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Physico-chemical Properties and Fatty Acids Composition of Bitter and Sweet Lupine Seed

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ABSTRACT

This study describes the constituents and properties of bitter lupine (BL) *(Lupinus termis)* and sweet lupine (SL) *(Lupinus albus)* seed oils in addition to oligosaccharides content in seed flour; each can be beneficial for consumers. There was a significant difference in saponnification number and ester value of both oils. The peroxide value was found (1.80 ± 0.20 meq/kg in BL seed oil compared to 1.89 ± 0.29 meq/kg in SL seed oil. Fatty acids (FA) composition showed that total unsaturated FAs were higher, (88.33 %) in BL seed oil than (87.25 %) in SL seed oil; both values markedly higher than saturated FA content. The major FA in both samples is oleic FA, (50.23 %) in BL and 45.00 % in SL. Essential FAs were found higher (39.80 %) in SL; compared to (36.11) in BL. Total phenols were significantly higher in BL seed oil (58.99 mg/kg); than.SL seed oil (50.95 mg/kg). The oil classes: triglycerides, free FA steroids and alcohols were found higher in SL seed oil; while hydrocarbons, mono-glycerides, di-glycerides and phospholipids were higher in BL seed oil. Total oligosaccharides were found significantly higher (9.74 ± 0.20 g/100 g) in BL flour compared to 8.99 ± 0.10 g/100 g in SL flour; with stachyose in concentrations of 3.9 ± 0.10 g/100 g and 3.88 ± 0.03 g/100 g; respectively.

Keywords: Bitter lupine, Sweet lupine, Physical properties, Fatty acids, Oligosaccharides, Oil classes, Oil properties.

INTRODUCTION

Lupine is a leguminous plant that grows in different climates and soils. It is a potential source of protein oil and pharmaceutical purposes[1,2]. Like other legumes, lupine seeds are characterized by higher values of protein, minerals and dietary fiber. Protein in lupine sees, (38 %)[3] is higher than other legumes and close to soy protein%[3].

Lupine is a neglected legume with high protein content and fat like sesame[4]. Lupine seeds, with high protein and dietary fibre, are a considerable human food and animal feed[5].

Lupine is lower in anti-nutritional factors than soya beans trypsin inhibitors and hemaglutinnins are practically absent[6,7,8, 9]. Lupine seed protein showed many essential amino acid deficiencies with

tryptophan being the most limiting. It also revealed a high amount of lysine and low amount of sulphur-containing amino acids with a chemical score (CS) of 47–59%[10]. CS is ratio of the amount of an essential amino acid in food protein to the amount of the same amino acid in a reference pattern[11].

Plant fats and oils are widely used in foods for salad dressing, frying, margarine, ice cream manufacture and cooking. Lupine oils are characterized by balanced fatty acid (FA) composition with total saturated FA of about 10% and total unsaturated FA 90%[12]. Lupine FA composition is mainly unsaturated with oleic and linoleic FA comprising 86% of whole oil[13]. The alkaloid content of lupine seed varies according to cultivar, soil type and cultivation season[14]. Lupine oils are characterized by balanced FA composition. Lupine seed flour is used in different cereal products as pasta, crisp, bread, cookie and cake[15].

MATERIALS AND METHODS

Bitter lupine *(Lupinus termis)* and Sweet lupine *(Lupinus albus)* seeds were collected from Cairo city Egypt. They were cleaned, and rendered free of dust and foreign bodies, stored in polyethylene bags and kept in refrigerator ready for use.

Preparation of Lupine seed flour

Lupine seeds were crushed using household mill (Braun, Germany); defatted by soaking in n-hexane (Boiling point 67°C) for 48 h with several changes of the solvent. The defatted flour was air dried at room temperature (27^{0}C); ground to pass through a 60 mesh and kept in refrigerator.

Preparation of Lupine seed oil

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 (ROT. VSAC. EVA. RVA. 64, Czechoslovakia). 0il samples stored in dark tightly fitting glass bottles and kept in refrigerator ready for analysis.

Physical properties of lupine seeds

1. **Seed index (Weight of 100–seeds):** Triplicate of random samples of 100 seeds were weighed. And the average was reported.
2. **Hulls and kernel percentages:** Dry seed samples were weighed and separated manually into hulls and kernels. The percentage of both kernels and hulls was determined on dry basis. Three samples were measured and the average was recorded.
3. **Relative density of seeds:** It was determined using the methods of Youssef 18 .100-seeds of known weight were placed in a graduated cylinder containing 200ml distilled water. The increase in volume was calculated as follows:

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

Thickness and diameter

Micrometer was used to measure both thickness and diameter.

Physical properties of lupine oil

1. **Specific gravity:** The specific gravity of oil samples was determined using 10ml pycnometer at 20^{0}C according to the method of AOCS[17].
2. **Relative index:** Relative index of the oil samples was measured using a Refractometer (CARI ZEISS JENA GDR) at 25^{0}C according to the method of AOCS[17].
3. **Melting point:** The melting point of oils samples was estimated using a hot plate microscope having thermometer, heating was adjusted to raise the temperature by 1^{0}C every 2 minutes.
4. **Viscosity:** The viscosity of oil samples was measured as cm pois using viscometer ICI Co Research equipment – London at 50^{0}C.
5. **Colour:** The colour of oil samples was determined by *Lovibond tintometer* using a 5.25 inch cell. The yellow filter was fixed at 35 and the intensity of red was measured according to the AOCS[17].

Proximate Composition of lupine seed flour

ICC Standard Methods[16] were used. Moisture content was determined by drying the sample flour at 105°C to a constant weight[16]. Ash content was determined by calcination at 900°C[16] Nitrogen content was determined using *Kjeldahil* method with factor of 5.7 to evaluate protein content[4]. The total lipid content was determined by extraction using hexane in soxhlet apparatus[16]. Starch content was determined using a polar metric method[17]. All measurements were made in triplicate and calculated on dry weight basis.

Chemical characterization of lupine seed oil

The values of acid, peroxide, saponnification and iodine were determined[19].

Fatty acids composition

Methyl esters of crude oils were prepared using 1% H_2SO_4 in absolute methyl alcohol[19]. A Perkin – Elmer gas chromatography (Model F22) with a flame ionization detector was use,d in the presence of nitrogen as a carrier gas. A glass column (2 mx 2.5 mm) packed with Chrome Q 80/100 mesh at a temperature of 270^{0}C was used. Standard fatty acids methyl esters, were used for identification. The area under each peak was measured and the percentage expressed in reference to total area.

Oligosaccharides content

Oligosaccharides, extracted from the flour samples (10 g of flour sample, placed in 150 ml of boiling distilled water and kept at boiling for 30 min) were determined using HPLC[20].

Statistical analysis

All results expressed as the mean of three determinations. The data were statistically analyzed using analysis of variance and least significant difference[21]. Significant difference was determined at the P‹0.05 level. Means ± standard deviations of three replicates were used[22]

RESULTS AND DISCUSSION

Physical Properties of lupine seed oil

Physical properties of BL and SL seeds such as seed index hull percentage, kernel percentage, relative density, seed dimensions (length, width, and thickness) are presented in Table (1). BL seeds were significantly (P≤0.05) higher in most physical properties (except kernel percentage) than SL seeds.

Table 1: Physical properties of bitter and sweet lupine seeds

Property	Bitter lupine seed	Sweet lupine seed	LSD
Seed index (g)	22.41^b ± 1.15	19.93^a ± 0.98	2.44
Hulls %	12.60 ^{b} ± 0.41	10.55^a ±0.31	0.85
Kernels %	88.39 ^{a} ± 0.44	89.09^b ± 0.75	0.98
Relative density (g/cm^3)	1.03 ^{b} ± 0.07	0.77^a ± 0.05	0.14
Seed length (mm)	10.33 ^{b} ± 0.35	8.10^a ± 0.46	0.94
Seed width (mm)	8.52 ^{b} ± 0.31	7.37^a ± 0.21	0.60
Seed thickness (mm)	4.56 ^{b} ± 0.11	3.71^a ± 0.20	0.37

Same letter in any raw (sample) signs for non-significant difference. *Mean ± standard deviation of 3 determinations. LSD = Least significant difference

Table 2 shows some physical properties of BL and SL seed oils. No significant (P≤0.05) difference was observed between BL and SL seed oils in their Relative index, specific gravity, melting point and Viscosity. These results agree well with those of lupinus termis seed oil[23] for relative index (1,471–1473), specific gravity (0.6169–0.9240) and colour (9R/1.3B).

Table 2: Physical properties of bitter and sweet lupine seed oils

Property	Bitter lupine seed	Sweet lupine seed	LSD
Relative index (25^{0}C)	1.4681^a ± 0.002	1.4585^a ± 0.002	0.003
Specific gravity (25^{0}C)	0.868^a ± 0.03	0.92^a ± 0.07	0.123
Melting point (^{0}C)	5.2^a ± 0.06	5.1^a ± 0.06	0.009
Viscosity (cm pois)	40.7^a ± 0.15	40.8^a ± 0.32	0.570
**Colour	9.0R/5,5 B	7.0R/3.7B	

Same letter in any raw (sample) signs for non-significant difference. Mean ± standard deviation of 3 determinations. LSD = Least significant difference. ** Means ± standard deviation of 2 determinations

Proximate analysis

Proximate analysis of whole BL seed flour showed 43.6 % total protein (N X6,25); 9.6 % crude lipids; 12.5% crude fiber; 3.2 % ash and 31.1% total carbohydrates (by difference). Proximate analysis of whole SL seed flour showed 38.6 % total protein (N X6,25); 12.4% crude lipids; 11.3% crude fiber; 3.3% ash and 34.4% total carbohydrates (by difference).

Chemical properties of lupine seed oil

Acid value (AV), an important indicator of vegetable oil quality, is expressed as the amount of KOH (in mg) necessary to neutralize free fatty acids found in 1 g of oil[23, 24].

Table 3 shows some chemical properties of lupine seed oils. There was no significant difference in both studies oils with respect to AV peroxide value and saponnification value. Howerver, there is a significant difference in total phenols, iodine value and ester value.

Table 3: Chemical properties of bitter and sweet lupine seed oils

Properties	Bitter	Sweet
Acid value	0.93^a ± 0.05	0.857^a ± 0.01
Peroxide value meq/kg	1.80^a ± 0.20	1.89^a ± 0. 29
Iodine value ml /100 g	115^b ± 2.0	110^a ± 2.60
Total phenols (mg / kg)	58.99^b ± 1.30	50.45^a ± 1.99
Saponification value	193.54^a ± 3.50	190.0^a ± 1.33
Ester value	190.00^a ± 3.60	185.05^b ± 1.44

Same letter in any raw (sample) signs for non-significant difference. .Mean + standard deviation of 3 determinations

In studies, 2012,[25] AV was reported as significantly higher in BL seed oil (0.935 ± 0.07) than SL seed oil, (0.835 ± 0.08); while ester value (in agreement with this study) was significantly lower in

SL seed oil as compared to BL seed oil. The peroxide values of both oils recorded in present study are well below the range of those reported by Codex Alimmantarius Commission (2001) i.e <10 meq/kg for soybean, rapeseed, cotton seed and coconut oils. The iodine values observed in both BL (115.4 ± 2.06) and SL (110.7 ± 2.15) seed oils were higher than that earlier reported[26] as 96%.

Fatty acids composition

Table 4 shows fatty acid composition of BL and SL seed oils There was a significant variation between the two types. Oleic acid was significantly higher in BL oil (50.23%) than SL seed oil (45%); while linoleic acid is significantly lower in BL (22%) and higher in SL (24.9%); with no significant difference in linolenic FA content: (13.5%) and (14.9%) for BL and SL seed oil, respectively. However, the content of linolenic acid in both types of lupine seed oils are far less than that reported in flax seed oil (47%)[27].

Lupine seed oil classes

No variations were observed in percentage of oil classes (like hydrocarbons, triglycerides, free fatty acids and steroids) in both BL and SL seed oils. However, BL seed oil showed higher content of diglycerides (Table 5).

Table 4: Fatty acids composition of bitter and sweet lupine seed oils

Saturated and unsaturated Fatty acids %	Lupine seed flours oil	
	Bitter lupine	Sweet lupine
Myristic $C_{14:0}$	0.140±0.03[a]	0.199±0.03[a]
Palmitic $C_{16:0}$	8.990±0.69[a]	7.612±0.69[a]
Stearic $C_{18:0}$	2.001±0.14[a]	1.711±0.14[b]
Arachidinic $C_{20:0}$	2.123±0.27[a]	2.657±0.27[a]
Oleic $C_{18:1}$	50.234±0.62[a]	45.00±0.62[b]
Linoleic $C_{18:2}$	22.546±1.18[b]	24.900±1.18[a]
Linolenic $C_{18:3}$	13.564±0.67[a]	14.897±0.67[a]
Palmitoleic $C_{16:1}$	0.440±0.11[a]	0.660±0.11[a]
Arachidic $C_{20:1}$	1.551±0.12[a]	1.789±0.12[a]
Total saturated fatty acids (TSFA)	13.251±0.54[a]	12.179±0.54[a]
Total unsaturated fatty acids (TUFA)	88.330±0.54[a]	87.246±0.54[a]
(TUFA)/ (TSFA)	6.67±0.23[b]	7.14±0.23[a]
Total essential fatty acid	36.114±0.84[b]	39.797±0.84[a]

Same letter in any raw (sample) signs for non-significant difference

Table 5: % of oil classes in bitter and sweet lupine seed oils

Class	Lupine seed oil	
	Bitter	Sweet
Hydrocarbons	1.00±0.045[a]	0.91±0.045[b]
Free fatty acids	9.00±0.11[a]	9.22±0.11[a]
Steroids	4.20±0.15[a]	4.50±0.15[a]
Monoglycerides	2.00±0.25[a]	1.50±0.25[a]
Diglycerides	8.00±0.50[a]	7.00±0.50[a]
Triglycerides	74.00±0.335[a]	74.67±0.335[a]
Phospholipids	2.20±0.045[a]	2.11±0.045[a]
Alcohols	0.21±0.16[b]	0.53±0.16[a]

Same letter in any raw (sample) signs for non-significant difference

Oligosaccharides content

Oligosaccharides are the most abundant soluble sugars; it was reported that sucrose and verbascose content in legume seeds are genetically affected whereas raffinose and stachyose content is mostly guided by environmental conditions[28].

As shown in Table 6 there is a significant difference between the content of each of raffinose and verbascose in flour of BL and SL seed. On the other hand, there is no significant difference between sucrose and stachyose content in the two studied samples. In Dolichos labab, legume seeds (as% of dry weight): stachyose (2.68 ± 0.13), raffinose (0.84 ± 0.03) and sucrose (1.49 ± 0.06) was reported[28].

Table 6: Oligosaccharides content of bitter and sweet lupine seed flours (mg/100 g sample)

Sugar	Bitter	Sweet	LSD
Sucrose	1.99a ± 0.10	2.00a ± 0.11	0.213
Stachyose	3.90a ± 0.10	3.88a ± 0.03	0.151
Raffinose	1.40 b ± 0.06	0.89as ± 0.07	0.166
Verbascose	2.45 b ± 0.05	2.22a ± 0.05	0.149
Total	9.74b ± 0.20	8.99 a ± 0.10	0.388

Same letter in any raw (sample) signs for non-significant difference. Mean + standard deviation of 3 determinations. LSD = Least significant difference

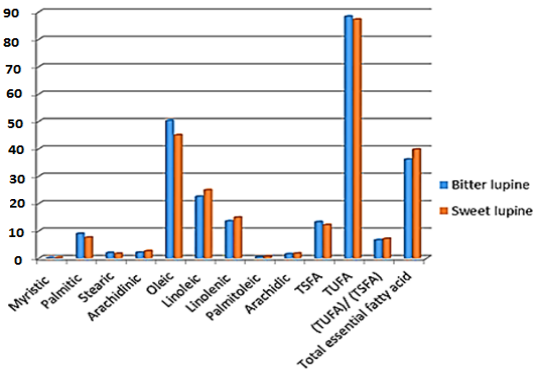

Fig. 1. Fatty acids composition of bitter and sweet lupine seed oils

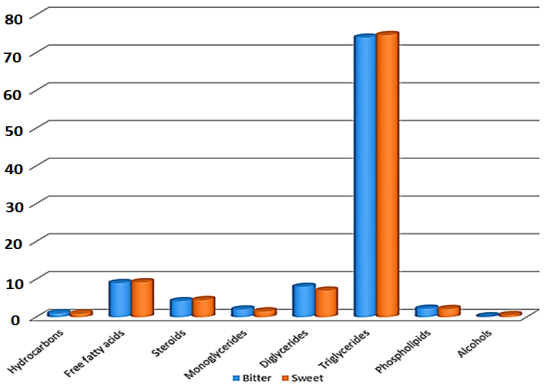

Fig. 2. Oil classes % of bitter and sweet lupine seed

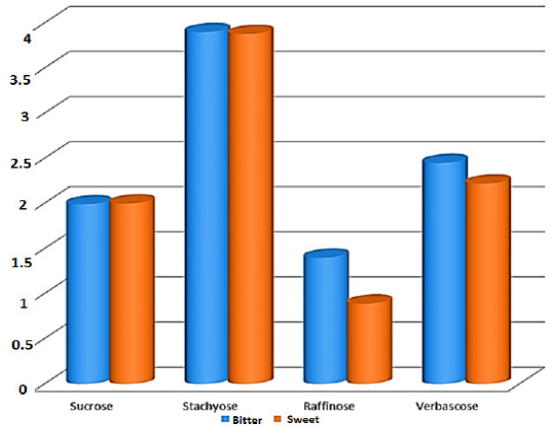

Fig. 3. Oligosaccharides content of bitter and sweat lupine seed flours (mg/100 g sample)

CONCLUSION

The BL seed weight was found (22.41b ± 1.15), significantly more than SL seed weight (19.93a ± 0.98); with hull % of (12.60 b ± 0.41) and (10.55 a ± 0.31); respectively. Essential FAs were found higher (39.80 %) in SL seed oil; compared to (36.11) in BL. The major FA in both samples is oleic FA, (50.23 %) in BL and 45.00 % in SL. Fatty acids (FA) composition showed that total unsaturated FAs were higher, (88.33%) in BL seed oil than (87.25%) in SL seed oil; both values markedly higher than saturated FA values. Total phenols were significantly higher in BL seed oil (58.99 mg/kg); than SL seed oil (50.95 mg/kg). Total oligosaccharides were found significantly higher (9.74 ± 0.20 g/100 g) in BL flour compared to 8.99 ± 0.10 g/100 g in SL flour; with raffinose concentrations of (1.40 b + 0.06 g/100 g) and (0.89as + 0.07 g/100 g); respectively.

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Confilicts of Interest

The authors declare no confilict of interest.

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