Isolation of protein and nutrient characteristics analysis from lentil

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Abstract:

The main purpose of this research work was to isolate the most refined form of protein from lentil bean for food processing. In this research work, lentils (*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, fiber content and energy value 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 ml H_2O/g. protein and oil absorption capacity of 1.95 ml oil/g. protein. It was found that emulsion stability of isolated lentil protein was 41%with foaming capacity was 22.67 %., The foam stability was preserved up to 150 min. 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: Lentil, Defatted flour, Soxhlet extraction, Isoelectric precipitation, Lentil protein isolate

Introduction

Plant proteins play significant roles in human nutrition, particularly in developing countries where average protein intake deficient. Because of inadequate supplies of food proteins, there has been a constant search for unconventional protein sources for use as both functional food ingredients and nutritional supplements. Legumes such as lentil contain a high concentration of proteins, carbohydrates and dietary fiber and make an important contribution to human diet in many countries [7].

Lentil is a protein calorie crop, its protein content is 22% to 35%. Lentil is deficient in the amino acids methionine and cysteine; it is an excellent supplement to cereal grain diets because of its good protein and carbohydrate content. Plant protein products are gaining interest as ingredients in food systems throughout many parts of the world; the final success of utilizing plant protein additives depends greatly upon the favorable characteristics that they impart to foods [7].

Beans are one of the most consumed legume worldwide. Beans reported to contain 17.6-23.62 % proteins, 1.27-3.62 % fat 2.86-5.00 % ash and 56.53-61.56 % carbohydrate [8] .They have a balanced amino acid composition while they are low in sulfur-containing amino acids (methionine and tryptophan) [9].

Isolates are the most refined form of protein products containing the greatest concentration of protein but unlike flour and concentrates contains no dietary fiber. They are very digestible and easily incorporated into different food products. Protein isolates are nowadays believed to have played a major role in the development of new class of formulated foods. It is high concentration of protein with the advantage of color, flavor and functional properties make it an ideal raw ingredient for used in beverages, infant foods and children milk food, textured protein products and certain types of specialty foods [6]. 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.

Materials and Methods

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.

Methods

Preparation of Lentil Flour

About 300g of lentil seeds. were washed with water to remove foreign materials and then the seeds were soaked in 1000 ml. of distilled water for 12 hr. and dehulled. After that, the seeds were crushed to smaller fragments with a blender and dried in an oven at 60℃ for 12 hr. They were powdered and sieved with 80 mesh screen and then stored in an air tight container.

Defatting the Lentil 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℃. In order to remove all ethanol, defatted lentil flour was dried in an oven at 60℃ for 12 hr. After that, it was ground in the grinder and sieved with 200 mesh screen. Then, defatted flour powder was packed with air- tight plastic bags.

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 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). 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 hr and then milled to pass 200 mesh screen. The isolated protein powder was stored in air- tight plastic bags.

Methods of Analysis

Physico-chemical properties of lentil flour, defatted flour and lentil protein isolate such as protein content, moisture, ash, fiber ,carbohydrate, fat content (AOAC- Method, 2000) [1] and also protein solubility, water absorption capacity, oil absorption capacity, emulsion capacity and stability, foaming capacity and stability of protein isolate were determined. The ED-XRF, Energy Dispersive X-ray Fluorescence Spectrometer (SPETRO XEPOS, Benchtop XRF Spectrometer) was used for the determination of elemental composition and FT-IR,Fourier Transform Infrared Spectroscopy(FT-IR, Perkin Elmer, 8400, Shimadzu) was examined the various functional groups of lentil protein isolate.

Results and Discussion

Physico-chemical properties of lentil flour were determined and presented in Table 1. It was observed that the protein content utilized local lentil flour (22.58 %) of local lentil flour was lower than that of the literature value, (31.12%) [5]. Fat content of utilized local lentil flour (1.17 %) was also lower than that of the literature value (1.81%) [5].The moisture content of local lentil flour was 9.62% to protect the greater danger of bacteria action and mould growth which produce undesirable changes. However, the crude fiber of local lentil flour, 0.68 % was significantly different from the literature value, 3.68 %. The high fiber content in literature 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. In the preparation of lentil protein isolate, the highest isolation of protein was related to the highest fiber removal and starch removal percentages from defatted lentil flour by using isoelectric precipitation method. The best defatted lentil flour was obtained by soaking it in ethanol solution for 16 hr, followed by soxhlet extraction (meal to solvent ratio were 1:5) at 60 ˚C. By combining the two processes, the highest fat removal of 29.03 % was achieved with relatively high protein content of 56.35 %. Isolation of protein from lentil was interrelated to the fat removal percentage.

Table 1. Physico-chemical Properties of Lentil Flour and Defatted Flour

Sr. No.	Composition (Dry Basis)	Lentil Flour	*Literature Values	Defatted Flour	**Literature Values
1	Protein content (%w/w)	22.58	31.12	56.35	53.5
2	Moisture content (% w/w)	9.62	9.14	10.14	8.7
3	Ash content (% w/w)	2.42	2.62	8.81	4.6
4	Fiber content (% w/w)	0.68	3.68	0.53	1.0
5	Carbohydrate content (% w/w)	63.53	51.63	23.73	30.0
6	Fat content (% w/w)	1.17	1.81	0.44	2.2
7	Energy value Kcal /100 g	357	353		-

*Qayyum, et al., 2012 [5]
**Mehmet, 2010 [4]

Table 2. Characteristics of Lentil Protein Isolate

Sr. No.	Characteristics		Lentil Protein Isolate	*Literature Values
1	Protein content	(% w/w)	84.12	84.46
2	Moisture content	(% w/w)	5.20	4.03
3	Ash content	(% w/w)	2.23	2.85
4	Fiber content	(% w/w)	0.16	0.18
5	Carbohydrate content	(% w/w)	7.87	7.88
6	Fat content	(% w/w)	0.42	0.60
7	Water absorption capacity	(ml.H_2O/g.)	1.82	1.90
8	Oil absorption capacity	(ml.oil/g.)	1.95	1.9
9	Emulsion stability	(%)	41	40-46
10	Foaming capacity	(%)	22.67	23.45

* Suliman, et al, 2006 [7]
**Note : The highest fiber removal, 69.81 % and starch removal, 66.89 % were achieved with relatively highest protein content, 84.12% from defatted flour at pH 4.7 (isoelectric point) by centrifugation at 3500 rpm, for 40min .

Characteristics of lentil protein isolate are described in Table 2. Lentil protein isolate was characterized by protein content 84.12 % and low content in fiber, 0.16 % and in ash, 2.23 %. By refinement, the carbohydrate level was substantially diminished to 7.87 %. Lentil protein isolate showed a water absorption capacity (WAC) of 1.82 mlH_2O/g protein. Water binding properties of protein is determined by their degree of interaction with water. 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 [7].The oil absorption capacity (OAC) of lentil protein isolate was 1.95 ml oil/g. proteins. Lentil protein isolate showed higher oil absorption capacity than chickpea[7].The mechanism of absorption as a physical entrapment of oil; several authors have related the oil absorption capacity to interaction of nonpolar side chain of the protein as well as to the conformation features of the proteins. High values of OAC are convenient in the protein isolates that are used as ingredients in the cold meat industry, particularly for sausages [7]. The WAC and OAC are determinants properties to develop a food of acceptable quality. The OAC is an important functional property because it improves mouth feel and flavor retention [7].Emulsion stability of isolated protein was 41 %. It was within the literature value [7].They [7] also reported that the emulsion stability depends primarily upon the water and oil absorption capacity. The foaming capacity of lentil protein isolate was 22.67% similar with the literature value [7]. Lentil protein foam had a lower capacity but highly stable compared to soy protein that studied [7].

The elemental compositions of lentil protein isolate was analyzed by ED-XRF. The data are shown in Table 3. It shows a rich source of chlorine. Chlorine is a component of all body secretions and excretions resulting from processes of building (anabolism) and breakdown (catabolism) body tissues.

Table 3. Elemental Compositions of Lentil Protein Isolate Analyzed by ED-XRF Method

Sr. No.	Elements	Compositions (%w/w)
1	Chlorine (Cl)	3.437
2	Phosphorus (P)	0.6384
3	Calcium (Ca)	0.3932
4	Potassium (K)	0.2090
5	Silicon (Si)	0.1647
6	Aluminum (Al)	0.1260
7	Sulfur (S)	0.09842
8	Iron (Fe)	0.05680
9	Manganese (Mn)	0.01418
10	Titanium (Ti)	0.00986
11	Vanadium (V)	0.00273
12	Chromium (Cr)	0.00142

Figure 1 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 reduction in solubility, at very low pH values could be due to the protein denaturation and insolubilization processes [2]. Lentil protein showed good solubility in both acid and alkaline pH region, which is an important characteristic for food formulation [7].

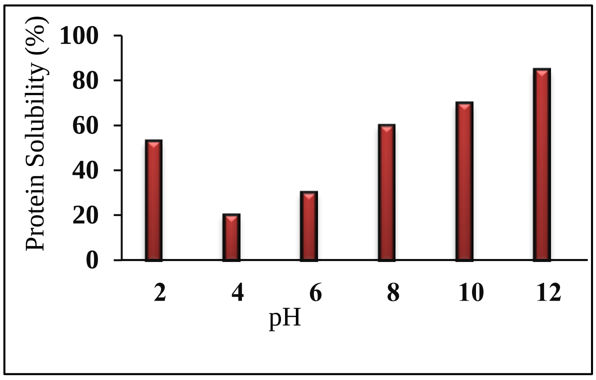


Figure 1. Effect of pH on the Protein Solubility of Lentil Protein Isolate

Emulsion capacity of lentil protein isolate at different pH are shown in Figure 2. Emulsion plays an important role in the manufacture of food products such as ice cream, mayonnaise, dressings and emulsified sausages [2]. The emulsifying capacity is an indicator used to evaluate the emulsion stabilizing properties of the lentil protein isolate. The ability of proteins to form stable emulsions depends on the size, charge, hydrophobic surface and flexibility of protein molecules. It was observed that emulsion capacity of protein was affected by environmental factor like pH [2].

Most vegetable proteins are globular proteins with low foaming properties [2].By contrast, the foam developed by lentil protein isolate still stable after keeping the foam for 150 minutes at room temperature, and the foam volume still retained 50.11% as show at Figure 3.

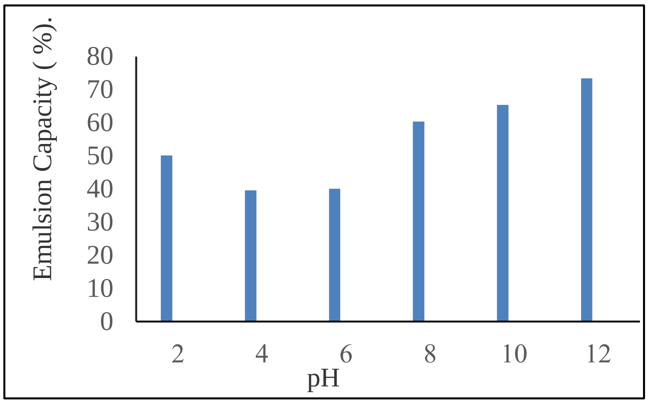


Figure 2. Effect of pH on the Emulsion Capacity of Lentil Protein Isolate

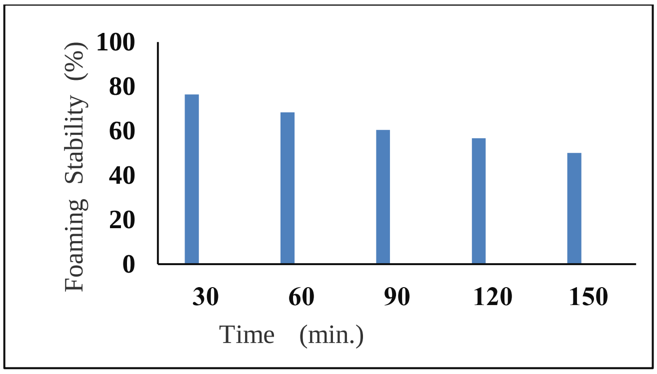


Figure 3. Effect of Time on the Foaming Stability of Lentil Protein Isolate

Various functional groups of lentil protein isolate was determined by FT-IR and the respective spectrum are shown in Figure 4 .The main absorption bands of peptide linkages are related to C=O stretching at 1635.69 cm^{-1} for lentil protein isolate represent amide primary, N-H bending and C-N stretching at 1528.64 cm^{-1} for lentil protein isolate represent amide secondary .With regard to the presence of primary amine group, the spectrum indicated by the vibrational frequencies for amine were at 3469.09 cm^{-1} for lentil protein isolate. In addition, the bands observed at 2864.39 cm^{-1} for lentil protein isolate is due to the presence of OH stretching. Thus, it was a normal lentil protein isolate consisting of, amide, carboxylic acids and carbonyl groups [3].

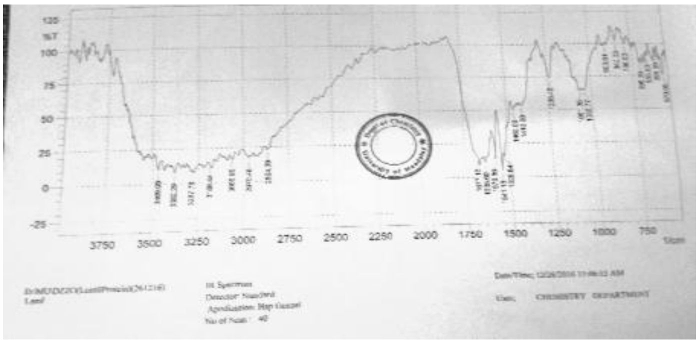
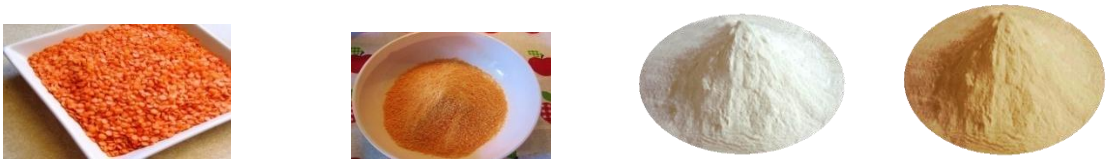


Figure 4. FT-IR Spectrum of Lentil Protein Isolate



(a) (b) (c) (d)

Figure 5. (a) Lentil (b) Lentil flour (c) Defatted flour (d) Lentil protein isolate

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 % was achieved with the highest protein content 56.35 %. 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 % 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 ml H_2O/g. protein and oil absorption capacity of 1.95 ml oil/g. protein. The foam developed by lentil protein isolate was found to be still stable up to 150 minutes at room temperature, and of foam volume retained 50.11 %. Having their excellent functional properties, lentil protein isolate can be further utilized for the supplementation of various food products.

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