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Chemical and Functional Characterizations of Chickpea Protein 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 evaluate the most refined form of protein from chickpea and to combat the problem of malnutrition. In this research work, chickpea (*Cicer arientum L.*) was collected from Monywa Township, Sagaing Region and nutritional values of chickpea flour like moisture content, ash content, protein content, crude fiber content, fat content, carbohydrate content and energy value were determined. The fat from chickpea 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 chickpea was studied. 46.15±0.04% protein content (defatted chickpea) was obtained by soaking in ethanol solution for 20 hr and followed by soxhlet extraction (meal to solvent ratio were1:6).

Keywords: Chickpea flour; chickpea protein concentrate; defatted flour; functional properties; soxhlet extraction.

1. Introduction

World demand for plant protein is increasing [5] because animal proteins are more expensive and scarce [8]. Myanmar was the 5th largest chickpea producing country after India, and Iran [6] during 2007.Chickpea (*Cicer arietium* L.) belongs to Family Fabaceae [4, 7, 8, 12 , 19] .Chickpea in Myanmar are cultivated in central dry zone of the country, especially Sagaing, Magway and Mandalay [18]. They are consumed widely throughout the world [17] and essential food resources which contribute to the nutritional health of manifold human diets [11].

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Human beings should depend on the chickpea proteins [1] due to low amounts of sulfur containing amino acids and low protein digestibility [14]. The quality of chickpea protein is better than other beans such as black bean and pigeon pea. Chickpea is a plentiful source of protein can help people make the nutritional quality of their foodstuffs. They involve protein and carbohydrates greater than other peas. Moreover, it reveals powerful nutritive value due to their high content in lysine and sulfur amino acids [3] and also an adequate source of minerals like potassium and phosphorus [7]. They were utilized protein rich instant foods because it is a great source of protein and exhibit desirable functional properties as food ingredients [13].

There are three principal methods to concentrate proteins depend on heat, acid or alcohol treatment [13]. The objectives of the present study were to remove the fat from chickpea flour and to determine the protein content of defatted chickpea flour for enhancement of protein isolation.

(a) **(b)**

Figure 1: (a) Chickpea (b) Chickpea production in Myanmar

2. Materials and Methods

2.1 Raw Materials

Chickpea was collected from Monywa Township, Saging Region. Ethanol was purchased from (BDH Chemicals Ltd), Able Chemical Store, Mandalay Region.

2.2 Preparation of Chickpea Flour

Chickpea seeds 300 g were washed with (Monywa Township, Saging Region) 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 hr and dehulled. After that, the seeds were crushed to smaller fragments with a blender and then dried in an oven (J.P.SELECTA,s.a, SPAIN) at 60℃ for 12 hours. And then, they were powered and sieved with 80 mesh screen using vibratory sieve shaker (J-VSS, NANOVA Ltd, KOREA) and then stored in an air tight container.

2.3 Defatting the Chickpea Flour

2.3.1 Soaking in the Solvent Ethanol

Chickpea flour (80 mesh) 100 g was soaked in 600 mL. of 95 % ethanol for (4 hr., 8 hr., 12 hr., 16 hr., 20 hr. and 24 hr) respectively. After soaking, the solvent was decanted and defatted chickpea 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 chickpea flour powder was packed with air- tight plastic bags.

2.3.2 Soxhlet Extraction Method

Chickpea 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 temperature provided the highest fat removal percentage was decided for that bean flour to solvent ratio 1:6. The extraction was again conducted for following bean flour to solvent ratios: 1:3, 1:4, 1:5, 1:6, and 1:7 at extraction temperature 65℃. The defatted chickpea flour powder were then prepared as described above.

2.3.3 Preparation of Chickpea Protein Concentrate

Chickpea flour 100 g was soaked in 600 ml. of 95 % ethanol for 20 hours and followed by soxhlet extraction (meal to solvent ratio were 1:6) at extraction temperature 65℃. In order to remove all ethanol, defatted chickpea flour 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, chickpea protein concentrate powder was packed with air- tight plastic bags.

2.4 Methods of Analysis

Physico-chemical properties of chickpea flour and defatted flour such as protein content, moisture, ash, fiber ,carbohydrate, fat content (AOAC- Method, 2000) [2] and also fat removal percentage 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 chickpea protein isolate.

2.4.1 Determination of Fat Removal Percentage

The fat removal percentage of chickpea protein isolate was determined.

$$\text{Fat Removal Percentage} = \frac{\text{A} - \text{B}}{\text{A}} \times 100$$

where, A= initial Fat content

B= final fat content

2.4.2 Statistical Analysis

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

3. Results and Discussion

The protein content, 19.94±0.03% of local chickpea flour was lower than that of the literature value [15], 22.83±1.07% due to species of chickpea, cultivation area and soil condition. Fat content of local chickpea flour was larger than that of the said literature value [15]. The moisture content of local chickpea flour was 8.21±0.01%. Excess of moisture content in chickpea flour can provide greater danger of bacteria action and mold growth which produce undesirable changes. Its moisture content should be controlled under 10 %. Furthermore, the ash content of chickpea flour was 3.01±0.02 % and it is an approximate measure of mineral and inorganic matter. However, the crude fiber of local chickpea flour, 1.00±0.03% was significantly different from the literature value [15], 3.50±0.16%. 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 chickpea, harvesting time and storage condition. The remaining lipids, mainly non polar compounds may still interact with proteins. High fat content may interfere protein isolation and protein may be denatured. So, fat should firstly be removed to isolate the protein.

Figure 2 postulates that, the protein content slightly increased from 19.98 % to 22.48% fat removal percentages of chickpea flour increased from 12.5 % to 21.53 % by soaking the chickpea flour in 95 % ethanol for 20 hr.

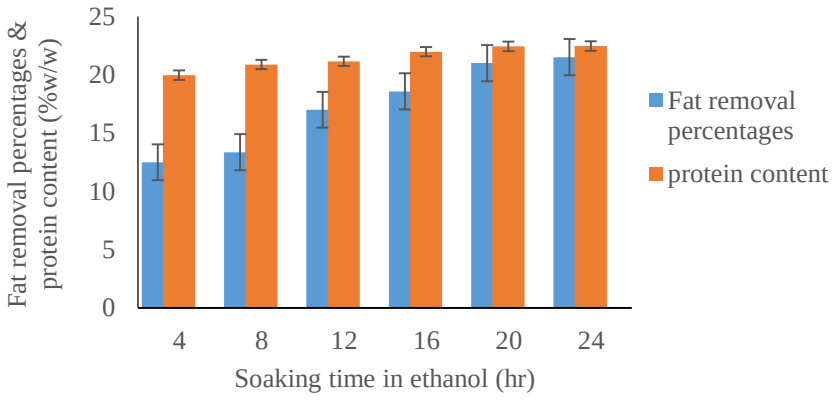


Figure 2: Effect of soaking time on the percentage of fat removal and protein content from chickpea flour

Figure 3 shows the effect of extraction temperature on the fat removal percentage, protein content of defatted chickpea flour by soxhlet extraction. It can be seen from the **figure 3** that, steadily increase in protein content from 22.79 % to 24.69 % whereas fat removal percentages increased from 34.38 % to 37.32% with increase in extraction temperature at extraction time of 6 hr. Increasing temperature from 65℃ to 70℃ did not bring about the increase on fat content and protein content. Moreover, high temperature may cause protein denaturing. Thus, 65℃ was found to be most suitable temperature for extraction of fat from chickpea flour.

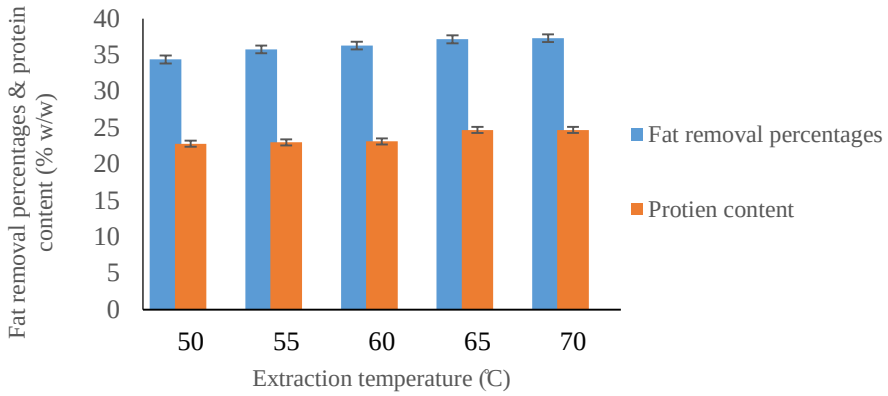


Figure 3: Effect of extraction temperature on the percentage of fat removal and protein content from chickpea flour

Table 1 describes the effect of ratio of ethanol soaked bean flour (partially defatted chickpea flour) to solvent on the percentage of fat removal and protein content from chickpea flour**.** It has been observed that combined effect of bulk soaking and soxhlet extraction influenced on the maximum removal of fat content as well as the higher yield of protein concentrate. The most suitable material to solvent ratio was 1:6 at the extraction temperature 65℃. By combining the two processes, the highest fat removal of 59.55 % was achieved with relatively high protein content of 46.15 %

Table 1: Effect of ratio of ethanol soaked bean flour (partially defatted chickpea flour) to solvent on the percentage of fat removal and protein content from chickpea flour

Meal to Solvent Ratio	Fat Removal Percentage (% w/w)	Protein Content (%w/w)
1:3	51.39±0.02	40.12±0.03
1:4	53.13±0.02	42.67±0.02
1:5	56.6±0.02	44.83±0.02
1:6	59.55±0.01	46.15±0.01
1:7	59.72±0.02	46.16±0.03

Physico-chemical properties of chickpea flour and chickpea protein concentrate were determined and the data were presented in **Table 2**. Chickpea protein concentrate was characterized by a protein content 46.15±0.01% and low content in fiber, respectively 0.69±0.03% and in ash, represented by 2.44±0.01%. By refinement, the carbohydrate level was substantially diminished to 40.74±0.01% level which is characteristic of the protein concentrate.

Table 2: Physico- chemical properties of chickpea flour and chickpea protein concentrate

Composition (Dry Basis) (%w/w)	Chickpea Flour (%w/w)	Chickpea Protein Concentrate (%w/w)
Protein content	19.94±0.03	46.15±0.04
Moisture content	8.21±0.01	7.65±0.02
Ash content	3.01±0.02	2.44±0.01
Fiber content	1.00±0.03	0.69±0.03
Carbohydrate content	62.08±0.02	40.74±0.01
Fat content	5.76±0.02	2.33±0.02

The elemental composition of chickpea protein concentrate was analyzed by ED-XRF. The data were presented in **Table 3**. It shows potassium, sulfur, iron, Zinc and copper. These minerals can effectively contribute towards the daily recommended allowances [16] for all groups. It was observed that chickpea protein concentrate is used for protein source but it can fulfill the micro nutrients deficiency as well.

Table 3: Elemental composition of chickpea protein concentrate analyzed by ED-XRF method

Elements	Compositions (%)
Potassium (K)	0.304±0.02
Sulfur (S)	0.058±0.03
Iron (Fe)	0.003±0.04
Zinc (Zn)	0.001± 0.03
Copper (Cu)	0.001±0.01

Various functional groups of chickpea protein concentrate were determined by FT- IRS and the respective spectrum is shown in **Figure 4** .The main absorption bands of peptide linkages are related to C=O stretching at 1651.12 cm^{-1} (amide primary), N-H bending at 1242.20 cm^{-1} (amide secondary) and C-N stretching. In addition, the band observed at 2928.04 cm^{-1} was due to the presence of OH stretching. There is normal chickpea protein concentrate consisted by amide and carboxylic acids groups [10].

Surface morphologies of chickpea flour and chickpea protein concentrate were illustrated in **figure 5**. It was examined by scanning electron microscopy (SEM) using magnification of 550. The microstructure of chickpea flour had typical kidney and ellipse shape and the surface of the chickpea flour appeared smooth, without pores and fissures. The SEM image of the ethanol leached chickpea protein concentrate showed the agglomeration into cluster or mass due to characteristic of less fat content.

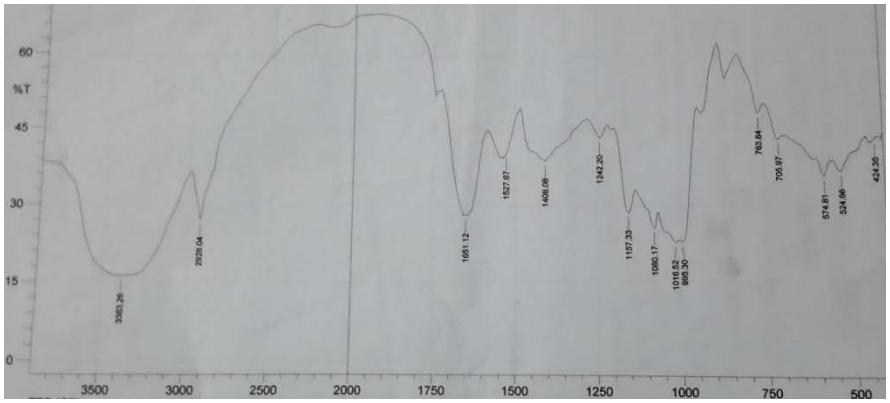
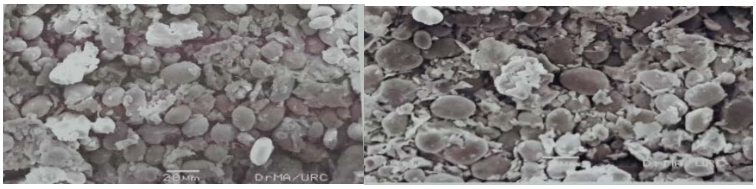


Figure 4: FT-IR spectrum of chickpea protein concentrate



(a) Chickpea Flour (b) Chickpea Protein Concentrate

Figure 5: Morphological nature of chickpea flour and chickpea protein concentrate

4. Conclusions

Combination of bulk soaking and soxhlet extraction accelerated the fat removal from chickpea. Isolation of protein from chickpea was interrelated to fat removal.

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