ISSN: 2230-9926

*Available online at http://www.journalijdr.com*

IJDR

*International Journal of Development Research*
*Vol. 07, Issue, 12, pp.17720-17724, December, 2017*

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

# PREPARATION AND CHARACTERIZATION OF CHICKPEA PROTEIN CONCENTRATE

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

[1]Industrial Chemistry Department, Yadanabon University, Mandalay, Myanmar
[2]Industrial Chemistry Department, University of Mandalay, Mandalay, Myanmar
[3]Industrial Chemistry Department, University of Yangon, Yangon, Myanmar

**ARTICLE INFO**

***Article History:***
Received 07th September, 2017
Received in revised form
24th October, 2017
Accepted 19th November, 2017
Published online 29th December, 2017

***Key Words:***
Chickpea Flour,
Defatted Flour
Soxhlet Extraction,
Chickpea Protein Concentrate,
Functional Properties.

**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 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 and carbohydrate content 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±0.01% 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).



**Citation: Zar Zar Oo, Thwe Linn Ko and Soe Soe Than. 2017.** "Preparation and characterization of chickpea protein concentrate.", *International Journal of Development Research*, 7, (12), 17720-17724.

## INTRODUCTION

World demand for plant protein is increasing (Eltayeb *et al.,* 2011) because animal proteins are more expensive and scarce (Ghribi *et al*., 2015). Myanmar was the 5th largest chickpea producing country after India, and Iran (FAOSTAT, 2007) during 2007.Chickpea (*Cicer arietium*L.) belongs to Family Fabaceae (Emami, 2007; George, 2014; Ghribi *et al.,* 2015; Lopez.O.Paredes, *et al*., 1991. Withan Gamage Thushan Sanjeewa, 2008) .Chickpea in Myanmar are cultivated in central dry zone of the country, especially Sagaing, Magway and Mandalay (Than Aung May, 2007). They are consumed widely throughout the world (Tharanathan, 2003) and essential food resources which contribute to the nutritional health of manifold human diets (Ladjal Ettoumi, 2015). Human beings should depend on the chickpea proteins (Abbas*et al*, 2015) due to low amounts of sulfur containing amino acids and low protein digestibility (Mubarak, 2004).

***Corresponding author:* Zar Zar Oo,**
Industrial Chemistry Department, Yadanabon University, Mandalay, Myanmar.

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 (Aurelia, I., *et al*, 2009) and also an adequate source of minerals like potassium and phosphorus (George *et al*., 2014). They were utilized protein rich instant foods because it is a great source of protein and exhibit desirable functional properties as food ingredients (Hui lui li, 1996). There are three principal methods to concentrate proteins depend on heat, acid or alcohol treatment (Hui lui li, 1996). 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.

## MATERIALS AND METHODS

### Raw Materials

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

**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 hr. 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

**Defatting the Chickpea Flour**

**Soaking in the Solvent Ethanol**

Chickpea flour (80 mesh) 100 gwas soaked in 600 mL of 95 % ethanol for (4 hr, 8 hr, 12 hr, 16 hr, 20 hr and24 hr) respectively. After soaking, the solvent was decanted and defatted chickpea 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 chickpea flour powder was packed with air- tight plastic bags.

**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.

**Preparation of Chickpea Protein Concentrate**

Chickpea flour 100 g. was soaked in 600 ml. of 95 % ethanol for 20hrand followed by soxhlet extraction (material 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 hr. 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.

**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 ) 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 of chickpea 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 20mL 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 (AOAC- 2000).

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

Protein content = % Nitrogen× 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) 250mL. 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)

### Determination of Fat Removal Percentage

The fat removal percentage of chickpea protein isolate was determined.

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

where, A= initial Fat content
B= final fat content

### 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 $p < 0.05$.

## RESULTS AND DISCUSSION

### Proximate Compositions of Chickpea Flour

Proximate compositions of chickpea flour was presented in Table 1. The protein content, 19.94±0.03% of local chickpea flour was lower than that of the literature value, 22.83±1.07% (Monywa Township, Sagaing Region) due to species of chickpea, cultivation area and soil condition (Quayyum *et al*., 2012). Fat content of local chickpea flour was larger than that of (Quayyum *et al*., 2012), 5.43 ± 0.26. 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 (Quayyum, *et al*., 2012), 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.

**Table 1. Proximate Compositions of Chickpea Flour**

| Composition (Dry Basis)(%w/w) | Chickpea Flour (%w/w) |
|---|---|
| Protein content | 19.94±0.03 |
| Moisture content | 8.21±0.01 |
| Ash content | 3.01±0.02 |
| Fiber content | 1.00±0.03 |
| Carbohydrate content | 62.08±0.02 |
| Fat content | 5.76±0.02 |

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

Figure 1 postulates that, the protein content slightly increased from 19.98± 0.03 % to 22.48± 0.04% and fat removal percentages of chickpea flour increased from 12.5±0.05 % to 21.53± 0.02 % by soaking the chickpea flour in 95 % ethanol. There was no sharply change in the percentage of protein and fat removal percentages between 20 hr and 24 hr soaking time in ethanol. So, the most suitable soaking time was found to be 20 hr.

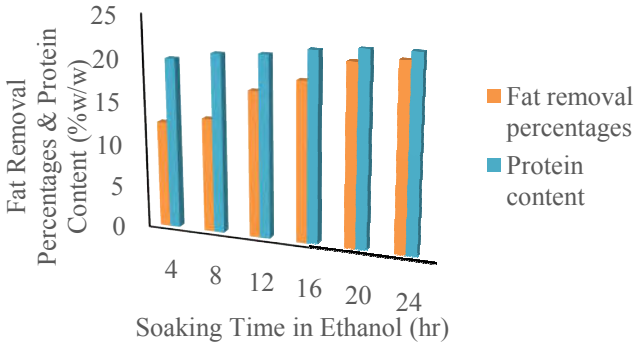


**Figure 1. Effect of Soaking Time on the Percentage of Fat Removal and Protein Content from Chickpea Flour**

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

Figure 2 shows the effect of extraction temperature on the fat removal percentage, protein content of defatted chickpea flour by soxhlet extraction.

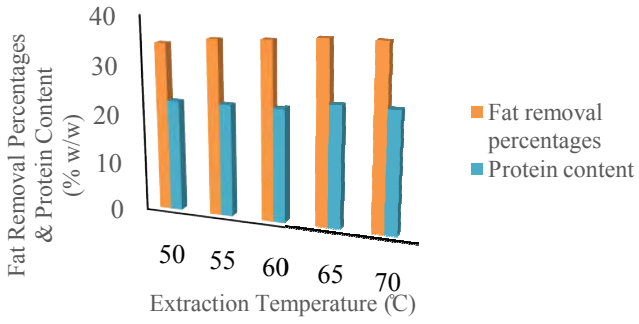


**Figure 2. Effect of Extraction Temperature on the Percentage of Fat Removal and Protein Content from Chickpea Flour**

It can be seen from the Figure 2 that, steadily increase in protein content from 22.79±0.02% to 24.69±0.03% whereas fat removal percentages increased from 34.38±0.02% to 37.32±0.04% 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.

**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**

Table 2 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**.**

**Table 2. 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**

| Material to Solvent Ratio | Fat Removal Percent (% 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 |

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±0.01% was achieved with relatively high protein content of 46.15 ±0.01%

**Physico-Chemical Properties of Chickpea Protein Concentrate**

Physico-chemical properties of chickpea protein concentrate was determined and the data were presented in Table 3.

**Table 3. Physico- Chemical Properties of Chickpea Protein Concentrate**

| Properties (%w/w) | Chickpea Protein Concentrate (%w/w) |
|---|---|
| Protein content | 46.15±0.01 |
| Moisture content | 7.65±0.02 |
| Ash content | 2.44±0.01 |
| Fiber content | 0.69±0.03 |
| Carbohydrate content | 40.74±0.01 |
| Fat content | 2.33±0.02 |

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.

**Elemental Composition of Chickpea Protein Concentrate**

The elemental composition of chickpea protein concentrate was analyzed by ED-XRF. The data are presented in Table 4. It shows potassium, sulfur, iron, Zinc and copper. These minerals can effectively contributes towards the daily recommended allowances (RDA, 1980) 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 4. 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 |

**Conclusion**

Chickpea 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 59.55±0.01 % was achieved with the highest protein content 46.15±0.01 %.Combination of bulk soaking and soxhlet extraction accelerated the fat removal from chickpea. Isolation of protein from chickpea was interrelated to fat removal.

**Acknowledgement**

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

Abbas, S., M.S.B, M.T.S, M.K. S, A.N.A and R.B.2015. "Nutritional and Functional Properties of Protein Isolates Extracted from Defatted Peanut Flour" *International Food Research Journal*, 22 (4), 153-1537.

AOAC, 2000. "Official Methods of Analysis of AOAC International",17th ed, Washington, DC: 5-15.

Aurelia, I., Iuliana, A., Aura, D., Gabriela, G., Cristina B. and Andrei, N. 2009. "Chemical and functional characterization of chickpea protein derivates", *Journal of Food Technology*, 5. 12-14.

Emami, 2007. "Hydrocyclone Fractionation of Chickpea Flour and Measurement of Physical and Functional Properties of Flour and Starch and Protein Fractions", University of Saskatchewan, Saskatoon, *Canada,*1-347.

Eltayeb, A.R.S.M, Ali. O.A, Abou-Arab, A.A. and Abu-Salem, F.M. 2011. "Chemical Composition and Functional Properties of Flour and Protein Isolate Extracted from Bambara Groundnut (*Vigna subterranean*)", *African Journal of Food Science*, 5 (2), 82-90, February.

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

Ghribi AM, Maklouf I, Blecker C, Attia. H and Besbes, S. 2015. "Nutritional and Compositional Study of Desi and Kabuli Chickpea (*Cicer Arietium* L.) Flours from Tunisan Cultiviars, *Advances food technology Nutritional Sciences*, 1 (2):38-47, 2015

Habibullah, Muhammad Abbas, and Hamid Ullah Shah, 2007. "Proximate and Mineral Composition of Mung Bean, *Sarhad Journal of Agriculture*", 23 (2).1-4.

Ladjal Ettoumi, Y. and Chibane, M. 2015. "Some Physico-Chemical and Functional Properties of Pea, Chickpea and Lentil Whole Flours", *International Food Research Journal*, 22 (3):987-996.

Lopez.O.Paredes, Ordorica- Falomir . C and Oliveres-Vazquez M.R. 1991. "Chickpea Protein Isolates: Physicochemical, Functional and Nutritional Characterization", *Journal of Food Science:* 56 (3), 726-729.

Hui lui li, 1996. "Chickpea Proteins For Food Applications", Ph.D thesis, University of Beijing, China, 1-3339.

Mubarak AE. 2004. "Nutritional Composition and Antinutritional Factors of Mung Bean Seeds (*Phaseolus Aureus*) as Affected by Some Home Traditional Processes", *Journal of Food chemistry,* 89: 489-495, January.

Quayyum, M.M.N., Butt, M.S., Anjum, F.M. and Hawaz, H. 2012. "Composition Analysis of Some Selected Legumes For Protein Isolate Recovery", *Journal of Animal and Plant Sciences*, 22(4).1156-1162.

Recommended Daily Dietary Allowances, 1980. "Food and Nutrition Board", *National Academy of Sicences-National Research Council*, USA, 1980.

Tharanathan, R.N. and Mahadevamma, S. 2003."Grain Legumes A Boon to Human Nutrition". *Trends in Food Science and Technology*, 14: 507-518.

Than Aung May, 2007. "Development and Adoption of Improved Chickpea Varieties In Myanmar", 5(1): 1-3, December

Withan Gamage Thushan Sanjeewa, 2008. "Physico-Chemical Properties of Chickpea Flour, Starch and Protein Fractions and their Utilization in Low-Fat Pork Bologn", University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1-223, August.

*******