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Amino Acid Composition and Physicochemical Properties of Bitter Lupine (*Lupinus termis*) Seed Flour

IKHLAS IBRAHIM KHALID[1], SIRELHATIM BALLA ELHARDALLOU[2] and ADIL ABDALLA GOBOURI[2]

[1]Faculty of Science, Shagra University, Saudi Arabia.
[2] Chemistry Department, Faculty of Science, Taif University, Saudi Arabia.
*Correspondance author E-mail: drhardallou@yahoo.com

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ABSTRACT

Physical properties, proximate composition, macro and micro minerals and amino acid profile of bitter lupine seed are investigated. The protein content of Whole Bitter Lupine Seed Flour (WBLSF) and De-hulled Bitter Lupine Seed Flour (DBLSF); 45.0 and 41.5% l, respectively; while fat content 2.9 and 1.2 %, respectively. Bitter lupine seed generally contains about twice the protein in normally consumed legumes. It is a good supplier of minerals. WBLSF contains, potassium, calcium, copper, iron, and zinc with concentration of 534 ± 2.89, 338 ± 3.60.; 1.04 ± 0.05; 5.30 ± 0.10 and 2.30 ± 0.03 (mg/100 g), respectively. WBLSF found rich in lysine, leucine and arginine: 4.5; 6.9 and 8.5 (mg/16 g N),respectively It can fulfill the essential amino acid requirement for human diet except for methionine (S-containing essential amino acid) and tryptophan: 0.34 and 0.99 (mg/16 g N), respectively. Net protein value (NPV) and Chemical Score of lupine seed was 0.153 and 12.186, respectively. The first limiting amino acid was cystine. Glutamic amino acid reported the highest amount (24.60 mg/16g N). Bitter lupine seed flour showed a relatively high concentration of indispensable amino acids, lysine and leucine.

Keywords: Bitter lupine seed, Physical properties; Proximate analysis; Macro and micro minerals; Amino acids concentration.

INTRODUCTION

Protein-energy malnutrition is a widespread problem throughout the world and has both health and economic consequences. It is the most available deficiency disease especially in third world countries[1]. The present feature of population growth shows that protein gap may continue to increase in the future and planned studies are needed to solve the problem. It is difficult and expensive to provide adequate proteins of animal origin. An alternative for improving nutrition status is to supplement the diet

with plant proteins. Recently, a special consideration given for the nutritional evaluation of proteins from plant sources[2]

Legumes (poor man's meat) are of significance in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins[3]. For food intake, in Afro-Asian states, legumes are the major contributors of protein and Calories for economic and cultural reasons. Food legumes are crops of the family *leguminosae* (also called *fabacae*). They are known as grain legumes, as they are grown for their edible seeds. Developing countries need protein-rich grain legumes for their food habit based on cereal diet and scarcity of fertile land[4, 5, 6].

Legumes are rich in protein, fiber, B-vitamins, iron, folate, calcium, potassium, phosphorus, and zin and low in fat. Legumes are similar to meat in nutrients, but with lower iron levels and no animal fats. The high protein and other nutrients content in legumes make them a good alternative for meat and dairy products. Vegetarians usually substitute legumes for meat.

Although legumes are rich of nutrients, they are low in calories, but make one feels full. They are of considerable value for diabetic people, as they relatively do not increase blood sugar. The body uses carbohydrates in legumes slowly, over time, providing steady energy. Legumes as part of a healthy diet, can help to lower blood sugar, blood pressure, heart rate, other heart diseases and diabetes risks. The fiber and other nutrients, in legumes, are of use to the digestive system, and may even help to hinder cancer[6].

Lupine is a valuable ancient legume, grown at different regions particularly the Mediterranean area and Indian highlands, with use of seeds as food[7, 8]. Lupine seeds, characterized of high protein content, rich in lysine, an essential amino acid and relatively poor in sulfur containing amino acids

Lupine is within twenty legumes used for human nutrition with cowpea (*Vigna sinensis* L.), kidney bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) as the most consumed[7, 10]. Although legume seeds contain a moderately high amount of protein, calories, certain minerals and vitamins; their use in food and feed is still limited by their low amount of sulfur-containing amino acids, low protein digestibility and the presence of anti-nutritional components[3, 11].

MATERIALS AND METHODS

Materials

Lupine (*Lupinus termis*) seed, obtained from local Cairo market, Egypt. The seeds, cleaned to remove husks and foreign matter then stored in polyethylene bags in the refrigerator until used.

Preparation of Lupine seed flours

Bitter lupine seeds whole or de-hulled, crushed using household mill (Braun, Germany). Fine flour, kept in refrigerator until used.

Hulls and kernel percentages

Dry seed samples, weighed and separated manually into hulls and kernels. The percentage of both kernels and hulls was determined on dry basis. The three samples measured and the average recorded.

Seed weight

Weight of 100 seeds taken at random, in triplicate. The average reported as 100 seeds weight..

Relative density of the seeds

The increase in volume of 200 ml of distilled water placed in a measuring cylinder after immersing 100 seeds of a known weight[12]. Relative density, calculated as follows:

$$\text{Relative density} = \frac{\text{The weight of seed (gm)}}{\text{The volume of seed (ml)}}$$

Oil extraction

Ground whole seeds in *n*- hexane (BP. 40 – 60^{0}C) at room temperature for 48 h with several solvent changes, followed by evaporation using rotary evaporator.

Specific gravity of extracted oil

Determined using 10 ml pycnometer at 20°C,[13].

Viscosity

Viscosity of oil samples was measured as Cm pois using viscometer ICI C of Research equipment –London at 50 ^{0}C.

Proximate chemical composition

Moisture content, protein, using Kjeldahel method; fat, using Soxhlet apparatus; ash (gravimetrically); crude fibre and carbohydrates (by difference)[14]. Means reported on dry weight basis.

Minerals content

Seeds flour samples were digested by concentrated HNO_3 and $HCLO_4$ (1:1, V/V) for 2 hours (till solution became colorless). Na, Ca and K were estimated using emission flame photometer (Model Corning 410). Mg, Mn, Fe, Zn and Cu; determined using atomic absorption spectrophotometer (Prekin – Elmer Instrument Model 2380).

Table 1: Physical properties of bitter lupine seeds

Property	Bitter Lupine seed	LSD
Seed index (g)	20.40 ± 1.10	2.50
Hull %	11.60 ± 0.038	0.80
Kernels %	80.30 ± 0.41	0.98
Relative density (g/cm^3)	1.01 ± 0.05	0.10

Amino acid analysis

Performed on bitter lupine seed flour using amino acid analyzer[15]

Each sample; hydrolyzed with 6 N HCl at 110 ^{0}C for 24 hr. Amino acid composition calculated considering the highest value for each amino acid. Sulfur-containing amino acids; determined after performing acid oxidation. Chemical score was calculated[16].

Amino acids calculated using the reference pattern[17]. The amino acid showing the lowest percentage (limiting amino acid) representing the chemical score. Essential amino acid index (EAAI) was calculated[18] using the amino acid composition of whole egg protein[19]. Protein efficiency ratio (PER) was estimated according to the following regression equation[20]

PER = - 0.488 +0.454 (Leucine) – 0.105 (Tyrosine).

The net protein value (NPV), calculated as follows:

NPV = (The lowest amino acid score ×% protein) /100

$$\text{Amino acid score} = \frac{\text{Test amino acid} \times 100}{\text{Reference amino acid}}$$

Statistical analysis

Data, based on three replicates, subjected to analysis of variance by complete block design (Gomez & Gomez, 1984). Standard deviation

Table 2: Proximate composition of whole and de-hulled bitter lupine seed flour (on dry basis)

Chemical constituents	Whole bitter lupine seed flour	De-hulled bitter lupine seed flour	LSD
Total protein (N X6,25)	41.5^b± 1.30	45.0^c ± 0.66	0.98
Crude lipids	2.9 ^{b}± 0.01	1.1 ^{a} ± 0.05	0.2
Crude fiber	18.0 ^{b}± 0.22	12.1^c± 0.11	0,46
Total ash	3.9 ^{a}± 0.05	3.7 ^{b}± 0.10	0.56
Total carbohydrates (by difference)	33.8 ^{a} ± 0.03	38.1^b± 0.10	1.45

Means ± standard deviation of three replicates. Means in the same raw with different letters are significantly different (P < 0.05). LSD = Least significan for t differences

evaluated. Least significance difference (LSD) mean computed and variations at 5% level; probability (P = 0.05).

RESULTS AND DISCUSSION

Physical properties of bitter lupine seeds flour

Seed index, hull percentage, kernel percentage, relative density, presented in Table (1). Generally, the values obtained in this study were higher than others[21].

Proximate composition

The moisture content of WBLSF, found 7.3 % and 8.0 % for DBLSF. Crude protein, fat content, crude fibre, ash and total carbohydrates, (on dry basis) (Table 2).

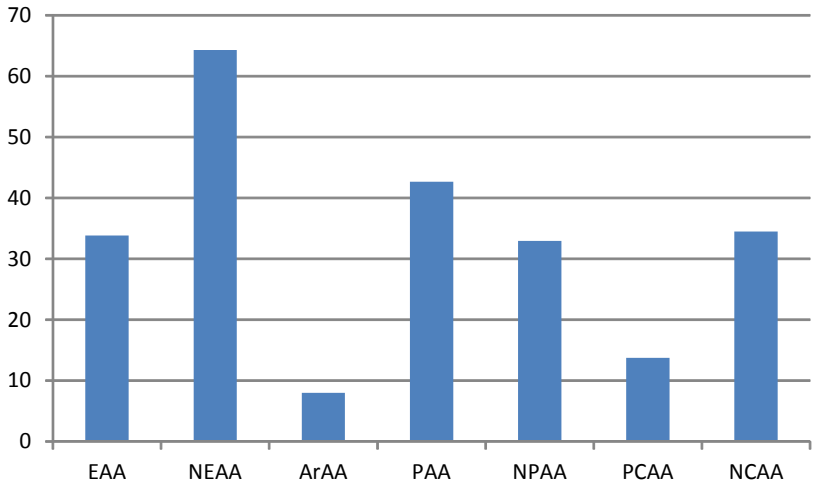

Fig. 1: Estimation of nutritional quality of bitter lupin seed flour based on amino acid composition

EAA: Essential amino acids NEAA:Non essential amino acids
ArAA: Aromatic amino acids PAA: Polar amino acids
NPAA: Non polar amino acids PCAA: Positively charged amino acids
NCAA: Negatively charged amino acids

Table 3: Macro and micro minerals content of whole and de-hulled bitter lupine seed flour (mg /100 g)

Element	Whole bitter lupine seed flour (WBLSF)	De-hulled bitter lupine seed flour (DBLSF)	LSD
Macro element:			
Potassium	534 ± 2.89	630 ± 5.10	8.12
Sodium	116 ± 2.34	189 ± 2.45	4.20
Calcium	338 ± 3.60	450 ± 3.50	5.80
Micro element:			
Zinic	2.30 ± 0.03	2.86 ± 0.05	0.12
Iron	5.30 ± 0.10	6.60 ± 0.13	0.20
Copper	1.04 ± 0.05	1.15 ± 0.06	0.05

Means ± standard deviation of three replicates. LSD = Least significant differences.

Table 4: Amino acids composition of de-hulled bitter lupine seed flour, (g/ 16 g nitrogen)

Amino acid	De-hulled bitter lupine seed flour (DBLSF)	FAO/WHO (1973)
Isoleucine	4.00	4.00
Leucine	6.90	7.00
Lysine	4.50	5.50
Cystine	1.00	–
Methionine	0.34	-
Total sulfur amino acids	1.34	3.50
Tyrosine	4.40	–
Phenylalanine	3.60	–
Total aromatic amino acids	8.00	6.00
Threonine	4.00	4.00
Tryptophan	0.99	1.00
Valine	4.10	5.00
Total essential amino acids	33.83	36.00
Histidine	3.93	–
Arginine	8.50	–
Aspartic acid	9.90	–
Glutamic acid	24.60	–
Serine	4.33	–
Proline	4.80	–
Glycine	4.20	–
Alanine	4.00	–
Total non – essential amino acids	64.26	–

Table 5: Classification of amino acids (g/ 16 g nitrogen) found in de-hulled bitter lupine seed flour (DBLSF)

Amino acid description	De-hulled bitter lupin seed flour
Total proteins	45
Total amino acids (TAA)	98
Total essential amino acids (TAA) with histidine	34.28
Total essential amino acids (TEAA) without histidine	29.9
Total non essential amino acids (TNAA)	64.26
Essential aromatic amino acid (EArAA)	8
Total acidic amino acids (TAAA)	34.5
Total basic amino acids (TBAA)	16.93
Total sulphur containing amino acids (TSAA)	1.34

Table 6: Estimation of nutritional quality of de-hulled bitter lupine seed flour(DBLSF); based on amino acids composition

Material	Protein (%)	First limiting Amino acid	Second limiting Amino acid	Third limiting Amino acid	Chemical score %	Net protein value (NPV)	Protein efficiency ratio (PER)
De-hulled bitter lupin seed flour	45.00	Methionine	Cystine	Tryptophan	12.186	0.153	2.67

Crude protein content of WBLSF and DBLSF, found 41.5 and 45.0 %; respectively (Table 2). This agrees with reported findings for protein in pea, common bean, chickpea and lentil (18.5 to 24%)[22]. Protein content in local and improved cowpea legume, widely consumed in Nigeria (22- 26%) [23]. Protein content of bitter lupine seed is clearly higher (Table 2); this difference can be attributed to genetic and environmental factors[24].

Fat content

The fat content 2.9 % for WBLSF and 1.2 % for or D, (Table 2); in agreement with (2.2 and 1.1 %), [25].

Crude fiber content

Fiber content, 18.0 % for WBLSF and 12. 1 % for DBLSF disagreeing with other values; 8.08%[27].

Ash content

Ash content, 3.9% for WBLSF and 3.7% for DBLSF (Table 2). Present results agree with those reported for faba bean legume seeds, 3.6%[28].

Total carbohydrates (calculated by difference)

(33.8 %) for WBLSF and 38.1 % for DBLSF (Table 2). A range of 24 to 37% starch content; reported for cowpea legume seeds[29].

Minerals content

Macro and micro mineral composition of WBLSF and DBLSF, presented in (Table 3). Significant ($P < 0.05$) differences, observed between WBLSF and DBLSF.

Legumes are a good source of minerals with more Ca content than most cereals[30]. Lupine seed flour, can be used as supplement for cereal flour to improve its Ca content.

Amino acid composition

Amino acid composition of the bitter lupine seed flours (BLF), reported as g/16 g N comparing to the[31].

Glutamic acid was the most abundant amino acid in DBLSF 24.60 g / 16 g nitrogen. The second most abundant amino acid, aspartic acid (9.90 g/16 g N). The amino acid composition of the four legumes

(chickpea, cowpea) indicated little variation in the contents of total essential and non-essential amino acids. The most concentrated essential amino acid in lupine seed was leucine with value (6.90 g/16 g N). Total amino acid and total essential amino acids in lupine seed were 98.09 and 29.90 g /16 g N; respectively. This is within the range of total essential amino acids without histidine.

Arginine content (8.3 %) agrees with that reported for chickpea seed[2]. Glutamic acid, found to be major non-essential amino acid in studied DBLSF (Table 4).

DBLSF showed a high % of total essential amino acid. The values of total sulphur containing amino acids in DBLSF: 1.34 g/16 g N (Table 4). The ratio of total acidic and basic amino acids in DBLSF found (34.50 : 16.93 g/16 g N); as reported for cowpea seed protein[32] suggesting protein in the legumes to be in acidic nature.

Further amino acid analysis, are shown in (Table5). Total essential amino acid (TEAA) 29.90 % in DBLSF without histidine; essential aromatic amino acid (EArAA) (8.00 g/16 g N). On the basis of chemical scores, low leucine : isoleucine ratio in DBLSF is desirable as it lead to amino acid balance in cereals that are already high in leucine and low in tryptophan and isoleucine. DBLSF is deficient in sulfur containing amino acids, as are most legume and vegetable proteins.

The first limiting amino acid was total sulfur amino acids and the second limiting amino acid was valine. The basic amino acid (BAA), (16.93 g/16 g N) found to be less than the total acidic amino acids (AAA), (34.5 g/16 g N) (Table 5) indicating that protein is acidic in nature. The Mitchel essential amino acids index (MEAAI) was 56.40%.

CONCLUSION

DBLSF showed high levels of crude protein, oil, crude fibre and ash than other legumes and wheat flour. Bitter lupine, promising legume seeds, especially for areas in the world where soya (which includes a high amount of both protein and oil) is not available. Bitter lupine seed is particularly relevant to compensate deficiencies in lysine and sulphur-containing amino acids, in cereals and grain legumes, respectively.

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