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PHYSICO CHEMICAL AND FUNCTIONAL PROPERTIES OF CHICKPEA PROTEIN ISOLATE

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Abstract:-

The main purpose of this research work was to isolate the most refined form of protein from chickpea for food processing. In this research work, chickpea (Cicer arietinum. 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 chickpea 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 chickpea protein isolate have been determined. The solubility curve corresponding to the chickpea protein isolate indicated the minimum solubility at pH 4 (protein solubility of 24 %) and maximum solubility at pH 12 (protein solubility of 89 %) respectively. The chickpea protein isolate had water absorption capacity of 1.65±0.12 mL H_2O/g protein and oil absorption capacity of 1.72±0.34 mL oil/g. protein. It was found that emulsion stability of isolated chickpea protein was 40.12 ±0.33 %with foaming capacity was 63.64±0.22 %. Isolated chickpea 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 chickpea flour, chickpea protein isolate, isoelectric precipitation*

INTRODUCTION

World demand for plant protein is increasing [Eltayeb A.,et al, 2011] because animal proteins are more expensive and scarce [Ghribi AM,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 Amponsah, 2014, Ghribi AM,et al, 2015, Lopez.O.Paredes, et al ,2016, Withan G.T.S., 2008] .Chickpea in Myanmar are cultivated in central dry zone of the country, especially Sagaing, Magway and Mandalay [Than Aung May]**.** They are consumed widely throughout the world [Tharanathan, R.N. & Mahadevamma, S., 2003] and essential food resources which contribute to the nutritional health of manifold human diets [Ladjal Ettoumi,Y.. & Chibane .M, 2015]. Human beings should depend on the chickpea proteins [Abbas- S., et al, 2015] due to low amounts of sulfur containing amino acids and low protein digestibility [Mubarak AE., 2004]. 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 Amponsah, 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].

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) [Akaerue BI & Onwuka GI, 2010].

The most refined form of isolated protein contains no dietary fiber [Jayasena V.,et al, 2011].They are high digestibility [Garba, U & Kaur G, 2014] and easily included into different food products [Shabnum Shaheen,et al, 2012] essentially beverages, infant foods and textured protein products [Seyam, A.A.,et al,1983]. The functional properties of processed protein isolates like protein content, protein solubility, water absorption capacity, oil absorption capacity, foaming capacity and emulsion stability were determined. The objectives of this research were to remove the fat, fiber and starch from chickpea flour for enhancement of protein isolation and to determine the characteristics of chickpea protein isolate.

2. Materials and Methods

2.1 Raw Materials

Chickpea 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. Chickpea

2.2 Methods

2.2.1 Defatting the Chickpea Flour

2.2.1.1 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.

2.2.2 Preparation of Chickpea Protein Concentrate

Chickpea flour 100 g was soaked in 600 mL of 95 % ethanol for 20 hr and 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, defatted flour powder was packed with air- tight plastic bags.

2.2.3 Preparation of Chickpea Protein Isolate

The protein isolate was obtained from defatted flour. Because the chickpea 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 10.5. Fiber and starch fractions were removed from the alkaline dispersion by centrifugation(DSC-200A-2, Digisystem Laboratory Instruments Inc., TAIWAN) at 3500 rpm, for 40 min. Solubilized proteins were collected as supernatant which subsequently was used for the protein fraction recovery by isoelectric precipitation (pH 4.5) 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 hr and then milled to pass 200 mesh screen. The isolated protein powder was stored in air- tight plastic bags.

2.4 Methods of Analysis

Physico-chemical properties of chickpea flour, defatted flour and chickpea protein isolate such as protein content, moisture, ash, fiber ,carbohydrate, fat content and also protein solubility, water absorption capacity, oil absorption capacity, emulsion capacity and stability, foaming capacity and stability of protein isolate were determined. The EDXRF, Energy Dispersive X-ray Fluorescence Spectrometer (SPETRO XEPOS, Benchtop XRF Spectrometer) was used for the determination of elemental composition of chickpea protein isolate.

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

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

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

2.4.2 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 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, w1= weight (g) of sample before drying, w_2= weight (g) of sample after drying

2.4.3 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: [AOAC, 2000]

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

2.4.4 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 [AOAC, 2000].

2.4.5 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: [AOAC, 2000]

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

2.4.6 Determination of Carbohydrate Content

Carbohydrate value of the sample was determined by using the following formula:
Carbohydrate (%) = 100 - (protein + fat + fiber + ash + moisture)

2.4.7 Protein Solubility

Protein solubility of the 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 [Aurelia, I., et al, 2009].

2.4.8 Water and Oil Absorption

Water absorption capacity was determined by centrifugation, according to the method described by [Sathe, S .K et al, 1982] 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 [Lin, M. J .Y et al, 1974]. 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.

2.4.9 Emulsion Stability

The procedure described by [Volkert, M. A & B. P. Kelin, 1979] was used for emulsion 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 stability using the following formulas:

$$\text{Emulsion Stability} = \frac{\text{Height of emulsion after heating}}{\text{Height of whole layer}} \times 100$$

2.4.10 Foaming Capacity

The foaming capacity was determined by the method of [Lawhon, J. T.et al, 1972]. 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.

$$\text{Foaming Capacity} = \frac{\text{Volume before whipping} - \text{Volume after whipping}}{\text{Volume before whipping}} \times 100$$

2.5 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 Physico-chemical Properties of Chickpea Flour

Physico-chemical properties of chickpea flour was determined and presented in Table 1. It was observed that the protein content utilized local chickpea flour 19.94±0.03 % of local chickpea flour was lower than that of the [Quayyum,M,M,N, 2012], 22.83 ± 1.07% . Fat content of local chickpea flour 5.76±0.02 % was larger than thatof the [Quayyum,M,M,N, 2012] 5.43 ±0.26 % . The moisture content of local chickpea flour was 8.21±0.01% to protect the greater danger of bacteria action and mould growth which produce undesirable changes. However, the crude fiber of local chickpea flour, 1.00±0.03% was significantly different from [Quayyum,M,M,N, 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. 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 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

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

Figure 2 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.

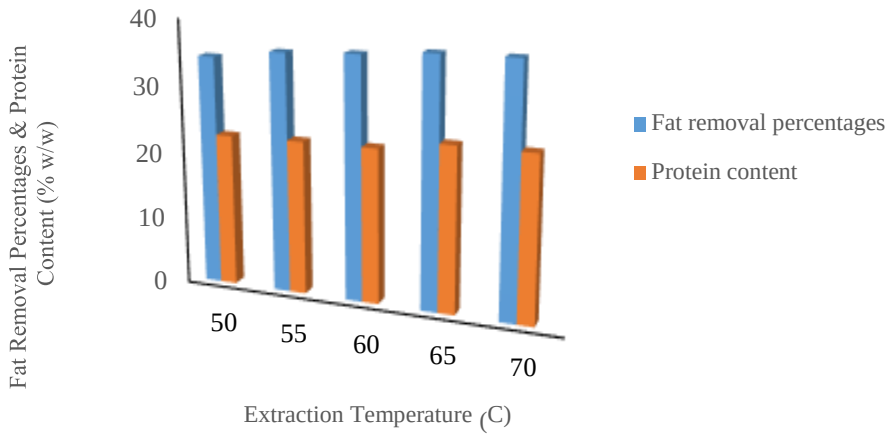

Figure 2. Effect of Soaking Time on the Percentage of Fat Removal and Protein Content from Chickpea Flour

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

Table 2 describes the effect of ratio of ethanol soaked bean 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. The most suitable material to solvent ratio was 1:6 at the extraction temperature 65°C. 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 % and also characteristics of defatted flour are presented in Table 3.

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

Meal to Solvent Ratio	Percentage of Fat Removal (% 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

Table 3 Characteristics of Defatted Chickpea Flour

Characteristics (%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

3.4 Effect of Different Acidic pH on the Protein Content, Fiber Removal 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 4. The highest fiber removal 30.43±0.4 % and starch removal 57.24 ±0.4 % were achieved with relatively highest protein content 73.10±0.3% at pH 4.5. So, pH 4.5 gave the highest yield of protein isolate from defatted chickpea flour due to insolubilization of protein at isoelectric point. The isoelectric pH of most of vegetable origin proteins correlate with values between 4 and 6 [Aurelia, I., et al, 2009].

Table 4. Effect of Different Acidic pH on the Protein Content, Fiber Removal Percentages

Different Acidic pH	Fiber Removal Percent (% w/w)	Starch Removal Percent (% w/w)	Protein Content (%w/w)
4.1	26.09±0.5	47.55±0.3	69.12±0.4
4.3	28.99±0.6	52.48±0.2	71.15±0.3
4.5	30.43±0.4	57.24 ±0.4	73.10±0.3
4.7	29.71±0.3	54.9±0.5	72.14±0.2
4.9	28.41±0.2	49.94±0.6	70.11±0.3

3.5 Physico-chemical Properties of Chickpea Protein Isolate

Physico-chemical properties of chickpea protein isolate was determined in Table 5.Chickpea protein isolate was characterized by protein content 73.10±0.3 % and low content in fiber, 0.48±0.4 % and in ash, 2.00±0.2 %. By refinement, the carbohydrate level was substantially diminished to 17.42±0.1%.

Table 5. Physico-chemical properties of Chickpea Protein Isolate

Properties	Chickpea Protein Isolate (%w/w)
Protein content	73.10±0.3
Moisture content	5.00±0.3
Ash content	2.00±0.2
Fiber content	0.48±0.4
Carbohydrate content	17.42±0.1
Fat content	2.00±0.3

3.6 Functional Properties of Chickpea Protein Isolate

Functional properties of chickpea protein isolate was shown in Table 5. They showed a water absorption capacity (WAC) of 1.65±0.12 mL H_2O/g protein. Water binding properties of protein is determined by their degree of interaction with water [Suliman, M. A., et al, 2006]. Chickpea protein isolate has a higher capacity of swelling, distortion and separation, that allows additional exposure of binding sites of water and increases water absorption [Suliman, M. A., et al, 2006].The oil absorption capacity (OAC) of chickpea protein isolate was 1.72±0.34 mL oil/g. proteins. 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 [Siddiq M, Ravi R & Harte J.B,Dolan K., 2010]. The OAC is an important functional property because it upgrades mouth feel and flavor retention [Josefina Porras-Saavedra, et al, 2013]. Chickpea protein foam had a lower capacity but highly stable compared to soy protein that studied [Suliman, M. A., et al, 2006].Emulsion stability of isolated protein was 40.12 ±0.33%. It was within [Suliman, M. A., et al, 2006].They [Suliman, M. A., et al, 2006] also reported that the emulsion stability responsible for the water and oil absorption capacity. The foaming capacity of chickpea protein isolate was 63.64±0.22 % the same as [Suliman, M. A., et al, 2006]. Chickpea protein foam owned a lower capacity but highly stable compared to soy protein that measured [Suliman, M. A., et al, 2006].

Table 6. Functional Properties of Chickpea Protein Isolate

Properties	Chickpea Protein Isolate
Water absorption capacity (mL H_2O/g)	1.65±0.12
Oil absorption capacity (mL oil/g)	1.72±0.34
Emulsion stability (%)	40.12 ±0.33
Foaming capacity (%)	63.64±0.22

3.7 Elemental Compositions of Chickpea Protein Isolate

The elemental compositions of chickpea protein isolate was analyzed by ED-XRF. The data are shown in Table 6. It shows chlorine, phosphorus, calcium and potassium. These minerals can effectively contributes towards the daily recommended allowances [RDA, 1980] for all groups. It was observed that chickpea protein isolate is used for protein source but it can fulfill the micro nutrients deficiency as well.

Table 7. Elemental Compositions of Chickpea Protein Isolate Analyzed by ED-XRF Method

Sr. No.	Elements	Compositions (%w/w)
1	Chlorine (Cl)	2.325 ±0.02
2	Phosphorus (P)	0.6632±0.01
3	Calcium (Ca)	0.6475±0.03
4	Potassium (K)	0.4302±0.01
5	Silicon (Si)	0.2967±0.02
6	Aluminum (Al)	0.1869±0.04
7	Sulfur (S)	0.1659±0.03
8	Iron (Fe)	0.08184±0.01
9	Manganese (Mn)	0.02788±0.02
10	Titanium (Ti)	0.01015±0.01

3.8 Protein Solubility of Chickpea Protein Isolate

Figure 3 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 chickpea protein isolate solubility was 82 % while at pH 2, the chickpea protein isolate solubility was 60 %. Also at pH 12, protein solubility was 89 % while at pH 4, the protein solubility was 24 %.The reduction in solubility, at very low pH values could be due to the protein denaturation and insolubilization processes [Aurelia, I., et al, 2009]. Chickpea protein showed good solubility in both acid and alkaline pH region, which is an important characteristic for food formulation [Suliman, M. A., et al, 2006].

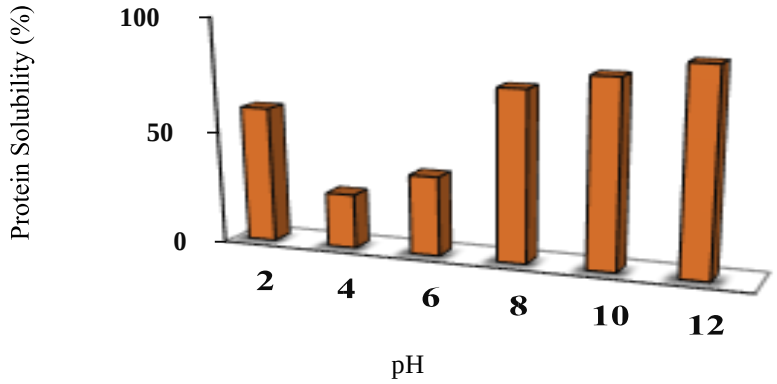

Figure 3. Effect of pH on the Protein Solubility of Chickpea Protein Isolate

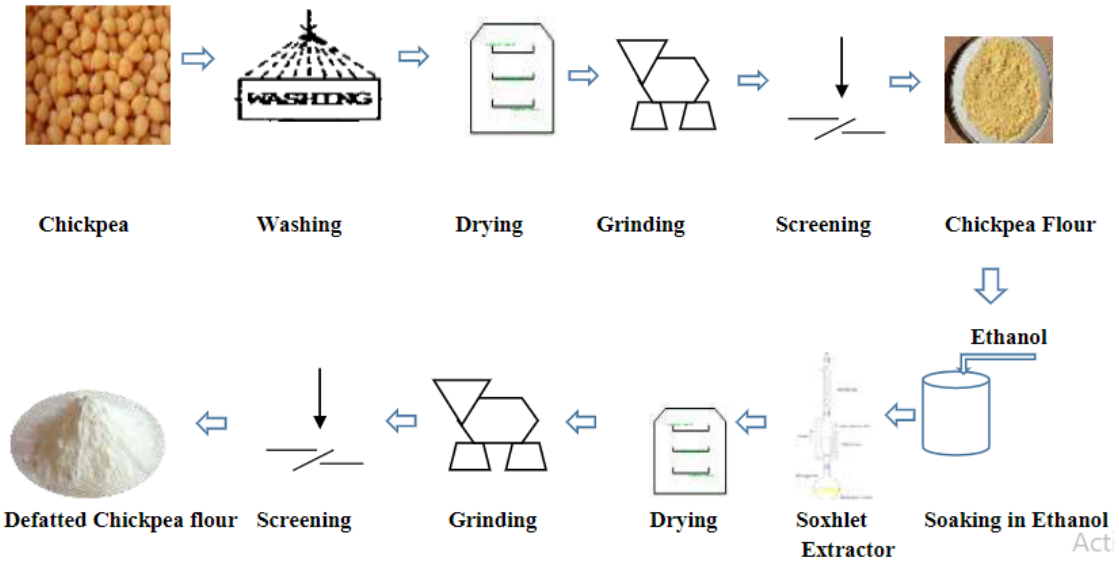

Figure 4.Process Flow Diagram for the Preparation of by Soxhlet Extraction from Ethanol Soaked Bean Flour

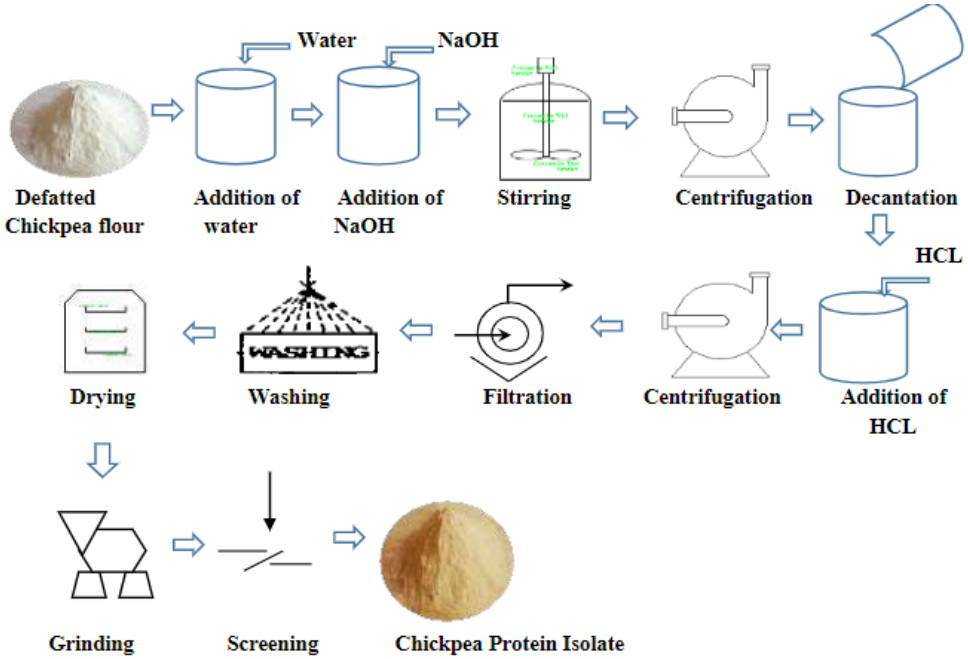

Figure 5. Process Flow Diagram for the Isoelectric Precipitation of Chickpea Protein Isolate

Conclusions

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 %. 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 73.10±0.3 % was achieved at pH 4.7. At pH 12, protein solubility was 89 % while at pH 4, the solubility was 24 %.The lentil protein isolate had water absorption capacity, 1.65±0.12 H_2O/g. protein and oil absorption capacity of 1.72±0.34 mL oil/g. protein. Having their excellent functional properties, chickpea protein isolate can be further utilized for the supplementation of various food products.

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