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Evaluation of the Nutritional Value of Bitter and Sweet Lupine Cultivars as Protein Source

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Abstract Protein flours and concentrates were prepared from two species of bitter and sweet lupine. Chemical composition was determined for flours and protein concentrates, while physical properties were determined including; solubility, also emulsion, foaming, water and oil absorption capacity in the two types of lupine protein concentrate. Non significant differences were found in chemical composition between the two lupine species in both flour and lupine protein concentrate, while higher content in total amino acids was observed in sweet than in bitter lupine protein, on the other hand non significant differences were found in the aforementioned physical parameters. Effect of casein replacement with bitter lupine protein concentrate (BPC) or sweet lupine protein concentrate (SPC) in basal diet on serum protein and liver function of rats was studied using 32 rats which were divided into four groups, each of eight rats as follow; normal control fed on basal diet, negative control fed on low protein basal diet and tested groups I and II fed on basal diet in which casein was replaced with BPC or SPC, feeding period was 28 days. Weight gain, food intake, FER, feces dry weight, and feces nitrogen were for normal control group in values of 85.0g, 3360 g, 0.20, 12.5g and 2.41%, which were higher than tested groups in values of 75.0g, 3320g, 0.18, 11.0g and 2.30% for group I and 79.0g, 3332g, 0.19, 11.30g and 2.32% for group II with non significant differences between the two groups in the above parameters respectively. Also significant decreases were found in protein efficiency ratio, net protein ratio and biological value in the tested groups in values of 1.80, 2.02 and 63.72 for group I and 1.89, 2.11 and 64.67 for group II and 2.03, 2.25 and 70.77 for control group in the three parameters respectively, while true digestibility showed non significant difference among the two rested and control groups in values of 95.3, 95.5 and 94.1%. On the other hand, non significant differences were found in biological evaluation among tested and control groups in serum of rats including; total protein, albumin, AST, ALT, uric acid and creatinine, in values of 7.03g/dl, 3.33g/dl, 40.3 mg/dl, 36.1mg/dl, 0.82 mg/dl and 5.40mg/dl for group I and 7.13g/dl, 3.43g/dl, 43.2mg/dl, 37.4mg/dl, 0.84 mg/dl and 5.42mg/dl for group II and 7.19 g/dl, 3.90g/dl, 44.0 mg/dl, 38.2 mg/dl, 0.88 mg/dl and 5.3mg/dl for normal control group in the above parameters respectively.

Keywords Lupine, Functional properties, Food Composition, Biological Availability, Biochemical Serum parameters, Rats

1. Introduction

The *Lupinus albus* L. species is a part of the leguminosae (fabaceae) family and the lupinus genus (Griffiths, et al., 2021). It has a high nutritional value and is commonly used in the food industry. (Gulewicz et al., 2008) in addition a good source of protein, fat, minerals, and dietary fibre, which is becoming more popular as a source of protein in the diet (Bartkiene et al., 2012). It has been utilised as a human and cattle food and emerged as a promising source of novel ingredients with a high protein content and an acceptable amino acid composition. Furthermore, lupine protein concentrates and isolates have a source of nutrition because of its high protein content, indigestible starches, and unsaturated fats and excellent technofunctional qualities that allow them to be used in a wide range of food products including baked goods and meat substitutes, caused by physical properties such as emulsification and foaming. (Abeshu & Kefale, 2017). In nature, there are about 400 species of lupine. Only a few species have been widely examined for their agronomical traits and nutritional values, including white lupin (*Lupinus albus*), blue lupin (*Lupinus angustifolius*), yellow lupin (*Lupinus luteus*), and pearl or Tarrwi lupin (Lupinus mutabilis) it was traditionally been used largely to feed livestock and aquaculture in most of the world, and this is still the case, People's interest in lupin as a food has developed but they've become more aware of its unique nutritional value and health benefits (Khan et al., 2015). White lupin's seed composition, particular particularly its high protein content, makes it great for animal feeding. It is commercially viable because to its adaptation to

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poor soil. Because to the presence of quinolizidine alkaloids and many antinutritional components, the crop has a notably bitter flavour, rendering it unfit for human consumption (Sun, et al., 2018). Sweet lupin (*Lupinus angustifolius*) is a flowering plant native to Australia, as well as numerous European, African, and South American countries. It has been determined that humans can safely consumed. because it contains only trace amounts of bitter and possibly toxic alkaloids despite the fact that lupin protein is of equivalent quality to regularly used soy protein, its utilisation in the food sector is limited. One explanation for this may be a lack of research on the functional qualities of lupine protein (Abraham et al., 2019). Food proteins' functional qualities, which refer to the underlying physicochemical characteristics that influence protein behaviour during processing and storage, as well as the quality of food formulations, are important determinants of their food lupine proteins have a good formation capacity, solubility and gel-formation abilities (Lara-Rivera et al., 2017 and Taglieri, et al., 2021) so that maximise the possibilities for food industry applications, it is important to determine and optimise the physico-functional properties of novel protein components (Feyzi et al., 2017). For the preparation of concentrated protein components from legumes, ultrafiltration has been used as an alternative to isoelectric precipitation, resulting in better protein recovery and physicofunctional characteristics (Šćiban, et al., 2021), It noticed that the low digestibility and nutritional quality of most vegetable protein lupin, however, studies have reported that the levels of undesirable constituents, such as alkaloids, phytic acid, lectins, saponins, trypsin inhibitors, and protease inhibitors, which can affect protein digestibility, are low compared to soybeans and other legumes (Thakur et al., 2019) the objective of this study was to determine chemical composition and the nutritional value for bitter and sweet lupine protein concentrate in rats.

2. Materials and Methods

2.1. Materials

All chemicals and kits were fine grade chemicals purchased from Sigma Chem. Co., (St. Louis, MO, USA), Merck (Germany), and bio dijonostic, Cairo, Egypt.

Bitter lupine (*Lupineus termis*) and Sweet lupine (*L. angustifolius*) were obtained from Agricultural Research Center, Field Crops Department.

2.2. Methods

Preparation of lupine protein concentrates.

Lupine seeds were crushed using high speed smashing machines (FW 100 model) 0.25 mm sieve to obtained lupine flour which was used for preparing protein concentrate according to the method of (Zheng et al., 2008). Lupine flour was soaked in aqueous ethanol 80% and stirred for 30 minutes at room temperature to dissolve alkaloids and non-protein components, then the slurry was filtered with Wathman paper No. 41, and the resultant cake was extracted for six time, the cake was pre-dried at room temperature for two hours, then kept overnight in forced oven at 45℃ for the final drying, the dried material was ground to pass through a 0.25 mm sieve to obtain protein concentrate.

2.2.1. Proximate Composition

All chemical analysis including; moisture, protein, fat and ash in lupine flour and protein concentrate were determined according to the methods of official methods of analysis (A.O.A.C, 2016) and carbohydrate content was calculated by difference.

2.2.2. Amino acids Analyses

The amino acids content of both bitter and sweet lupine protein concentrate were determined by high-performance liquid chromatography (HPLC, Agilent 1100) according to Terrell (1992) and Henderson, et al., (2000) and compared to the human amino acid requirements for an adult (Food and agriculture Organization/World health Organization 1991).

2.3. Evaluation of Functional Properties in Protein Concentrate

2.3.1. Protein Solubility

Protein solubility was determined according to Vogelsang-O'Dwyer (2020) by dispersions of 1% (w/v) lupine protein concentrate, and pH was adjusted in range from 2.0 to 8.0. Protein solubility was expressed as percentage of original protein content remaining in the supernatant.

$$\textbf{\textit{Protein solubility \%}} = \frac{amount\ \ of\ nitrogen\ \ in\ the\ supernatant}{amount\ \ of\ nitrogen\ \ in\ the\ sample} \times 100$$

2.3.2. Emulsifying Capacity

Emulsifying capacity was carried out as reported by Bader et al., (2011) at different pH values of 2.0, 4.0, 4.5, 6.0, and 8.0/min using hand blender (THB-1000S, 220V-50/60Hz, Turkey) at high speed. Corn oil was added until the emulsion produced collapsed. The emulsifying ability of the sample was determined by the amount of oil used up to this point.

2.3.3. Foaming Capacity

Foaming capacity was measured using the method described by (Jayasena et al., (2010) in suspensions of sample at different pH values which was whipped using a Moulinex mill machine at high speed for 5 minute. After 30 seconds, the volume of the resulting foam layer was measured. Foam capacity was calculated as a percentage of the liquid's original volume increased after it was whipped.

$$\textbf{\textit{Foaming capacity \%}} = \frac{vol.after\ \ whipping\ \ - vol.before\ \ whipping}{vol.before\ \ whipping\ .} \times 100$$

2.3.4. Water and Oil absorption

Water and Oil absorption were determined according to Rodríguez-Ambriz et al., (2005). One gram of each sample was mixed with 10 ml distillated water or corn oil and standing for 30 min/ 25℃. then centrifuged at 1600 Xg **(**Heraeus instruments, made in Germany, 230V, 50/60 Hz, 270W) The difference between the amount of added water or oil and recovered one was used as a measure of absorption.

2.4. Biological Evaluation

Male rats were obtained from animal house of National Organization for Drug Control and Research Giza, Egypt. Rats were kept one week under standard laboratory conditions and housed in stainless steel cages in an air conditioned room with temperature 22±3℃ and relative humidity 30-70% for acclumization. The investigation complies with the guide for the care and use laboratory animals (NODCAR /II/01/2021). The experimental protocol was approved by the Institutional Ethics Committee of NODCAR, Giza.

2.4.1. Experimental Design

Thirty-two rats weighing 90± 10g were randomly divided into four groups each of eight animals. The formula of the basal diet used in the present study was as follows: 10% protein, 10% corn oil, 4% salt mixture, 1% vitamin mixture, 5% cellulose and 70% starch (AOAC, 2016). Negative control group (control^{-}) was fed on basal diet containing low protein 4%, positive control group (control^{+}) was fed on basal diet, group I and II were fed on basal diet in which casein replacement with bitter or sweet lupine protein concentrate respectively feeding period was 28 days (da Silva et al., 2020).

2.4.2. Protein Quality Measurement

Dietary intake and body weight were recorded weekly during the experimental period for 28 day. Feces were collected in individual containers and dried in an air oven at 105℃ then weighed, ground and estimated to determine the total nitrogen content. These data were used to calculate feed Efficiency Ratio and protein Efficiency Ratio as follow:

$$\mathbf{FER} = \frac{WG}{FI}$$

$$\mathbf{PER} = \frac{WG}{PI}$$

Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER), weight gain (WG), food Intake (FI), protein intake (PI) (Hegsted, 1997 and Martínez et al., 2007).

Net protein Ratio (NPR) was determined in the 14th day of the experiment, weight gain of the test group plus the weight loss of the group fed on low protein diet, regarding the protein intake of the test group by the method of (Bender and Doell 1957; Martínez et al., 2007). The relative of protein efficiency ratio (PER-R) and Net protein Ratio (NPR-R) were determined to be 100% of the PER and NPR results of the basal diet (Aletor, 2012).

$$NPR = \frac{WG\ (g) + CTR - 14th\ day}{PI}$$

True digestibility was also determined as follow:

$$TD = \frac{100[I-(F-FK)]}{I}$$

True digestibility (TD), the amount of nitrogen intake (I), the amount of nitrogen excreted in feces (F), the nitrogen fecal loss of the low protein diet group (FK) (Phillips, et al., 1981). At the end of experiment blood samples were taken from the orbital plexus of eye of each rat (Shermer and Jones, 1967) serum was separated and its biochemical parameters were determined.

2.4.3. Serum Biochemical Analysis

Total protein and albumin were determined according to Doumas, (1971), creatinine was determined according to Bartles, et al., (1972), Uric acid was determined according to Bartham and Tinder (1972) Alanine aminotransferase activity (ALT) and Aspartate aminotransferase activity (AST) were determined according to Bergmeyer and Harder, (1986).

2.5. Statistical Analysis

The data can be displayed as mean ± standard error and analyzed using one-way ANOVA, followed by Tukey post-hoc test, using Graph Pad Prism data analysis program Graph Pad software, Inc., San Diego, CA, USA). A value of $p \leq 0.05$ was considered statistically significant.

3. Results and Discussions

3.1. Chemical Composition of Lupine Flours and Lupine Protein Concentrate

Proximate chemical compositions of flour and protein concentration in bitter and sweet lupine are given in Table (1). Moisture content in percentage were 8.2, 7.3 in flour also were 6.9 and 6.3 in protein concentrate in bitter and sweet lupine respectively. The percentage of crude protein were lupine flour 36.0, 37.2 and in protein concentrate 61.0, 62.8 in the two types of the aforementioned lupine respectively, these results were similar to those obtained by (Martínez-Villaluenga et al., 2007) lupine family has a protein content range of 30 to 40%. Furthermore, protein content of *L. albus* flour ranged from 33 to 47% and lupine seeds have a high protein content, ranging from 28 to 48% depending on the species Jezierny, et al (2010).

Also, lipid content of bitter and sweet lupine flours were 10.5 and 9.7% which were higher than that of protein concentrate in percentage values of 8.1 and 7.4%, respectively (table 1). These results were in agreement with those obtained by Erbaş et al., (2005) who found that Lupine has a fat content ranging from 6 to 13%, indicating a high concentration of polyunsaturated fatty acids.

Ash contents of flour and protein concentrate were 3.8, 4.2, 2.7 and 2.4%. Also carbohydrate contents of flour and protein concentrate were 49.7, 48.9, 28.2 and 27.4 in better

and sweet lupine respectively. Carbohydrate content equivalent to 48% of the seed weight for *L. albus* and 43.8% for *L. angustifolius* as obtained by (Glencross et al., 2008, and (Banti and Bajo, 2020).

Table 1. Chemical composition of flours, bitter and sweet lupine protein concentrate (g/100gm)

Component	Lupine flour		Lupine protein concentration	
	Bitter	Sweet	Bitter	Sweet
Moisture	8.2± 0.11	7.3± 0.19	6.9±0.24	6.3±0.31
Protein	36.0±1.14	37.2±0.56	61.0±1.81	62.8±0.98
Fat	10.5±0.31	9.7± 0.44	8.1± 0.34	7.4± 0.29
Ash	3.8± 0.40	4.2± 0.28	2.7± 0.10	2.4± 0.14
Carbohydrate	49.7±1.70	48.9±0.93	28.2±0.41	27.4±0.54

Mean ±SE, n = 3

3.2. Amino Acids of Lupine Protein Concentrate

Table 2. Essential amino acids (EEA) composition of bitter and sweet lupine protein concentrate (mg/g)

Amino acid	Essential amino acids content	
	BPC	SPC
Histidien	15	19
Leucine	59	66
Isoleucine	33	40
Lysine	19	20.3
Theronine	55	43
Tryptophan	15	14
valine	22	24
Tyrosine&Phenylalanine	46	58
Methionine& Cysteine	12	15
Total EAA	276	299.3

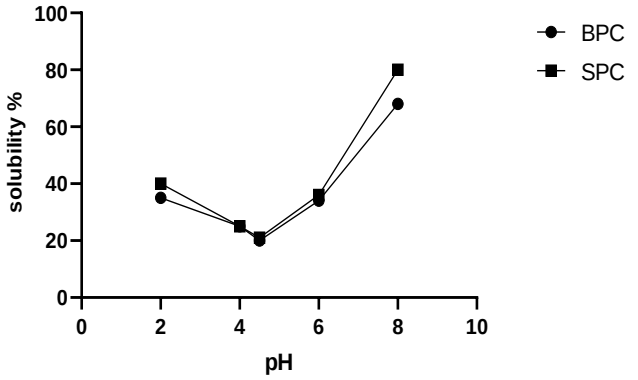
BPC: Bitter lupine protein concentrate
SPC: Sweet lupine protein concentrate, Mean ±S.E, n= 3

The essential amino acids of bitter lupine protein concentrate (BPC) and sweet lupine protein concentrate (SPC) are shown in Table 2. Their essential amino acids profiles are nearly equivalent and they cover the requirements of adult human for all essential amino acids except the cysteine and methionine, which were slightly deficiency legumes and other lupine species have been found to be deficient in sulphur amino acids such as methionine, cysteine and cystaine (Iqbal, et al., 2006 and Vogelsang-O'Dwyer, et al., 2020). Lupine protein revealed methionine which was the first limiting amino protein acid quality in all lupine cultivars (Lopez and Mohiuddin, 2021). The amino acids profile of protein from yellow and blue lupine seeds was slightly high in lysine level that was only slightly higher in yellow lupine. On the other hand, BPC was higher than SPC in all essential amino acids except threonine and tryptophan, also Starkute, et al., (2016) reported that Statistical analysis revealed that lupine variety has a major effect on essential amino acids content (EAA).

4. Evaluation of Physic Functional Properties in Protein Concentrate

4.1. Solubility of Protein

The effect of pH on the protein solubility of bitter and sweet lupine protein concentrates (BPC and SPC) lupine is illustrated in Figuer 1. Both tested protein had similarly solubility trends, showing maximum solubility at pH 2 and 8 and minimal solubility at pH 4–5. The minimum protein solubility of BPC and SPC was 20 and 21%, respectively, at pH 4.5 and increase with increasing pH. This result indicating that the isoelectric point (Ip) of all tested protein concentrates was at pH 4.5 because pH affects the molecule's net charge, protein carries positive charges at the amino group ($NH3^{+}$) in an acidic media and negative charges at the carboxylic group (COO^{-}) in an alkaline media, on the other hand, protein's net charge is zero at Ip and the repulsive forces between proteins are reduced, increasing protein aggregation and even precipitation (Sá, et al., 2020) the protein concentrate produced by ultrafiltration technique appears to have provided a protein concentrate with improved solubility. Also Akharume, et al., (2021) reported that over 90% protein solubility is important for optimal functional uses of vegetable proteins, also, it is an important quality for many food and beverage applications and typically required for various functional properties such as emulsification, gelation and foam production (Agarwal, et al., 2015).



BPC: bitter lupine protein concentrate
SPC: sweet lupine protein concentrate, Mean ±S.E, n= 3

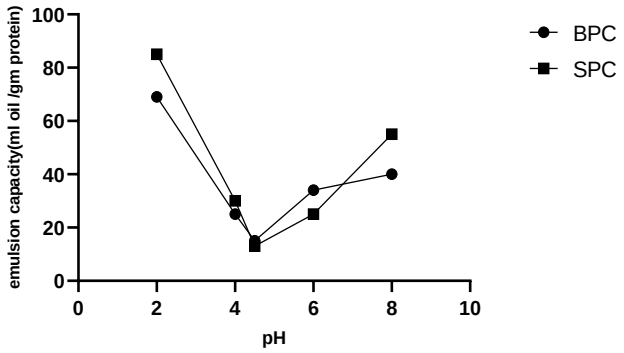
Figure 1. Solubility of bitter and sweet lupine protein concentrates

4.2. Emulsion Capacity

Emulsion capacity (EC) of the protein samples at pH 2, 4, 6 and 8 is presented in Figure 2 Bitter and sweet lupine protein concentrates (BPC and SPC) had similar(EC), which tended to decrease as pH increased. It was noticed that (EC) increased when pH moved away from the isoelectric point, either higher or lower. The maximum emulsion capacity of BPC and SPC was 69 and 85 ml oil / g protein respectively, at pH 2. while at pH 8, the minimum values are 40 and 55 ml oil / g protein, respectively, on the other hand at pH 4.5 (Ip) BPC and SPC had emulsion capacity of 15 and 13ml oil / g

protein, respectively.

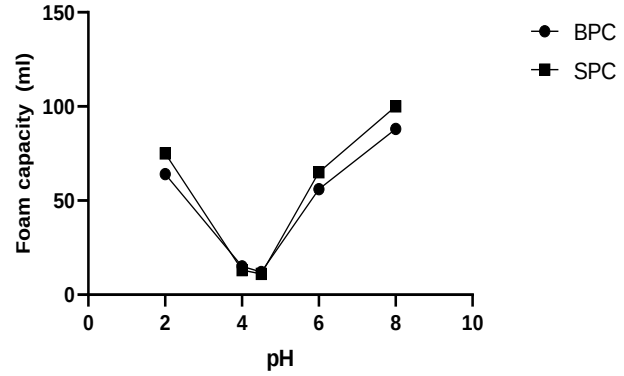
The isoelectric point and ultrafiltration of lupine protein concentrates' had high emulsification capacity at low pH, suggests that they may be particularly suitable as vegetable protein emulsifiers to substitute animal-derived emulsifiers in foods, particularly those that are very acidic in nature Sá, et al., (2020). The emulsifying capacity was improved more by alkaline pH than acidic pH. The hydrophilic-lipophilic balance, which was affected by pH, was a factor in emulsifying capacity (Lafarga et al., 2019).



BPC: Bitter lupine protein concentrate
SPC: Sweet lupine protein concentrate, Mean ±S.E,, n= 3

Figure 2. Emulsion capacity of bitter and sweet lupine protein concentrates

4.3. Foaming Capacity



BPC: Bitter protein lupine concentrate
SPC: Sweet protein lupine concentrate, Mean ±S.E, n= 3

Figure 3. Foam capacity of bitter and sweet lupine protein concentrates

Foaming capacity of BPC and SPC depended on the used pH which was gradient from 2 to 8 including the isoelectric point (4.5) as seen in Figure 3. The lowest foaming capacity of BPC and SPC were recorded at the isoelectric point increasing pH to 8 led to remarkable increasing in foaming capacity. It was noticed that BPC and SPC had lower foaming capacity in values of 12 and 11 ml respectively at pH 4.5 (Ip), The highest foaming capacity was recorded at pH 8 by sweet protein concentrate (100ml) followed by bitter protein concentrate (88 ml). When pH of protein approaches its isoelectric point, the net charge on the protein structure is low wherefore the protein is less soluble and has less unfolding flexibility, causing an increasing in surface tension. As a result, there is less protein adsorbed to the air-water surface, leading to decrease foaming capacity. On the other hand, as the pH increasing towards the alkaline region the net charge of the protein is increased, the molecular interaction is enhanced and the foaming capacity is improved (Burger and Zhang, 2019). Highest foam stability for lupine Protein was at pH 4.5 and that stability progressively declined as the pH become more alkaline (Piornos et al., 2015).

4.4. Water and Oil Absorption Capacity

Data in Table 3 showed that sweet protein concentrate had higher water absorption capacity compared to bitter protein concentrate in values of 3.5 and 3.3 ml water /g protein respectively, which indicating the increase of hydrophilic groups (Kar, et al., 2019). Also, The flour's low water absorption capacity may be due to the low protein content.

On the other hand, BPC had higher oil absorption capacity than SPC in values of 2.8 and 2.7ml oil/g protein respectively. High oil absorption capacity may be attributed to the high protein content. The different proportions of non-polar side chains of amino acids on the surface of protein molecules may led to variation in, oil absorption capacity, the oil absorption capacity of protein to non-polar side chains as well as various physical features (Kar, et al., 2019).

Table 3. Water and oil absorption capacity of sweet and bitter protein concentrate

Raw material	Water absorption capacity (ml/g)	Oil absorption capacity (ml/g)
BPC	3.3	2.8
SPC	3.5	2.7

BPC: Bitter lupine protein concentrate.
SPC: Sweet lupine protein concentrate, Mean ±S.E, n= 3.

5. Assessment of Protein Quality

5.1. Effect of Bitter and Sweet Protein Concentrate on Weight Gain, Food Intake, FER, Feces Weight Dry and Feces Nitrogen

Data in Table 4 showed gradually decreasing in body weight gain and total food intake in both tested groups I and II which were feeding with basal diet in which protein replaced with bitter or sweet lupine protein concentrate at the end of experiment compared to normal control group, non significant difference between them was found in values of 75, 79 and 85g for body weight, 3320, 3332 and 3360 g for food intake respectively, on the other hand Stanek, et al., (2015) reported that decreased consumption of lupine may be attributed to the presence of alkaloids in the experimental diets, which may be responsible for reduced feed intake in rats because rats are sensitive to alkaloids. Also, *Lupines albus* contains low alkaloids than other lupine species, the species, however, has increased manganese levels, which might cause loss of appetite in birds and sheep (Sobotka,

et al., 2016).

Concerning feed efficiency ratio (FER), The groups feeding on basal diet containing bitter lupine protein concentrate (I) or sweet protein lupine concentrate (II) showed decrease changes when compared to control group was found and non significant changes between them in values of 0.18,0.19 and 0.20% in the three groups respectively.

The tested groups I and II showed also gradually significant decreases in feces dry weight and nitrogen content compared to normal control group, in values of 11.0, 11.30g and 2.30, 2.32% for the two parameters respectively, Sobotka, et al., (2016) reported that urinary and fecal nitrogen losses from the breakdown of amino acids not consumed for protein synthesis, as well as decreased protein synthesis and increased catabolism, and the production of urea that is excreted in the urine.

5.2. Effect of Bitter and Sweet Protein Concentrate on Protein Efficiency Ratio, Net Protein Ratio and True Digestibility

Results in Table 5 showed significant decrease in Protein efficiency ratio (PER) and Net protein ratio (NPR) in the two tested groups I and II which were fed on basal diet in which casein was replaced with BPC or SPC respectively compared with control group, while non significant between groups were found, the greatest decrease value was found in group I in values of 1.80 and 2.02 followed by group II in values of 1.89 and 2.11 compared to control group in values of 2.03 and 2.25 respectively. Also Sá, A.G.A., et al., (2020) suggested that NPU in lupine protein concentrates is similar to that of many other legumes such as peanuts, broad beans, and lentils, and it is lower than of soy. Use of limiting amino acids as DL Methionine has been shown to improve protein quality, this could rats fed the lupine protein concentrate had lower NPU and Biological value scores (Lukuyu, et al., 2014). Protein sources with a PER of less than 1.5 are of low quality (Hassan et al., 2014).

Results in table 5 showed that the average percentage of adequate casein as measured by PER ratio (PER%) and relative NPR ratio (NPR%) ranged from 89.10 to 88.50% and 93.57 to 93.70% respectively.

Table 4. Weight gain, food intake, FER, Feces dry weight, and Feces nitrogen of rats fed on bitter and sweet lupine protein concentrate

Groups	Weight gain (g)	Total food intake (g)	FER	Feces dry weight (g/rat/28 days)	Feces nitrogen%
Control	85 ^{a} ± 1.10	3360 ^{a} ± 4.04	0.20 ^{a} ± 0.04	12.5 ^{a} ± 0.34	2.41 ^{a} ± 0.14
I	75 ^{b} ± 0.74	3320 ^{b} ± 3.44	0.18^{b} ± 0.03	11.0 ^{b} ± 0.22	2.30^{b} ± 0.19
II	79 ^{b} ± 0.82	3332 ^{b} ± 4.20	0.19^{ab}± 0.03	11.30 ^{ab} ± 0.24	2.32^{b} ± 0.20

Control: rats fed on basal diet, Group I: rats fed on basal diet containing bitter lupine protein concentrate, Group II: rats fed on basal diet containing sweet lupine protein concentrate.
Mean ± SD, n=6 rats.
a, b: Significantly different from control, I and II, respectively at P<0.05 using one way ANOVA followed by Tukey as post-hoctest

Table 5. Protein efficiency ratio (PER), Net protein ratio (NPR), biological value (B.V) and True digestibility (TD) of rats fed on bitter and sweet lupine protein concentrate

Groups	PER	PER%	NPR	NPR%	B.V	TD
Control	2.03^{a} ± 0.17	100 ^{a}± 1.14	2.25 ^{a}± 0.23	100 ^{a}± 2.02	70.77	94.1^{a}± 1.46
I	1.80^{b}± 0.16	89.1^{b}± 1.2	2.02 ^{b} ± 0.13	88.50^{b}± 1.55	63.72	95.3 ^{a}± 1.50
II	1.89^{b}± 0.15	93.57 ^{c} ± 1.1	2.11^{ab}± 0.17	93.70^{c}± 2.15	64.67	95.5 ^{a}± 1.54

Control: rats fed on basal diet, Group I: rats fed on basal diet containing bitter lupine protein concentrate, Group II: rats fed on basal diet containing sweet lupine protein concentrate.
Mean ± SD, n=6 rats.
a, b: Significantly different from control, I and II, respectively at P<0.05 using one way ANOVA followed by Tukey as post-hoctes

Table 6. Biological effect of bitter and sweet lupine protein concentrate on serum;protein and albumin, AST,ALT, creatinine and uric acid in rats

groups	T.protein (g/dl)	Albumin (g/dl)	AST (mg/dl)	ALT (mg/dl)	Cretininea (mg/dl)	Uric acid (mg/dl)
control	7.19^{a} ± 0.27	3.90^{a} ±0.17	44.0^{a} ±0.77	38.2^{a} ±0.87	0.88 ^{a} ± 0.03	5.31 ^{a} ± 0.28
I	7.03^{a} ± 0.40	3.33^{a} ± 0.19	40.3^{a} ± 0.60	36.1^{a} ± 0.50	0.82^{a}± 0.05	5.40^{a}± 0.27
II	7.13^{a} ± 0.27	3.43^{a} ± 0.15	43.2^{a} ± 0.45	37.4^{a} ± 0.59	0.84^{a}± 0.05	5.42^{a}± 0.19

Control: rats fed on basal diet, Group I: rats fed on basal diet containing bitter lupine protein concentrate, Group II: rats fed on basal diet containing sweet lupine protein concentrate.
Mean ± SD, n=6 rats.
a, b: Significantly different from control, I and II, respectively at P<0.05 using one way ANOVA followed by Tukey as post-hoctes

Non significant changes were found in true digestibility (TD) at (P<0.05) among control and tested groups also, Lestingi et al., (2016) reported that anti nutritional compounds such as lectins and protease inhibitors, which can reduce protein digestion, may led to the increased digestibility of lupine protein concentrates.

Lupine varieties demonstrated good digestibility, comparable to cereals such wheat, rice and oats, and both varieties were better digestible than legumes such as soybeans and beans (Martínez, et al., 2007) which have digestion values of less than 80% (Drulyte and Orlien, 2019).

5.3. Effect of Bitter and Sweet Protein Concentrate on Biochemical Parameters

Results presented in Table 6 demonstrated the effect of feeding on Bitter or sweet protein concentrate on serum biochemical parameters in rats; the results showed non significant changes in total protein and albumin among normal control and tested groups in values of 7.03 and 3.33 mg/dl in group I which was fed on BPC followed by group II which was fed on SPC in values of 7.13 and 3.43mg/dl while the control group values were 7.19 and 3.90 mg/dl in the aforementioned parameters respectively at the end of feeding period. It was suggested that this can be explained by the thermos seeds' functional protein efficiency and the balance of essential amino acids that function on improving digestion, which chemically affected the maintenance of normal total protein, albumin, and globulin in blood plasma (Alwan et al., 2019). Also Viveros, et al., (2007) found a decrease of albumin in chickens fed on white lupine.

Non significant change were found (P<0.05) in AST and ALT activity in the two tested groups I and II compared to control group as seen in table 3 with the greatest reduction values in group I which was fed on BPC in values of 40.3 and 36.1 mg/dl followed by group II which was fed on SPC in values of 43.2 and 37.4mg/dl, respectively compared to control group which was fed on basal diet contains casein in values of 44.0 and 38.3 mg/dl in the two parameters respectively. The concentration of lupine protein and the amino acid balance play a role in reducing liver activity, and consuming 7% protein from soybean or sweet lupine tempeh significantly (P<0.05) reduced the effect of lupine seeds on plasma and liver AST, ALT, and LDH activities, ALT activity significantly decreased in rats fed the hypercholesterolemia diets supplemented with bitter or sweet lupine seeds (Hassan, et al., 2014). Also, alloxan-diabetic rats which were given *Lupinus albus* for 28 days, their AST, ALT, and LDH activity were restored to normal levels, the effect of lupine seeds on the activities of AST, ALT, and LDH in plasma and liver suggests that these seeds may help to prevent hypercholesterolemia-induced liver damage (Straková, et al., 2021).

Non significant increases in creatinine and uric acid were found in tested group I and II at the end of experimental period in values of 0.82, 0.84 mg / dl in group I and 5.40, 5.42 mg/dl in group II compared to control group 0.88 and 5.31mg/dl respectively for the two parameters at the end of experiment as seen in table (4). lambs fed on pea had lower levels of serum uric acid than those fed pea + sweet lupine or only sweet lupine, with the differences attributed to sweet lupine's higher rumen degradability (Lestingi et al., 2016), also, Similar creatinine concentrations were found in lambs fed concentrates containing 25% pea, 12% soybean meal, or 25% lupine. The cause of the creatinine concentration difference is unknown (Facciolongo, et al., 2014).

The results showed that long-term feeding with white lupine had no effect on uric acid molar concentrations or alanine aminotransferase activity (Straková, et al., 2021) Also, the tempeh produced from soybeans or sweet lupine at a high concentration (7%) significantly improved lipid profiles, liver enzyme activity, uric acid and urea nitrogen concentrations, and caused a stronger protective effect in hepatocytes (Hassan et al., 2014).

6. Conclusions

Based on the above results, it could be concluded that protein concentrate from bitter and sweet lupine could be used to produce different kinds of functional food, for people suffering from; diabetes, obesity, hypertension and cardiovascular disease, also it could be used in vegetarian diet and free gluten diet for people with celiac disease, so lupine was more valuable than other species of legume as a source of protein with high biological value, which affect the physiological value of the human body.

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