1.82	1.86	1.70
Valine	6.84	6.92	5.50
Total essentially amino acids	45.23	45.86	46.00
Arginine	7.61	7.53	
Aspartic	9.53	9.98	
Glutamic	16.76	15.64	
Serine	4.14	4.23	
Porline	5.07	5.19	
Glycine	5.54	5.47	
Alanine	6.12	6.10	
Non-essential amino acids	54.77	54.14	

3.4. Chemical scores for the essential amino acids of the garden cress meal and protein isolate.

The results shown in Table 4 show that histidine scored the highest chemical protein score of 144.85 and 140.38 for garden meal and garden protein isolate, based on the FAO/WHO/UNU (1989) reference. The data also indicated relatively high chemical protein scores of garden cress meal and protein isolate 124.36 and 125.82 for valine, 117.75 and 119.86 for phenylalanine+tyrosine, and 107.06 and 109.41 for tryptophan, respectively. In contrast, isoleucine, leucine and threonine showed relatively low chemical protein scores for both garden meal and protein isolate. Lysine and methionine + cystine showed the lowest score of representing the limiting amino acid (LAA) of the garden cress meal and protein isolate.

Table (4). Chemical scores for the essential amino acids of the garden cress meal and protein isolate.

Amino acids	Chemical score*	
	Garden meal	Garden isolate
Histidine	144.85	140.38
Isoleucine	88.48	92.61
Leucine	89.35	88.60
Lysine	78.48	81.67
Cysteine+Methionine	78.33	82.86
Tyrosine+phenylalanine	117.75	119.86
Threonine	84.19	82.56
Tryptophan	107.06	109.41
Valine	124.36	125.82

*The chemical score compared with the provisional values for the amino acids of the FAO/WHO/UNU standard (1989).

The garden meal and garden cress protein isolate had a relatively high protein efficiency ratio (PER). The PER value for the garden meal and garden cress protein isolate were 1.18 and 1.35 as compared to 1.46 for the control diet (Table 5), showing the high nutritional quality of the jojoba protein isolate and no significantly with casein. These data of garden cress protein could be used as a complement for proteins deficient in some essential amino acids. **Sumangala *et al.*, 2004** reported that the essential amino acid score of garden cress flour was 28.53% with methionine as the most limiting amino acid. These play a very important role in human nutrition. Lysine helps to maintain proper nitrogen balance. The body uses methionine to derive the brain food, choline. It also aids in digestion, as well as serving as a fat burner. It can interact with other substances to detoxify harmful agents, and is essential for the production of cysteine and taurine. L-Tryptophan acts as a sleep aid. It is also necessary for the production of niacin and is used by the body to make the neurotransmitter, serotonin **(Reeds, 2000)**.

Table 5. Food intakes, protein consumption, gain in body weight and PER values of experimental rats.

Parameters	Groups		
	Control	Garden meal	Garden protein isolate
Initial body weight	115.3±3.42^{a}	115.4±3.25^{a}	115.9±3.56^{a}
Final body weight	184.1±4.17^{a}	169.8±5.10^{c}	178.5±4.29^{b}
Body weight gain	68.8±2.82^{a}	54.4±2.48^{c}	62.6±3.14^{b}
Food intake	312.6±5.11^{a}	306.7±5.31^{b}	309.8±4.68^{ab}
Protein consumption	46.89±2.24^{a}	46.01±2.07^{a}	46.47±2.36^{a}
PER	1.46±0.10^{a}	1.18±0.16^{b}	1.35±0.12^{a}

3.4 Biochemical evaluation of the jojoba protein isolate

The biological experiment was performed to elucidate the effect of administering the garden cress meal and garden protein isolate, on the rat kidney functions, and the results of this experiment were taken as a guide to evaluate the safety of the garden meal and protein isolate. The results shown in Table 6 indicate that the administration of garden meal and protein isolate did not cause any significant change in the activities of uric acid, urea and creatinine functions. Regarding the effects of administering 15% of garden meal and garden protein isolate on the total bilirubin the results also indicated no significant changes.

Table 6. Serum urea, uric acid, creatinine and total bilirubin of experimental rats.

Parameters	Groups		
	Control	Garden meal	Garden protein isolate
Serum uric acid (mg.dL–1)	4.98±0.19	4.87±0.21	4.91±0.16
Serum urea (mg.dL–1)	35.46±3.48[a]	34.19±2.56[a]	35.17±2.94[a]
Serum creatinine (mg.dL–1)	1.74±0.13	1.76±0.15	1.81±0.18
Total bilirubin (mg.dL–1)	1.06±0.09	1.12±0.10	1.16±0.11

Conclusions

The present study reveals that *L. sativum* seeds with high nutritional value can be exploited as a functional food ingredient. Garden cress meal and protein isolate can be used as source of minerals and protein rich in essential amino acids after extracting the fat. These studies are required the bioavailability of meal and protein isolate and the possibility of using this as a functional food ingredient apart from using it as a source of dietary fiber.

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