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Process isolation of protein isolate from chick pea (*Cicer arietinum*) with the solubility and color characteristics

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Abstract
In the present study alkaline extraction method used as it was found that alkaline reagents were more effective in extraction of protein from food legumes. Chick pea flour defatted with the Soxhlet extraction method, pH was adjusted to 9 with NaOH, Centrifugation has to be carried out at 8000 rotations per minutes then proteins were precipitated. Finally protein isolate extraction performed with the freeze drying method. The color of both the protein isolates were dark this may be due to the isolation of protein from whole chick pea flour instead of deskin, defatted chick pea flour. Protein solubility profiles showed a decreasing solubility with increasing pH until it reached a minimum at the isoelectric point (pH 4.0–5.0). For the experiment two cultivars of chick pea variety JAKI -9218 (Desi) and Kabuli variety PKV K-4 were used from local market of Nagpur city.

Keywords: chick pea, alkaline extraction, flour, freeze drying, centrifugation, color, solubility

Introduction
Chick pea is major winter pulse crop grown in India. Among the pulses, chickpea occupies 30 per cent of area with 38 per cent of annual production in India. The average productivity is 1129 kg/ha which is far below the potential expected from improved technologies. (R.K.S. Tomar, 2010) [21]. The higher protein contents indicate that chickpeas are truly called “meat of the poor”. The water soluble, salt soluble and total salt soluble protein fractions from the chickpea seed flour were also obtained. No significant variation among the varieties was observed for protein contents in case of each fraction. This variation may be attributed to climatic and varietal differences by Muhammad Aslam Shad and *et. al* (2009).
Fractionation of proteins and starches can be done by wet processing or dry processing. Wet processing is used for the preparation of highly purified protein and starch. This method requires high level of energy for drying and refining of the effluent. The most common wet processing methods include alkaline extraction, isoelectric precipitation, ultrafiltration and salting out. Dry processing is effluent-free and a cheaper method, but the purity of starch and protein fractions from this method is less than wet processing. Dry processing mainly includes dehulling, dry milling and air classification to obtain starch-rich and protein-rich fractions. (S. Emami 2002) [23].
Application of protein isolate regards, the high solubility of these isolate in the acidic pH range indicates that the isolate may be useful in the formulation of acidic food like protein rich carbonated beverages. Since protein solubility affects other functionalities like emulsification, foaming and gelation, the high solubility of the proteins indicates that they could have promising food applications. Optimal conditions have been obtained in the preparation of protein isolates from Mucuna bean (Mugendi J.B.*et al*., 2010) [18]. Y.A. Adebowale (2008) [3] stated industrial application of proteins such as in the production of fibers, adhesives, ingredients of coating, emulsifiers, food additives, and different food products depend upon bringing proteineous materials into solution. Hence the knowledge of protein solubility will be an important factor in selecting particular vegetable proteins for possible industrial application. The solubility of the protein isolates from pea, faba bean and soya beans and revealed similar patterns. A Fernandez *et al*. (1997) Lower solubility was detected at a pH range between 4.0 and 6.0. On the other hand, maximum solubility was obtained at pH values of 8.0 and 9.0 for soybean and faba bean protein isolates, respectively. However, the pea protein isolates had lower solubility than those in the other protein isolates at basic pH values. Hunter (L, a, b)

values of soya bean protein isolate (standard), mucuna bean protein isolate (MBPI) and dehulled mucuna bean flour were observed by Mugendi J.B.*et al* (2010) [18]. The L (lightness) and 'b' (yellowness) values for processed MBPI were significantly lower than for both mucuna flour and SBPI. However, the 'a' (redness) value for MBPI was significantly higher than for SBPI but lower than for mucuna flour. Total color difference (ΔE) between SBPI and MBPI was significantly higher than for mucuna flour. Processed MBPI was very dark as evidenced by low "L" value compared to SBPI and mucuna flour.

Materials and Methods

The study was carried out in the Department of Food Technology, Laxminarayan Institute of Technology; Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur (Maharashtra), during the year 2010-2011. The details of materials used and methods adopted during the present investigation are presented in this chapter.

Materials

Chick peas

Two cultivars of chick pea variety JAKI -9218 (Desi) and Kabuli variety PKV K-4 were procured from local market of Nagpur city. The peas were cleaned, ground in mixture grinder and stored properly at room temperature prior to their use in actual experiment.

Chemicals and Glass wares

In the present investigation analytical grade chemicals from Himedia, Emerck, BDH and Glass wares from Borosil were used.

Proximate Analysis

The proximate analysis of Chick pea flour and Defatted chick pea flour was carried out according to the standard A.O.A.C (2000) procedures.

Moisture content:

Moisture

The moisture content of flour was determined according to method No. 44-15 A of (A.O.A.C, 2000). 5g of flour sample was taken in tarred crucible and dried in a hot air oven at 100 ± 5 ºC till to a constant weight. The moisture content was calculated by the formula given below.

$$\% \text{ Moisture} = \frac{\text{Initial weight} - \text{final weight}}{\text{Total weight of sample}} \text{ X } 100$$

Ash

The ash content was determined as a total inorganic matter by incineration of the samples at 600ºC according to method No. 08-01 of (A.O.A.C., 2000). Remaining inorganic materials are reduced to their most stable form, oxides or sulphates and are considered as 'ash'.

Procedure

Oven dried 5 g sample was taken in a pre-weighed crucible and charred on the burner. Then it was ignited in the muffle furnace at 550-600ºC for 5-6 hours or till constant weight of grayish ash was obtained. The ash of sample was calculated through following formula.

$$\% \text{ Ash} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty Crucible}}{\text{Total weight of sample}} \times 100$$

Fat

The method employed was that of solvent extraction using a Soxhlet extraction as described in method No. 30-10 (A.O.A.C., 2000). 2 g of flour was taken in a thimble and placed in extraction tube of Soxhlet apparatus. About 250 ml of Hexane was added in 500 ml bottom flask of the apparatus and connected to Soxhlet apparatus. The fat was extracted by running Hexane over the sample at the rate of 3-4 drops per sec for about 5 hr. The solvent was recovered and the flask was kept in hot air oven for 10 min at 40-50ºC. The flask was cooled in desiccator and weighed. Fat percentage was calculated according to the following formula.

$$\% \text{ fat} = \frac{\text{Final weight of flask} - \text{Empty weight of flask}}{\text{Weight of sample}} \times 100$$

Protein

The protein was determined by the Kjeldhal's method as described in method No. 46-10 of (A.O.A.C., 2000). This is based on the fact that on digestion with concentrated sulphuric acid and catalysts, organic compounds are oxidized and the nitrogen is converted to ammonium sulphate. Upon making the reaction mixture alkaline, ammonia is liberated, removed by the steam distillation, collected and titrated.

Procedure

The nitrogen content of samples was determined by using micro Kjeldhal's method. The sample was first digested in digestion flask with H_2SO_4 in presence of digestion mixture for 3-4 hr till the contents of digestion flask get transparent color. The samples were then diluted with distilled water up to 250 ml in a volumetric flask. The ammonia from the samples was liberated through distillation after adding 40% NaOH solution and collected in flask containing 4 % boric acid solution using methyl red as an indicator. The nitrogen content in the samples was determined by titrating against standard 0.1 N H_2SO_4 solution and the crude protein percentage was calculated by using following formula

$$\%N = \frac{(\text{Sample-Blank}) \times \text{N of } H_2SO_4 \times 0.014 \times \text{D.F.}}{\text{Wt. of sample (g)}} \times 100$$

% Protein = % Nitrogen × 6.25

Crude fiber

Crude fiber content was determined by following the method No. 32-10 as described in (A.O.A.C., 2000). 2 g fat and moisture free sample was taken and placed in 1000 ml beaker. 200 ml solution of 1.25 % H_2SO_4 was added in the beaker. The sample was then digested by boiling for 30 min. Then it was filtered by using suction apparatus. The residue was washed with hot water until become acid free. The residue was then again transferred to 1000 ml beaker and boiled with 200 ml solution of 1.25 % H_2SO_4 for 30 min. It was again filtered and the residue was transferred to pre-weighed crucible and dried in an oven at 100ºC of 24 hr till constant weight was obtained. Then the dried residue was charred on a burner and ignited into muffle furnace at 550-600ºC for 5-6 hr, cooled in desiccator and weighed. The loss in weight during incineration represents the weight of crude fiber in

sample. The crude fiber percentage was calculated by using the following formula.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Preparation of protein isolate

Protein isolates from different chickpea cultivars were prepared using following method. Firstly both the chick peas were cleaned and dry milled or ground through mixer grinder. It is then sieved through sieve. Further the chickpea flour is defatted by using soxhlet apparatus. Dispersions of defatted chickpea flours (5%, w/v) in distilled water were made. The pH of dispersions was adjusted to pH 9 with 0.1 N NaOH at room temperature (30°C). It is then shaken for nearly 1 hr and centrifuged at 8000g for 15 min. In order to obtain increased yields, the extraction and centrifugation procedures were repeated twice on the residue. The extracts were combined and the pH adjusted to 4.5 with 1 N HCl to precipitate the protein. The proteins were recovered by centrifugation at 8000 rpm (rotations per minutes) for 15 minutes followed by removal of the supernatant by decantation. Protein curd was washed twice with distilled water and centrifuged at 8000 rpm for 10 minutes. The washed precipitate was then freeze dried as protein isolate.

Process Flow sheet

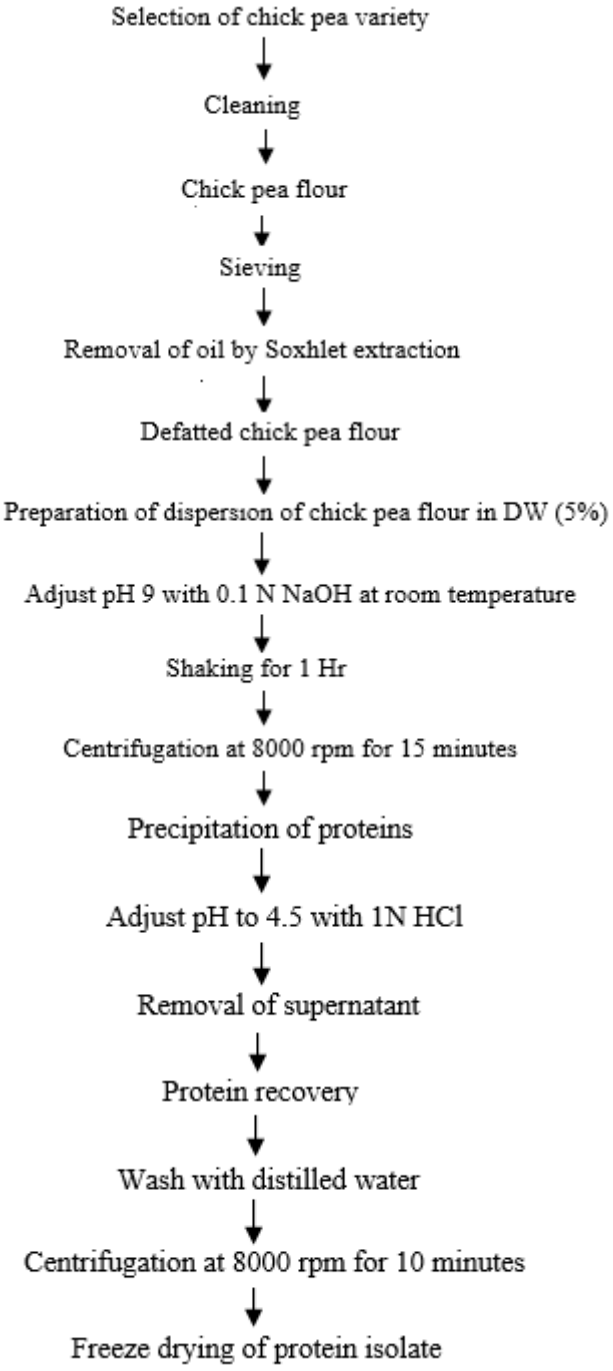

Fig 1: Flow diagram for the Preparation of protein isolate by alkaline extraction method

Protein solubility

Protein solubility at different pH may serve as a useful indicator for the performance of protein isolates in the food system and also an extent of protein denaturation because of chemical treatment. Protein solubility of samples was studied in the pH range of 3.0–7.0. Sample (100 mg) for each pH was

suspended in 20 ml distilled water and the pH of the suspensions was adjusted to a specific value using 0.1 N HCl or NaOH solutions. These suspensions were agitated over a metabolic shaker for 1 hr at room temperature. The pH was checked and adjusted, then centrifuged at 8000g for 15 min. The protein content of supernatant was determined by the method of Lowry *et al*. using Bovine Serum Albumin as standard. Duplicate determinations were carried out and solubility profile was obtained by plotting averages of protein solubility (percent) against pH. Solubility was expressed as the percentage of the total protein of the original sample that was present in the soluble fractions.

Color characteristics

The color of the powder was measured for all samples using Hunter Lab Colorimete (Model DP 9000 D25A), (Hunter Associates Laboratory, Reston, VA, USA) in terms of L value (lightness, ranging 0-100 indicating black to white), a value and b. A glass cell containing sample was placed above the light source, covered with a white plate and L*, a* and b* color values were recorded. The L* value indicates the lightness, 0–100 representing dark to light. The* value gives the degree of the red-green color, with a higher positive a* value indicating more red. The b* value indicates the degree of the yellow-blue color, with a higher positive b* value indicating more yellow.

Total color difference (ΔE) was calculated by applying the equation

$$\Delta E = \{(Ls-L)2 + (as-a)2 + (bs-b)2\}1/2$$

Where Ls, as and bs are reference tile against which instrument was calibrated.

Result and Discussion

Proximate composition of the chickpea seed flours and that of defatted seed flour were analyzed. The presented varieties are prominent in middle part of India that is east Maharashtra and most of Madhya Pradesh. The protein content of chickpea isolates in the present study were found to be slightly lower than those reported before as far as average values are concerned, as it was 16.10% in Desi and 17.80% in Kabuli chickpea flour. Investigated varieties had higher carbohydrate content which was higher, as per previously research reported. It was found that Desi (Jaki 9218) variety had more carbohydrate content than Kabuli (PKV K-4). The Desi variety has 70.61% and Kabuli has 66.33% carbohydrate. The protein isolates prepared from defatted flours showed fat content of 0.49–0.98% (Manindar kaur 2005). These lipids mainly of a polar nature interacted with proteins so need to remove. Both type of flour had higher lipid content. The fat content of chick pea flour of both Desi and Kabuli varieties were found to be 2.94 % and 5.92 % respectively. After removal of fat prior to protein isolation the fat content of defatted chick pea flour was 0.3% in Desi and 0.4% in Kabuli chick pea flour. The ash content of chickpea flours and defatted chickpea flours were found as 2.33, 2.80% in Desi variety and 2.82, 2.91% in Kabuli variety respectively. The crude fiber content in Desi chick pea was 3.54% and in PKV K-4 was 2.42%. The investigated varities has shown low moisture content. The moisture content of Jaki 9218 was 4.48% and that of PKV K-4 was 4.73%. The defatted samples were also analyzed for moisture content and have shown very less decrease in moisture content as it was found 4.20 % in JAKI 9218 and 4.70 % in PKV K4. On an average moisture content of dry seeds ranges from 7 to 8% in this case the low moisture indicates the long post harvest life span of given varieties.

Color characteristics

Hunter Lab color values (L*, a*, b* and ΔE) of protein isolates from different chickpea cultivars are shown in Table No 5.2. The varietal difference was observed for various Hunter color parameters. The Hunter values showed that chickpea isolates were significantly darker and reddish in color, with given L* value. ΔE, which indicated total color difference, for chickpea protein isolates were ranged to 63.1 for Desi and 69.9 for Kabuli chick pea protein isolate. Protein isolate from Kabuli chickpea showed the highest L* (59.36.33) and ΔE (63.18) value, indicating its lighter color as compared to isolates from Desi types. It is observed L*, a*, b* and ΔE value of 66.58, 5.19, 21 and 63.18 respectively for Kabuli chickpea protein isolates. The results obtained for kabuli chick pea protein isolate were matched with (Manindar Kaur 2007) and desi chik pea protein isolate found to be more darker comapared to previous research.

Table 1: Color characteristics of chick pea isolates

Chickpea protein	L	a*	b*	ΔE
Desi	66.585	3.935	20.91	69.92
Kabuli	59.36	5.19	21	63.18

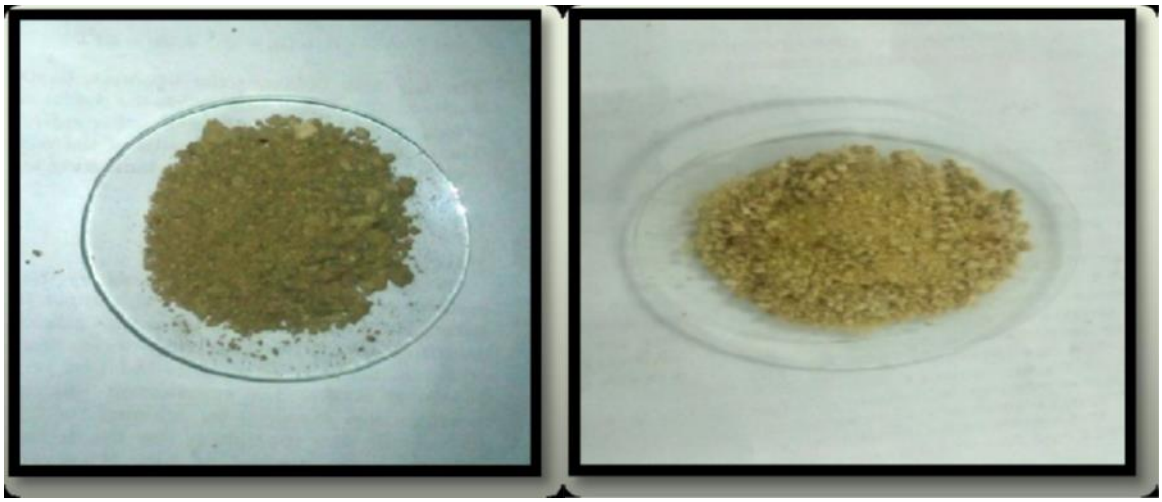

Fig 2: Protein isolate (Desi) Protein isolate (Kabuli)

Protein solubility

Protein solubility at different pH may serve as a useful indicator of the performance of protein isolates in the food systems, and also the extent of protein denaturation because of heat or chemical treatment. The solubility profiles of Desi and Kabuli chickpea protein isolates is slightly similar. The protein isolates had minimum solubility in the pH range 4.0–5.0, as it is the isoelectric pH range and maximum solubility at pH 3 and 7.0 (Fig. 5.1). Most of the plant proteins have isoelectric pH at 4.0–5.0. At the isoelectric point, there is no net charge on the protein; so they get precipitated.

At low pH, large net charges are induced and repulsive forces increase, resulting in unfolding of proteins. Above pH 6 all proteins had solubility greater than 50%. Profiles with low solubility over a broad range of pH are indicative of severe protein denaturation and insolubilization which have been shown to markedly affect the functional properties of proteins.

Table 2: Shows the protein solubility of two verities

pH	Solubility of Desi chickpea protein Isolate (%)	Solubility of Kabuli chickpea protein isolate (%)
3	53	48
4	26	24
5	22	21
6	55	50
7	62	59

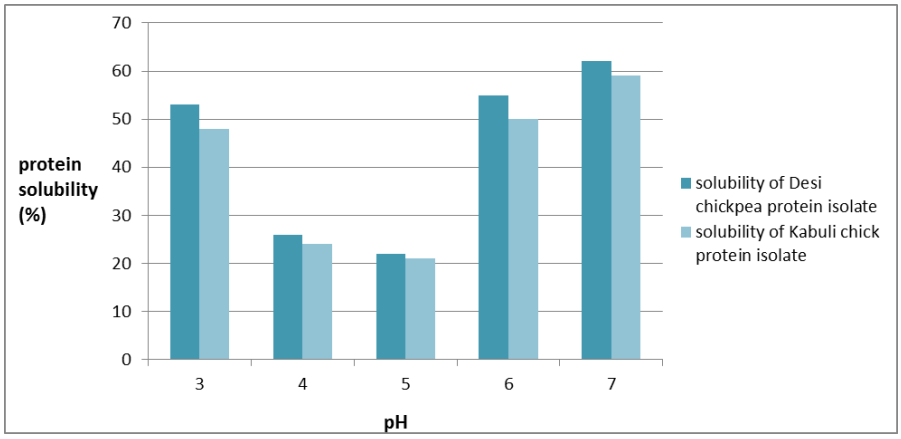

Fig 3: Solubility profile of chick pea protein isolate from two varieties

Increased solubility at low and high pH may be attributed to decreased protein-protein interaction owing to the charged nature of proteins outside their isoelectric point. Similarly, Mugendi *et al.* (2010) [18] reported a minimum nitrogen solubility for cowpea protein isolates at pH 4 and 5 and increased solubility at low and high pH. The solubility profile of a protein provides some insight into the extent of denaturation or irreversible aggregation and precipitation which might have occurred during the isolation process. It also gives an indication of the types of foods or beverages into which the protein could be incorporated. Factors such as concentration, pH, ionic strength and the presence of other substances influence the solubility of protein. The characteristics described above can be understood on the basis of the overall ionic charge of the protein with the pH. At low pH values, most of the carboxyl and amino groups from the lateral amino acid chains are protonated in the –COOH and –NH3 + forms respectively, and the overall charge of most protein molecules is positive. As the pH increases some of the carboxyl groups are dissociated into –COO and –H+, according to their dissociation constants, and the positive charges associated with the proteins diminish up to the isoelectric point, where these are neutralized.

At this point, the protein cannot be hydrated by water molecules, due to the modification of its tertiary and quaternary structures and its solubility reaches a minimum value. As the pH increases even more, the amino groups dissociate into –NH2 and –H+, and the overall protein charge becomes negative due to the presence of –COO groups and can consequently be hydrated and dissolved in water.

Summary and Conclusion

Legumes are valuable protein sources for both humans and animals. However, their nutritive value is lower than expected on the basis of their chemical composition. Thus, protein isolation has been proposed as an important alternative within chemical treatments in order to improve the legume nutritive value. Both the varieties had long post harvest storage period within which moisture was decreased to near about 4.48 % in Desi and 4.73% in Kabuli. Investigated verities had more carbohydrate content than protein so the physiological properties of isolates might have affected. Both the varieties had significant oil content i.e. Kabuli and Desi variety had near about 5.92% and 2.94% oil content. Both these varieties contains good amount of crude fiber. The defatted flours of present varieties were also analyzed prior to protein isolation. The most of fat was removed. The color of both the protein isolates were dark this may be due to the isolation of protein from whole chick pea flour instead of deskin, defatted chick pea flour. Protein solubility profiles showed a decreasing solubility with increasing pH until it reached a minimum at the isoelectric point (pH 4.0–5.0).

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