American Journal of Food Science and Technology, 2017, Vol. 5, No. 6, 238-244
Available online at http://pubs.sciepub.com/ajfst/5/6/3

DOI:10.12691/ajfst-5-6-3

Isolation of Protein from Defatted Lentil Flour

Thwe Linn Ko[1], Soe Soe Than[2], Zar Zar Oo[3,*]

[1]Industrial Chemistry Department, University of Mandalay, Mandalay, Myanmar
[2]Industrial Chemistry Department, University of Yangon, Yangon, Myanmar
[3]Industrial Chemistry Department, Yadanabon University, Mandalay, Myanmar
*Corresponding author: mazarzaroo99@gmail.com

Abstract The main purpose of this research work was to isolate the most refined form of protein from lentil for food processing. In this research work, lentil (*Lens culinaris* L.) was collected from Monywa Township, Sagaing Region and nutritional characteristics such as moisture content, ash content, fat content, carbohydrate content, protein content and fiber content were determined. The fat of raw bean flour was removed by bulk soaking in ethanol and also by soxhlet extraction using ethanol as solvent before isolating the protein. In addition, the fiber and starch from defatted lentil flour was removed by alkaline extraction and acid precipitation method to isolate the protein (isoelectric precipitation). Protein solubility, water and oil absorption capacity, emulsifying capacity and stability, foaming capacity and stability of lentil protein isolate have been determined. The solubility curve corresponding to the lentil protein isolate indicated the minimum solubility at pH 4 (protein solubility of 20 %) and maximum solubility at pH 12 (protein solubility of 85 %) respectively. The lentil protein isolate had water absorption capacity of 1.82± 0.07 mL H_2O/g. protein and oil absorption capacity of 1.95±0.06 mL oil/g. protein. It was found that emulsifying activity and stability of isolated lentil protein were 73.44± 0.03 % and 41± 0.07% while foaming capacity and stability were 22.67± 0.06 % and 50.11± 0.05 %. Isolated lentil protein improved texture appearance and taste than the lentil flour and thus it can better be used as nutrition and functional ingredients in many food products.

Keywords: *defatted lentil flour, solvent extraction, isoelectric precipitation, functional properties, lentil protein isolate*

Cite This Article: Thwe Linn Ko, Soe Soe Than, and Zar Zar Oo, "Isolation of Protein from Defatted Lentil Flour." *American Journal of Food Science and Technology*, vol. 5, no. 6 (2017): 238-244. doi: 10.12691/ajfst-5-6-3.

1. Introduction

In the developing countries, protein malnutrition is one of the major important issue because animal proteins are high cost and scarce than plant sources. So, plant protein is commonly consumed by vegetarian as a replacement for meat [1,2,3,4]. Legumes are the second largest source of human food [5] and play significant role in alleviating protein-energy nutrition [1]. Human beings should depend on the legume proteins to meet the protein requirement in their diet [6].

Dry Legumes like beans, peas and lentils are nutritious source of high quality plant- based protein and its family (*Leguminosae*) *alsocalled Fabacae* [2,3,4,7]. Legumes are consumed widely throughout the world [7] and essential food resources which contribute to the nutritional health of manifold human diets [8]. They contain 17.6-23.62 % proteins, 1.27-3.62 % fat, 56.53-61.56 % carbohydrate [9] and also plentiful sources of some vitamins and minerals [3]. Moreover, they have a good proportions of amino acid although they are low in sulfur – containing amino acids like methionine and tryptophan [10]. Indeed, several studies suggest that increasing consumption of legumes may provide protection against diseases such as cancer, diabetes, osteoporosis, and cardiovascular diseases, among others [7].

In order to produce the greatest concentration of protein [9] which may have to be concentrated and isolated from the legume [11].

Isoelectric precipitation is one of the commonly applied methods to produce isolated protein and this method depends on the application of different solubility. Higher solubility is occurred at the alkaline and acid pH range whereas lower solubility exists at the isoelectric point (around pH 4-5) [12].

The most refined form of isolated protein contains no dietary fiber [13]. They are high digestibility [14] and easily included into different food products [15] essentially beverages, infant foods and textured protein products [16]. The functional properties of processed protein isolates like protein content, protein solubility, water absorption capacity, oil absorption capacity and foaming properties, emulsion capacity, least gelation concentration and yield percent were determined.

The objectives of this research were to remove the fat, fiber and starch from lentil flour for enhancement of protein isolation and to determine the characteristics of lentil protein isolate.

2. Materials and Methods

2.1. Raw Materials

Lentil was collected from Monywa Township, Saging Region, Myanmar. Ethanol from (BDH Chemicals Ltd), Sodium hydroxide and hydrochloric acid of analar grades were used.

Figure 1. Lentil

2.2. Methods

2.2.1. Preparation of Lentil Flour

About 300 g of lentil seeds were washed with water to remove foreign materials and then the seeds were soaked in 1000 mL of distilled water using automatic water distiller (LWD-3004, DAIHAN LABTECH Co., LTD, KOREA) for 12 hours and dehulled. After that, the seeds were crushed to smaller fragments with a blender and dried in an oven (J.P.SELECTA,s.a, SPAIN) at 60°C for 12 hours. They were powdered and sieved with 80 mesh screen using vibratory sieve shaker (J-VSS, NANOVA Ltd, KOREA) and then stored in an air tight container.

2.2.2. Preparation of Defatted Flour

Lentil flour 100 g was soaked in 600 mL of 95 % ethanol for 16 hr. and followed by soxhlet extraction (material to solvent ratio were 1:5) at extraction temperature 60°C. In order to remove all ethanol, defatted lentil flour was dried in an oven at 60°C for 12 hours. After that, it was ground in the grinder and sieved with 200 mesh screen. Defatted flour powder was obtained.

2.2.3. Preparation of Lentil Protein Isolate

The protein isolate was obtained from defatted flour. Because the lentil proteins display a higher solubility for pH>10, the pH of the defatted flour dispersion prepared in water was adjusted, by using 2N NaOH, to 11.3. Fiber and starch fractions were removed from the alkaline dispersion by centrifugation (DSC-200A-2, Digisystem Laboratory Instruments Inc., TAIWAN) at 3000 rpm for 30 min. Solubilized proteins were collected as supernatant which subsequently was used for the protein fraction recovery by isoelectric precipitation (pH 4.7) using pH meter (HANNA, pH-300). For pH adjustment, 2N HCl solution was used. After precipitation, the proteins were separated by centrifugation at 3500 rpm, for 40 min. The precipitate was washed with distilled water (pH 7.0) for three times, to achieve a complete removal of any existing contaminant. The precipitate was allowed to dry at room temperature for 10 hours and then milled to pass 200 mesh screen.

2.3. Methods of Analysis

Physico-chemical properties of lentil flour, defatted flour and lentil protein isolate such as protein, moisture, ash, fiber, carbohydrate, fat contents and also protein solubility, water absorption capacity, oil absorption capacity, emulsifying activity and stability, foaming capacity and stability of protein isolate were determined.

2.3.1. Determination of Protein Content

(2) g of sample was transferred to a digestion flask followed by the addition of 3 g of catalyst mixture (K_2SO4:CuSO4:SeO_2 in 100:20:2.5) and 20 mL of concentrated sulphuric acid. The content was then digested till transparent liquid was obtained. The volume of digested material was made up to 100 mL with distilled water. Carry out a blank digestion without the sample and make the digest to 100 mL. Measured aliquot of digested material was distilled with excess of 40% NaOH solution and the liberated ammonia was collected in 20 mL of 2% boric acid solution containing 2-3 drops of mixed indicator (10 mL of 0.1 percent bromo cresol green + 2 mL of 0.1 percent methyl red indicator in 95 percent alcohol). The entrapped ammonia was titrated against 0.01 N hydrochloric acid. A reagent blank was similarly digested and distilled. Nitrogen content in the samlple was calculated as follows and a factor of 6.25 was used to convert nitrogen to protein [17].

$$\%N_2 = \frac{\text{Sample titre} - \text{Blank titre} \times \text{Normality of HCl} \times 14 \times \text{vol.made of digest} \times 100}{\text{Aliquot of the digest taken} \times \text{Weight of sample} \times 1000}$$

$$\text{Protein content} = \%\ \text{Nitrogen} \times 6.25.$$

2.3.2. Determination of Moisture Content

3 g of sample was weighed in a petri dish and dried for 4 hours at 110°C in hot air oven and it was cooled in a desiccators and weighed. The process of heating, cooling and weighing was repeated. Moisture content was calculated as follows: [17]

$$\text{Moisture}(\%) = \frac{W_1 - W_2}{W_1} \times 100.$$

where, W_1= weight (g) of sample before drying
W_2= weight (g) of sample after drying

2.3.3. Determination of Ash Content

Accurately weighed 1g of sample was introduced into the porcelain crucible. The crucible and sample were carefully ignited over hot plate and heated until the sample was thoroughly charred. Then, it was placed in the muffle furnace at 550°C for 5 hours until residue was free from carbon. The crucible and ash were then cooled in the desiccator and weighed. The weighing, heating in the furnace and cooling were repeated until the constant weight was obtained. The ash content of sample was calculated as follow: [17]

$$\text{Ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100.$$

2.3.4. Determination of Crude Fiber Content

The sample was weighed into 500 mL beaker and 200 mL of boiling 0.255 N sulphuric acid (1.25 percent w/v) was added. The mixture was boiled for 30 min keeping the volume constant by the addition of hot water at frequent intervals (a glass rod stirred in the beaker helps smooth boiling). At the end of this period, the mixture was filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200 mL of boiling 0.313 N (1.25 percent w/v) NaOH was added. After boiling for 30 min., the mixture was filtered to a crucible, dried overnight at 80-100°C and weighed. The crucible was kept at in a muffle furnace at 550°C for 3 hours. Then it was cooled in desiccators and weighed again. The difference in residue weights and ash represents the weight of crude fiber [17].

2.3.5. Determination of Fat Content

Accurately weighed (5) g of sample was introduced inside the thimble and a piece of cotton was placed at the open and of the thimble. The thimble containing the sample was kept inside soxhlet apparatus fixed with round bottom flask (500) mL containing petroleum ether (B.P 40-60°C) 250 mL. The extraction flask was heated on the heating mantle for 14 hours at the boiling point of petroleum ether. After the extraction was completed, the ether dissolving oil was transferred into the beaker. Then, the ether was removed by evaporation. Fat content was calculated as follows: [17]

$$\text{Fat}(\%) = \frac{\text{Fat weight}}{\text{Sample weight}} \times 100.$$

2.3.6. Determination of Carbohydrate Content

Carbohydrate value of the sample was determined by using the following formula:

$$\text{Carbohydrate content } (\%) = 100 - (\text{protien} + \text{fat} + \text{fiber} + \text{ash} + \text{moisture}).$$

2.3.7. Determination of Fat, Fiber and Starch Removal Percentages

The fat removal percentages of defatted flour, fiber and starch removal percentages of lentil protein isolates were determine.

$$\text{Fat removal percent} = \frac{\text{Initial fat} - \text{Final fat}}{\text{Initial fat}} \times 100 \quad (1)$$

$$\text{Fiber removal pecentages} = \frac{\text{Initial fiber} - \text{final fiber}}{\text{Initial fiber}} \times 100 \quad (2)$$

$$\text{Starch removal percentages} = \frac{\text{Intial starch} - \text{final starch}}{\text{Initial starch}} \times 100. \quad (3)$$

2.3.8. Protein Solubility

Protein solubility of the lentil protein isolate was studied at pH values ranging from 1.8 to 11.8. Initially, suspensions with 5% protein derivate in 0.1 N NaOH, were obtained. For a better solubilization, the suspensions were stirred for 2 hours, at room temperature, using a magnetic stirrer. Aliquot parts from the suspension were sampled for the determination of protein solubility at different pH values achieved after the adjustment with a 2M HCl solution and 1 hour agitation. Before mineralization, the samples were centrifuged at 3000 rpm, for 30 min. From the resulted supernatant, the total nitrogen was determined according to the semi micro Kjeldahl method. Protein solubility curve was constructed by using the average values obtained for each considered pH value [18].

2.3.9. Water and Oil Absorption

Water absorption capacity was determined by centrifugation, according to the method described by [19] which was slightly modified. 3 g of protein isolate was first dried for 24 hours at 104°C and afterwards placed into pre-weighed centrifuge tubes and dispersed into 25 ml of distilled water. The obtained dispersions were occasionally stirred. After 30 min of storage at 22 ± 1°C, the samples were centrifuged for 30 min at 3500 rpm. The supernatant was removed and the moisture excess was released by drying for 25 min at 50°C. The tubes containing the samples were reweighed. The water absorption capacity was determined for a genuine pH of the protein suspension and expressed as mL absorbed water/g of protein derivate.

Oil absorption was determined according to the method of [20]. The protein isolate (0.5 g) was homogenized with 6 mL of sunflower oil into a pre-weighed centrifuge tube. Aiming for a better proteins dispersion in oil, the content of the tubes was stirred for 1 min, afterwards, the samples were centrifuged at 3000 rpm, for 25 min, 30 min later the oil separated being removed. Oil absorption capacity was expressed as mL oil/g of protein isolate.

2.3.10. Emulsifying Activity and Emulsion Stability

The procedure described by [21] was used for both emulsification activity and stability of isolated protein. Emulsions were prepared with 1g of protein, 50 mL of distilled water at room temperature (25°C) and 50 mL of corn oil. The mixture was emulsified for 30 min. Each emulsified sample was divided equally into 50 mL centrifuge tubes. Content of one tube was directly centrifuged at 3000xg for 30 min while the other centrifuged under the same conditions after heating in a water bath at 80°C for 30 min and cooling to 15°C. The height of the emulsified layer, as a percentage of the total height of material in the unheated tubes was used was used to calculate the emulsifying activity and stability using the following formulas:

$$\text{Emulsifying activity}(\%) = \frac{\text{Height of emulsion}}{\text{Height of whole layer}} \times 100$$

$$\text{Emulsion stability}(\%) = \frac{\text{Height of emulsion layer after heating}}{\text{Height of whole layer}} \times 100$$

2.3.11. Foaming Capacity and Stability

The foaming capacity was determined by the method of [22]. About 100 mL of distilled water were added to 3 g protein isolate. The mixture was homogenized for 5 min in a blender set at high speed at room temperature (25°C) and then transferred to 250 mL measuring cylinder. The volume of foam at 30 second was calculated, and the increase in volume is expressed as a percent foam capacity. After 30 min, the volume of foam was measured and expressed as foaming stability.

$$\text{Foaming capacity}(\%) = \frac{\text{volume after whipping} - \text{volume before whipping}}{\text{volume before whipping}} \times 100$$

$$\text{Foaming stability}(\%) = \frac{\text{Foam volume after time(t)}}{\text{initial foam volume}} \times 100.$$

2.4. Statistical Analysis

Statistical analysis was carried out using a one way analysis of variance (ANOVA) and the significant difference between the samples were determined using LSD test at $p < 0.05$.

3. Results and Discussion

3.1. Proximate Composition

Proximate composition of lentil flour was determined and presented in Table 1. It was observed that the protein content, 22.58±0.07 % of local lentil flour was lower than that of the (31.12±1.68%) [23], and fat content, 1.17±0.04 % of local lentil flour was larger than that of the (0.81±0.04 %) [23]. The moisture content of local lentil flour was 9.62± 0.05% to protect the greater danger of bacteria action and mold growth which produce undesirable changes. However, the crude fiber of local lentil flour, 0.68±0.17 % was significantly different from the 3.68±0.43 % [23]. The high fiber content in [23] may be due to bean's hulls. Thus, dehulling can reduce the fiber. The proximate composition of bean flour can be varied depending on the weather and soil conditions, cultivation area, and species of lentil, harvesting time and storage condition. High fat content may interfere protein isolation and protein may be denatured. Therefore, fat should firstly be removed to isolate the protein.

Table 1. Proximate Composition of Lentil Flour

Composition (% w/w)	Lentil Flour
Protein content	22.58 ±0.07
Moisture content	9.62 ±0.05
Ash content	2.42±0.06
Fiber content	0.68±0.17
Carbohydrate content	63.53±0.03
Fat content	1.17±0.04

Table 2 describes the effect of ratio of ethanol soaked bean flour to solvent on the percentage of fat removal and protein content from lentil flour. It has been observed that combined effect of bulk soaking and soxhlet extraction influenced on the maximum removal of fat content. The most suitable material to solvent ratio was 1:5 at the extraction temperature 60°C. By combining the two processes, the highest fat removal of 29.03 ± 0.03 % was achieved with relatively high protein content of 56.35 ± 0.02% and also characteristics of defatted flour are presented in Table 3.

Table 2. Effect of Ratio of Ethanol Soaked Bean Flour to Solvent on the of Fat Removal and Protein Content

Material to Solvent Ratio	Fat Removal Percent (% w/w)	Protein Content (%w/w)
1:3	17.74 ± 0.02	49.35 ±0.01
1:4	19.97 ± 0.01	52.77 ±0.01
1:5	29.03 ± 0.03	56.35 ±0.02
1:6	25.26 ± 0.03	51.39 ± 0.01
1:7	23.87 ±0.01	49.41 ±0.03

Table 3. Characteristics of Defatted flour

Characteristics (% w/w)	Defatted Flour
Protein content	56.35 ±0.02
Moisture content	10.14 ± 0.01
Ash content	8.81 ± 0.06
Fiber content	0.53 ± 0.06
Carbohydrate content	23.73 ± 0.14
Fat content	0.44 ± 0.06

3.2. Effect of Amount of 2N NaOH Solution on the pH, Fiber Removal and Starch Removal Percentages from Defatted Flour

To isolate the protein, the effect of amount of 2N NaOH solution on the pH, fiber removal and starch removal percentages and protein content from defatted flour are described in Table 4. The optimum pH of protein solulibilzation was 11.3 by using 2.6 mL of 2N NaOH solution at 3000 rpm. This was in agreement with [18] the lentil protein display higher solubility at pH >10.

Table 4. Effect of Amount of Alkaline Solution on the pH, Fiber Removal and Starch Removal Percentages

Volume of NaOH (mL)	pH	Fiber Removal Percent (% w/w)	Starch Removal Percent (% w/w)	Protein Content (%w/w)
2.0	9.3	16.98±0.01	34.26±0.04	76.11±0.03
2.2	10	20.76±0.03	43.95±0.02	78.43±0.05
2.4	10.6	24.52±0.01	52.29±0.02	80.43±0.02
2.6	11.3	30.19±0.03	55.58±0.01	81.24±0.06
2.8	12	31.13±0.02	55.92±0.01	81.33±0.02

3.3. Effect of Centrifugation Speed on the Protein Content, Fiber Removal and Starch Removal Percentages from Defatted Flour

Table 5 shows effect of centrifugation speed on the protein content, fiber removal and starch removal percentages from defatted flour. It was observed that, fiber removal percentage increased from 24.52±0.04 % to 49.06±0.03 % and starch removal percentage increased from 44.67±0.03 % to 65.49±0.01 % of lentil protein isolate by changing centrifugation speed. And thus, the protein content slightly increased from 78.62±0.04 % to 83.69±0.03 %.There was no sharp change in fiber removal and starch removal percentages between 3500 rpm and 4000 rpm. So, the most suitable centrifugation speed was found to be 3500 rpm.

Table 5. Effect of Centrifugation Speed on the Fiber Removal and Starch Removal Percentages

Centrifugation Speed (rpm)	Fiber Removal Percent (% w/w)	Starch Removal Percent (% w/w)	Protein Content (%w/w)
2000	24.52±0.04	44.67±0.03	78.62±0.04
2500	26.41±0.02	53.27±0.02	80.67±0.03
3000	30.19±0.03	55.58±0.01	81.24±0.06
3500	47.17±0.02	65.44±0.02	83.67±0.05
4000	49.06±0.03	65.49±0.01	83.69±0.03

3.4. Effect of Centrifugation Time on the Protein Content, Fiber Removal and Starch Removal Percentages from Defatted Flour

Besides, to decide the most suitable centrifugation time on the protein content, the fiber removal and starch removal percentages from defatted flour, centrifugation time was varied from 10 min, to 50 min at 3500 rpm. The resultant data are described in Table 6.The protein content steadily increased by changing the centrifugation time. The highest protein content 84.10±0.04 % with highest fiber removal 64.15±0.05 % and starch removal 66.87±0.07 % were observed for 40 min centrifugation time. Increasing centrifugation time did not bring about the increase on protein content and hence also on the fiber removal and starch removal percentages. So, the most suitable centrifugation time was 40 min.

Table 6. Effect of Centrifugation Time on the Fiber Removal and Starch Removal Percentages

Centrifugation Time (min)	Fiber Removal Percent (% w/w)	Starch Removal Percent (% w/w)	Protein Content (%w/w)
10	28.30±0.07	59.88±0.04	82.25±0.06
20	39.62±0.03	63.25±0.03	83.11±0.02
30	47.17±0.02	65.44±0.02	83.67±0.05
40	64.15±0.05	66.87±0.07	84.10±0.04
50	66.04±0.06	66.88±0.05	84.11±0.07

3.5. Effect of Different Acidic pH on the Protein Content, Fiber and Starch Removal Percentages

Furthermore, to get the largest protein precipitation at the most suitable acidic pH, the pH were changed from 4.1 to 4.9. Effect of different acidic pH on the protein precipitation, fiber and starch removal percentages from the most solubilized protein concentrated solution are shown in Table 7. The highest fiber removal 69.81±0.04 % and starch removal 66.84±0.05 % were achieved with relatively highest protein content 84.12 ± 0.06 % at pH 4.7. So, pH 4.7 gave the highest yield of protein isolate from defatted lentil flour due to insolubilization of protein at isoelectric point. Yield percentage of lentil protein isolate was 20.11%. The isoelectric pH of most of vegetable origin proteins correlate with values between 4 and 6 [18].

Table 7. Effect of Different Acidic pH on the Protein Precipitation, Fiber and Starch Removal Percentages from the Most Solubilized Protein Solution

Different Acidic pH	Fiber Removal Percent (% w/w)	Starch Removal Percent (% w/w)	Protein Content (%w/w)
4.1	60.38±0.02	33.38±0.03	76.13±0.04
4.3	64.15±0.04	37.42±0.05	77.11±0.05
4.5	67.92±0.04	44.21±0.02	78.74±0.03
4.7	69.81±0.04	66.84±0.05	84.12±0.06
4.9	66.04±0.03	42.29±0.02	78.43±0.03

Physico-chemical properties of lentil protein isolate was determined in Table 8. Lentil protein isolate was characterized by protein content 84.12±0.06 % and low content in fiber, 0.16±0.10% and in ash, 2.23±0.09 %. By refinement, the carbohydrate level was substantially diminished to 7.87±0.09 %.

Table 8. Physico-Chemical Properties of Lentil Protein Isolate

Properties (% w/w)	Lentil Protein Isolate
Protein content	84.12 ±0.06
Moisture content	5.20 ±0.04
Ash content	2.23±0.09
Fiber content	0.16±0.10
Carbohydrate content	7.87±0.09
Fat content	0.42 ±0.24

3.6. Functional Properties

Functional properties of lentil protein isolates are shown in Table 9. They showed a water absorption capacity (WAC) of 1.82 ± 0.07 mL H_2O/g protein. Water binding properties of protein is determined by their degree of interaction with water [11]. Lentil protein isolate has a higher capacity of swelling, distortion and separation, that allows additional exposure of binding sites of water and increases water absorption [11]. The oil absorption

capacity (OAC) of lentil protein isolate was 1.95±0.06 mL oil/g. proteins. Lentil protein isolate showed higher oil absorption capacity than chickpea [11].The process of absorption as a physical entrapment of oil; several authors have related the oil absorption capacity to interplay of nonpolar side chain of the protein as well as to the shape characteristics of the proteins [9]. The OAC is an important functional property because it upgrades mouth feel and flavor retention [24]. Emulsifying activity and stability of isolated protein were 73.44± 0.03 % and 41± 0.07. Emulsions properties are very important properties that proteins and other amphoteric molecules play a part in the development of novel foods [8]. They [11] also reported that the emulsion stability based on the water and oil absorption capacity. Most vegetable proteins are globular proteins with low foaming properties [18]. The foaming capacity and stability of lentil protein isolate were 22.67± 0.06 % and 50.11± 0.05 %. Lentil protein foam owned a lower capacity but highly stable compared to soy protein that studied [11].

Figure 2 showed the minimum solubility was observed at pH 4 to 6 and maximum solubility occurred at the extreme pH. Therefore, the lack of electrical charge for pH 4.7, influenced negatively the water binding and the solubility of protein. For extreme pH values, the net electrical charges are high, and allow rejection forces between the protein chains and thus the protein solubility increases. At pH 10, the lentil protein isolate solubility was 70% while at pH 2, the lentil protein isolate solubility was 53%. Also at pH 12, protein solubility was 85 % while at pH 4, the protein solubility was 20%. The decreasing in solubility, at very low pH values could be due to the protein denaturation and insolubilization processes [18]. Lentil protein exhibited good solubility in both acid and alkaline pH region, which is an important characteristic for food formulation [11].

Table 9. Functional Properties of Lentil Protein Isolate

Functional Properties		Lentil Protein Isolate
Water absorption capacity	(mL H_2O/g)	1.82 ± 0.07
Oil absorption capacity	(mL oil/g)	1.95 ±0.06
Emulsifying activity	(%)	73.44 ± 0.03
Emulsion stability	(%)	41 ± 0.07
Foaming capacity	(%)	22.67 ± 0.06
Foaming stability	(%)	50.11 ± 0.05

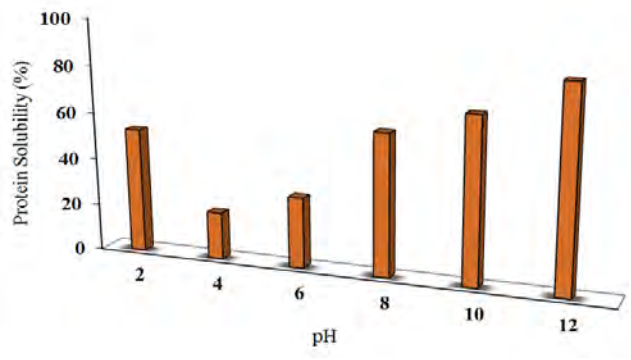

Figure 2. Effect of pH on the Protein Solubility of Lentil Protein Isolate

4. Conclusions

Lentil flour could be effectively defatted by using the combination of soaking in ethanol solution followed by soxhlet extraction. It was found that the highest fat removal percentage 29.03 ±0.03 % was achieved with the highest protein content 56.35± 0.02 %. The highest isolation of protein was related to the highest fiber removal and starch removal percentages from defatted flour by using isoelectric precipitation. The highest protein content 84.12±0.06 % was achieved at pH 4.7. At pH 12, protein solubility was 85 % while at pH 4, the solubility was 20 %.The lentil protein isolate had water absorption capacity, 1.82± 0.07 mL H_2O/g. protein and oil absorption capacity of 1.95±0.06 mL oil/g. protein. Having their excellent functional properties, lentil protein isolate can be further utilized for the supplementation of various food products.

Acknowledgements

I wish to acknowledge to my supervisor Dr. Soe Soe Than, Professor, Industrial Chemistry Department, University of Yangon and co-supervisor Dr. Thwe Linn Ko, Professor, Industrial Chemistry Department, University of Mandalay, Myanmar, for their invaluable guidance, and kind advice throughout the research period.

References

[1] Kudre,T.G,Benjakul ,S.& Kishimura, H. "Comparative study on chemical compositions and properties of protein isolates from Mung bean, Black bean and Bambara groundnut", *Journal of Sciences and Food Agriculture*, 93 (10). 2429-2436.Feburary. 2013.

[2] Butt, M. S. & Batool, R. "Nutritional and functional properties of some promising legumes protein isolates". *Pakistan Journal of Nutrition*, 9. (4), 373-379. 2010.

[3] Habib Ullah, *et al.* "Proximate and mineral composition of Mung bean, *Sarhad Journal of Agriculture*", 23 (2).1-4.2007.

[4] George Amponsah, Zjen Ma & Joyce Irene Boye. "Crop- legume", *Food Processing: Principles and Applications*, 305-337. 2014.

[5] Berrios, J.D.J, "Extrusion cooking of legumes: Dry bean flours". *Encyclopedia of Agricultural, Food and Biological Engineering*, 1-8. 2006.

[6] Sibt-e-Abbas., *et al*, "Nutritional and functional properties of protein isolates extracted from defatted peanut flour" *International Food Research Journal*, 22 (4). 153-1537. 2015.

[7] Tharanathan, R.N. & Mahadevamma, S., "Grain legumes-a boon to human nutrition". *Trends in Food Science and Technology*, 14. 507-518, 2003.

[8] Ladjal Ettoumi,Y.. & Chibane .M. "Some physico-chemical and functional properties of pea, chickpea and lentil whole flours", *International food Research Journal*, 22 (3). 987-996. 2015.

[9] Siddiq M, Ravi R, Harte J.B,Dolan K.D, "Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.)", *Food Science and Technology*. 232-237.2010.

[10] Wani.I.A,Sogi D.S.Shivhare U.S,Grill B.S., "Physico-chemical and functional properties of native and hydrolyzed kidney bean (*Phaseolus vulgaris* L.) Protein Isolates", *Food Science and Technology*.53 (1). 278-284. 2013.

[11] Suliman, M. A., *et al*, "Solubility as influenced by pH and NaCl concentration and functional properties of lentil proteins isolate". *Pakistan Journal of Nutrition*. 5(6).589-593. 2006.

[12] Akaerue BI, Onwuka GI, "Evaluation of the yield, protein content and functional properties of Mung bean [*Vigna radiate* (L.) Wilczek] protein isolates as affected by processing", *Pakistan Journal of Nutrition*, 9 (8). 728-735, August, 2010.

[13] Jayasena V., Chih H.J, Nasar-Abbas S.M, "Efficient isolation of lupin protein", *Food Australia*, 63 (7). 306-309. 2011.

[14] Garba, U, Kaur G, "Protein isolates: Production, functional Properties and applications", *International Research Journal of Chemistry (IRJC)*, 6 (3). 35-45.January, 2014.

[15] Shabnum Shaheen,*et al*. "Comparative nutritional analysis between *Vigna radiata* and *Vigna mungo* of Pakistan", *African Journal of Biotechnology*,11 (25). 6694-6702. March. 2012.

[16] Seyam, A.A., Banank, O.J & Breen, M.D, "Protein isolates from navy and pinto Beans: their uses in macaroni products", *Journal of Agricultural Food and Chemistry*. 31. 499-502. 1983.

[17] AOAC, Association of Official Analytical Chemists, Official Method of Analysis.17th ed., Washington, DC. 5-15, 2000.

[18] Aurelia, I., *et al*, "Chemical and functional characterization of chickpea protein derivates", *Journal of Food Technology*, 5. 12-14. 2009.

[19] Sathe, S .K., Deshpande, S .S. and Salunkhe, D. K., "Functional properties of winged bean(psophocarpus tetragonolobus L.DC) proteins", *Journal of Food Science*, 47, 503-509, 1982.

[20] Lin, M. J .Y., Humbert, E. S and Sosulki, F. W., "Certain funciton al properties of sunflower meal products". *Journal of Food Science*. 39. 368, 1974.

[21] Volkert, M. A and B. P. Kelin, "Protein dispersibility and emulsion characteristics of flour soy products". *Journal of Food Science*, 44, 43-96, 1979.

[22] Lawhon, J. T., C. M. Gater and K. F .Mattil, "Whippable extract from glandless cottonseed flour", *Journal of Food Science*, 37, 317-321, 1972.

[23] M.Qayyum, M. Butt, "Composition analysis of some selected legumes for protein isolate recovery", *Journal of Animal and Plant Sciences*, 22(4). 1156-1162. 2012.

[24] Josefina Porras-Saavedra, *et al*, "Comparative study of functional properties of protein isolates obtained from three Lupinus species", *Advanced in Bioresearch*, 4 (4). 106-116. December, 2013.