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RESEARCH ARTICLE

CHEMICAL AND FUNCTIONAL CHARACTERIZATIONS OF LENTIL PROTIEN CONCENTRATE

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ABSTRACT

Many protein concentrates have been developed for providing different functional or physical properties to meet the requirement of various food systems. The main purpose of this research work was to isolate the most refined form of protein from lentil and to combat the problem of malnutrition. In this research work, lentil (*Lens Culinaris*L.) was collected from Monywa Township, SagaingRegion and nutritional values of lentil flour like moisture content, ash content, protein content, crude fiber content, fat content and carbohydrate content were determined. The fat from lentil flour was removed by soaking in ethanol and also by soxhlet extraction using ethanol as solvent before isolating the protein. The fat removal efficiency of these two methods were investigated. Moreover, combined effect of these two methods on the removal percentage of fat from lentil was studied. 56.35 ± 0.02% protein content (defatted lentil) was obtained by soaking in ethanol solution for 16hr and followed by soxhlet extraction (meal to solvent ratio were1:5).

INTRODUCTION

World demand for plant protein is increasing (Eltayeb *et al*, 2011) because animal proteins are more expensive and scarce (Ghribi *et al*, 2015). 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 (Kudre, 2013; Butt and Batool, 2010; Habib Ullah, *et al*., 2007; George Amponsah, 2014). Legumes are the second largest source of human food (Berrios, 2006) and play significant role in alleviating protein-energy nutrition (Kudre, 2013). Human beings should depend on the legume proteins to meet the protein requirement in their diet (Sibt-e-Abbas., *et al*, 2015). Dry Legumes like beans, peas and lentils are nutritious source of high quality plant- based protein and its family (*Leguminosae*) *alsocalled Fabacae* (Butt, M. S. and Batool, R, 2010, Habib Ullah, *et al*., 2007, George Amponsah *et al*., 2014, Tharanathan and Mahadevamma, S, 2003). Legumes are consumed widely throughout the world (Tharanathan, R.N. and Mahadevamma, S, 2003) and essential food resources which contribute to the nutritional health of manifold human diets (Ladjal Ettoumi and Chibane, 2015).

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They contain 17.6-23.62 % proteins, 1.27-3.62 % fat, 56.53-61.56 % carbohydrate (Siddiq Ravi *et al*, 2010) and also plentiful sources of some vitamins and minerals (Habib Ullah, *et al*., 2007). Moreover, they have a good proportions of amino acid although they are low in sulfur – containing amino acids like methionine and tryptophan (Wani *et al*, 2013). Indeed, several studies suggest that increasing consumption of legumes may provide protection against diseases such as cancer, diabetes, osteoporosis, and cardiovascular diseases, among others (Tharanathan, R.N. andMahadevamma, S, 2003). In order to produce the greatest concentration of protein (Siddiq Ravi *et al*, 2010) which may have to be concentrated and isolated from the legume (Suliman *et al*, 2006). The objectives of this research were to remove the fat from lentil flour for enhancement of protein isolation and to determine the characteristics of lentil protein concentrate.

MATERIALS AND METHODS

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.

Methods: 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.

Defatting the Lentil Flour

Soaking in the Solvent Ethanol

Lentil flour (80 mesh) 100 g was soaked in 600 mL of 95 % ethanol for (4 hr, 8 hr, 12 hr, 16 hr and 20 hr) respectively. After soaking, the solvent was decanted and defatted lentil was dried in an oven at 60℃ for 12 hours. After that, it was ground in the grinder and sieved with 200 mesh screen. Then, defatted lentil flour powder was packed with air- tight plastic bags.

Soxhlet Extraction Method

Lentil flour (80 mesh) 100 g was placed inside a thimble and loaded into the main chamber of the soxhlet extractor. 600 mL of 95 % ethanol was placed in a round bottom flask and extraction was started at different temperatures 50℃, 55℃, 60℃, 65℃ and 70℃ respectively. The defatted lentil flour powder were then prepared as described above.

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.

Methods of Analysis

Physico-chemical properties of lentil flour and defatted flour such as protein, moisture, ash, fiber, carbohydrate, fat contents of protein concentrate were determined. The ED-XRF, Energy Dispersive X-ray Fluorescence Spectrometer (SPETRO XEPOS, Benchtop XRF Spectrometer) was used for the determination of elemental composition, FT-IR, Fourier Transform Infrared Spectroscopy (FT-IR, Perkin Elmer, 8400, Shimadzu) was examined the various functional groups and SEM, Scanning Electron Microscope was determined the morphological nature of lentil protein concentrate.

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 sample was calculated as follows and a factor of 6.25 was used to convert nitrogen to protein.

$$\%N_2 = \frac{\text{Sample titre} \times \text{Blank titre} \times \text{Normality of HCl} \times 1.04 \times \text{vol.made of digest} \times 100}{\text{Aliquot of the digest taken} \times \text{Weight of sample} \times 1000}$$

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

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: (AOAC, 2000).

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

where, w_1= weight (g) of sample before drying , w_2= weight (g) of sample after drying.

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: (AOAC, 2000).

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

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 (AOAC, 2000).

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: (AOAC, 2000).

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

Determination of Carbohydrate Content

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

Carbohydrate (%) = 100 - (protein + fat + fiber+ash + moisture)

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 $p < 0.05$.

RESULTS AND DISCUSSION

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%) (M.Qayyumand M. Butt,2012), and fat content, 1.17±0.04 % of local lentil flour was larger than that of the (0.81±0.04 %) (M.Qayyumand M. Butt, 2012). 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 % (M.Qayyumand M. Butt,2012). The high fiber content in (M.Qayyumand M. Butt,2012) 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

Effect of Soaking Time on the Percentage of Fat Removal and Protein Content from Lentil Flour

Figure 1 shows the effect of soaking time on the percentage of fat removal and protein content from lentil flour. The protein content slightly increased from 24.19 ± 0.04 % to 27.69 ± 0.01 % by soaking the lentil flour in 95 % ethanol for 16 hr. There was no sharp change in the percentage of protein content and fat removal between 16 hr. and 24 hr. soaking time. So, the most suitable soaking time was found to be 16 hr.

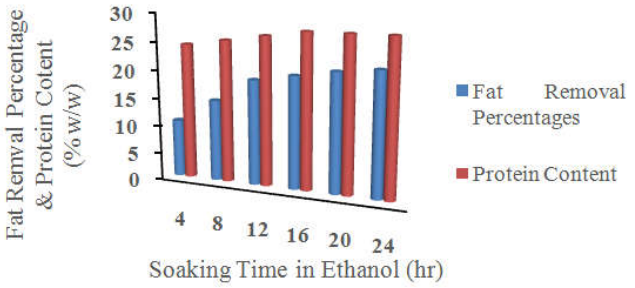

Figure 1. Effect of Soaking Time on the Percentage of Fat Removal and Protein Content from Lentil Flour

Effect of Extraction Temperature on the Percentage of Fat Removal and Protein Content from Lentil Flour

Figure 2 shows the effect of extraction temperature on the fat removal, protein content of defatted lentil flour by soxhlet extraction. . It can be seen from the figure 2 that, steadily increase in protein content from 27.77 ± 0.01%to 29.57 ± 0.02 % with increase in extraction temperature at extraction time of 6 hr. Increasing temperature from 60°C to 70°C did not bring about the increase on fat removal and protein content. Moreover, high temperature may cause protein denaturing. Thus, 60°C was found to be most suitable temperature for extraction of fat from lentil flour.

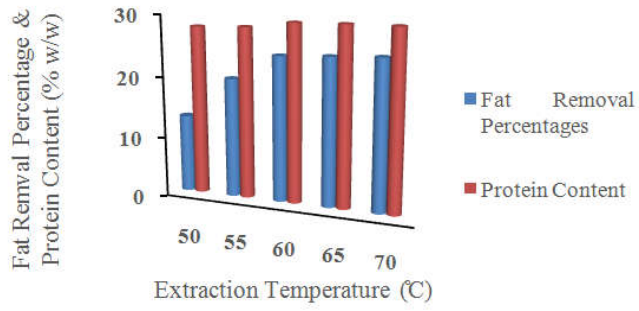

Figure 2. Effect of Extraction Temperature on the Percentage of Fat Removal and ProteinContent from Lentil Flour

Effect of Ratio of Ethanol Soaked Bean Flour to Solvent on the Percentage of Fat Removal and Protein Content from Lentil Flour

Table2 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 of fat removal. 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.

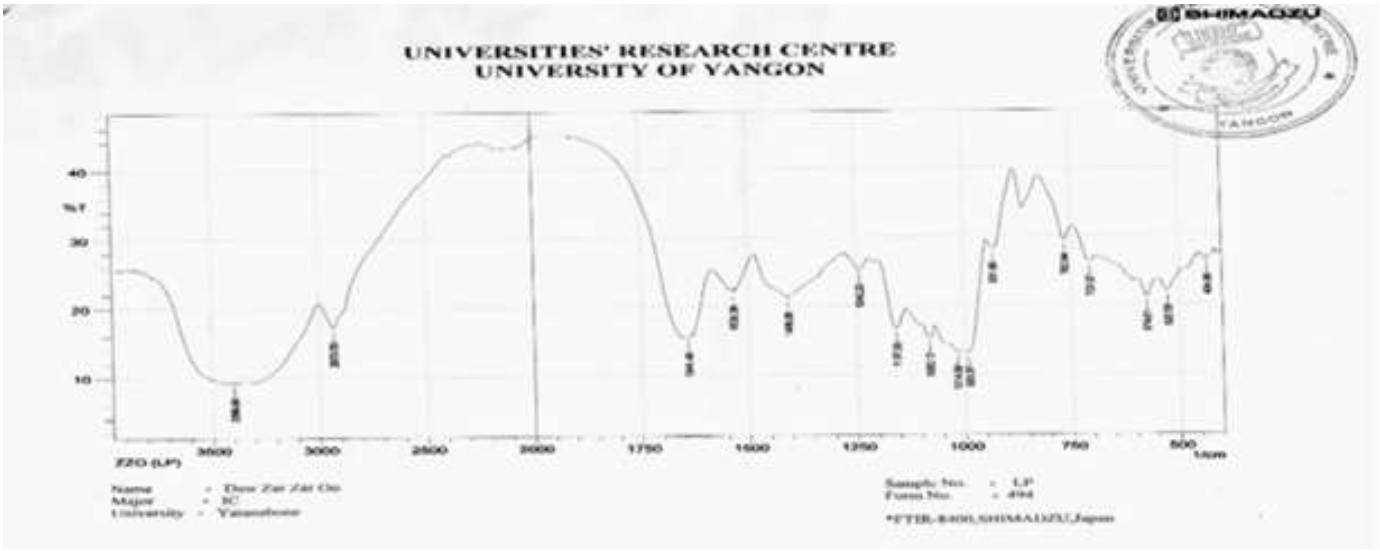

Figure 3. Various Functional Groups of Lentil Protein Concentrate

Table 2. Effect of Ratio of Ethanol Soaked Bean Flour to Solvent on the of Fat Removal Percentage 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 Lentil Protein Concentrate

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

Elemental Composition of Lentil Protein Concentrate

The elemental composition of lentil protein concentrate was analyzed by ED-XRF. The data are presented in Table 4. It shows potassium, calcium, iron, Zinc and copper. These minerals can effectively contributes towards the daily recommended allowances (RDA, 1980) for all groups. It was observed that lentil protein concentrate is used for protein source but it can fulfill the micro nutrients deficiency as well.

Table 4. Elemental Composition of Lentil Protein Concentrate Analyzed by ED-XRF Method

Elements	Compositions (%)
Potassium (K)	0.0194
Calcium (Ca)	0.020
Iron (Fe)	0.005
Zinc (Zn)	0.001
Copper (Cu)	0.001

Various Functional Groups of Lentil Protein Concentrate

Various functional groups of lentil protein concentrate were determined by FT-IR and the result is described in Figure 3. The main absorption bands of peptide linkages are related to C=O stretching at 1641.48 cm^{-1} (amide primary), N-H bending at 1242.20 cm^{-1}(amide secondary) and C-N stretching With regard to the presence of amino group, the spectra indicated by the vibrational frequencies for amine was at 3398.69 cm^{-1}. In addition, the band observed at 2933.83 cm^{-1} was due to the presence of CH and OH stretchings. Thus, it was normal lentil protein concentrate consisted by amine, amide, carboxylic acids and carbonyl groups (Kudre *et al*, 2013).

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 %.

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