



## Recent Advances in Isolation and Characterization of Protein Concentrate and Isolate from Seeds

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### ABSTRACT

*Protein concentrate is defined as the non-oil fraction of the amino acid rich components obtained from defatting plant part followed by extraction and isoelectric precipitation. In this paper, an attempt has been made to review some existing methods used in the isolation and characterization of protein concentrate and isolate from plant origin. The methods were also compared based on the properties of their resulting concentrate and their percentage yields as reported by many researchers. Among these methods, enzyme-solvent extraction methods yielded highest amount of concentrate followed by alkaline filtration and lastly enzymatic extraction. Similarly, types of extraction method employed were observed to have significant impact on the functional properties of the concentrate.*

**Keywords:** Protein, Concentrate, Isolate, Seeds and extraction

### 1. 0 INTRODUCTION

Animals and plants play major roles in the supply of nutrients for human consumption, although more than 70% of the proteins based nutrients are of animal origin which is inadequate especially in the developing countries where the average protein intake is always less than the required, as such there has been a constant search for unconventional legumes as new protein sources (Aladesanmi, 2015; Ayodele & Aladesanmi, 2015). There are many protein

sources of which the most commonly utilized in nutritional products are casein and whey proteins. To compliment those from animal origin, plant proteins are now used commercially in number of formulated foods (Alfonso Clemente a, 1999).

Soya beans is widely used as plant source of protein concentrate and isolate, although other sources such as Groundnut (Mustapha et al., 2015), peas and chickpeas (Alfonso Clemente a, 1999), *Adenopus breviflorus* (Ayodele & Aladesanmi, 2015) *Cereus*

jamacaru (Costa, Souza, Jr., Zárata, & Campos, 2001) Lupin seed (Duranti, Consonni, Magni, Sessa, & Scarafoni, 2008), Indian Chickpea (Kaur & Singh, 2007), Wal nut (Mao & Hua, 2012), and Guard Melon seed (Ogundele, 2013) had been used for long as sources of protein.

Isolation and characterization of protein concentrate is done through different methods, among which are: ultrafiltration and isoelectric precipitation technique, alkaline extraction, acid extraction, enzymatic extraction, enzymatic solvent extraction and jet cooking.

## **2.0 DEFINITION OF PROTEIN CONCENTRATE AND ISOLATE**

Proteins are enormously complex polymers based on up to 20 different amino acids. The amino acids are the building block of proteins, their different mode of connection through peptide bonds leads to their structural and functional differences, their size and type and the size of the peptide chain. Therefore, in theory, there is a limitless number of proteins with unique properties. They are made up of 50–55% carbon, 6–7% hydrogen, 20–23% oxygen, 12–19% nitrogen and 0.2–3% sulfur (Rodrigues, Jorge, Coelho, Graça, & Carvalho, 2012). Due to their above nature food proteins are defined “as those which are easily digested, non-toxic, nutritionally adequate, functionally useful and abundant” (Rodrigues et al., 2012). Traditionally, food proteins are present in milk, meat (including fish and poultry), eggs, cereals, legumes and oilseeds. Since the functional properties of the proteins are related to their structural and physico-chemical characteristics, their behavior in food can only be improved if

their physical, chemical, nutritional and functional properties are known, in addition to the changes undergone during processing” Thus, protein concentrate and isolate can be broadly defined as the non-oil fractions of amino acids rich components obtain when plant parts (i.e. seeds, leaf, or tubes) are defatted into flour, followed by alkaline extraction and Isoelectric precipitation or ultrafiltration (Ogundele, 2013) and so on.

## **3.0 METHODS OF ISOLATION AND CHARACTERIZATION OF PROTEINS CONCENTRATE AND ISOLATE.**

The industrial production and purification of protein concentrate and isolate from vegetable seeds depends solely on the processing medium. As protein concentrate is produced, when neutral, acidic or alkaline medium is used for extraction and purification. Resulting in up to 48-70% protein yield. While, the protein isolate (85-90% protein) is extracted and purified using alkaline medium (Moure, Sineiro, Domínguez, & Parajó, 2006).

The choice of method for the characterization of proteins concentrate and isolate is always based on the nutritional and antinutritional values of the resulting concentrate or isolate as well as the economic factors such: yield, time, condition and medium of the production. Below is the description of various methods employed by different authors, using different plant seeds in isolation of proteins concentrate and isolate. This will clearly explain the Recent Advances in Isolation and Characterization of Protein Concentrate and Isolate from Seeds.

(i) **i Ultra filtration and isoelectric precipitation techniques,**

After dehulling and milling of seeds obtained from different plants Boye et. al., (2010) described the simple and strait forward methods for ultrafiltration and isoelectric precipitation as shown in the Figure 1:

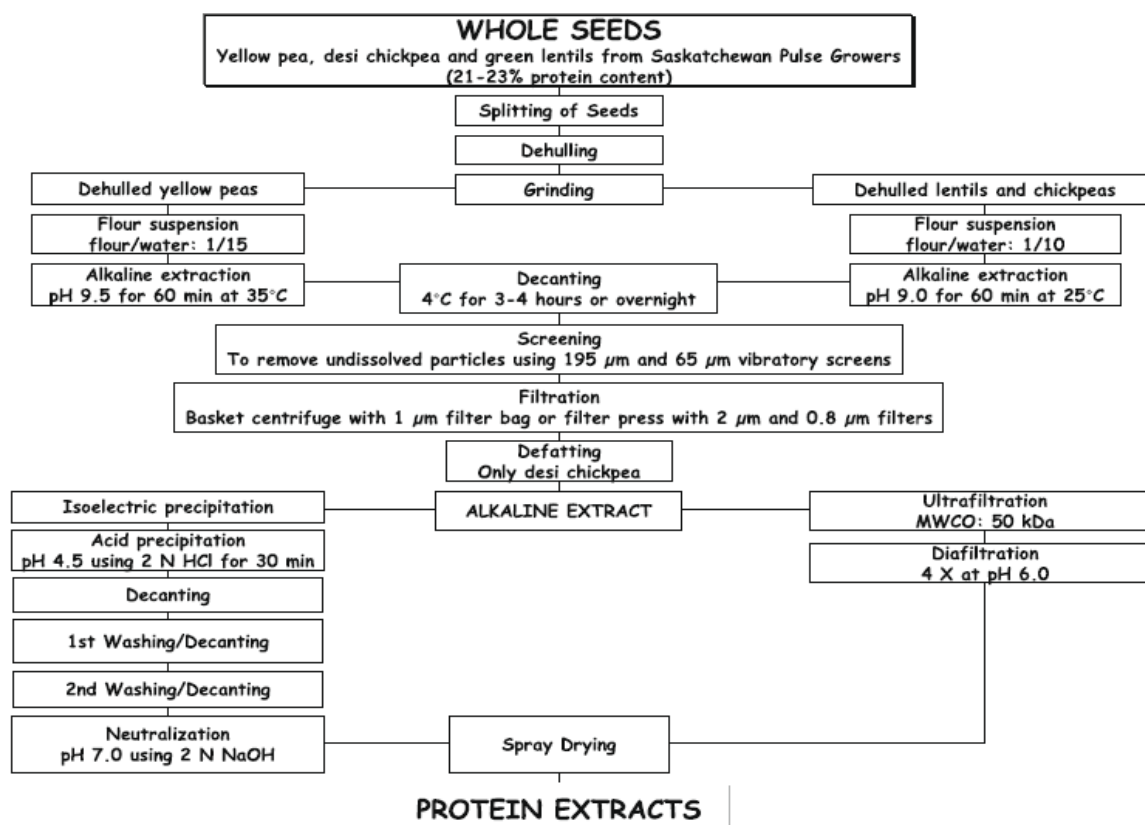


Figure 1.0: The Schematic Diagram of Ultra filtration and Isoelectric Precipitation Determination. Source (Boye et al., 2010)

Ultrafiltration and Isoelectric Point processing techniques allowed the isolation of different plants protein concentrates with protein contents varying between 63.9% and 88.6% (Boye et al., 2010). This clearly indicates the effectiveness of the methods in production of protein isolate and concentrate. In the same vein, and (J.O Ogundene, 2015), employed the

isoelectric precipitation method in their respective investigations.

Similarly, and Ogundene, (2015) observed that, the use of alkaline extraction followed by isoelectric precipitation method improves the protein content of extract better than direct isoelectric precipitation process.

## (ii) ii. Alkali Extraction and Isoelectric Precipitation

In this method, defatted flour is mixed with water at flour to water ratio of 1/10 and a solubilizing pH of 8.5 adjusted with 1.0N NaOH. The flour suspensions will then be stirred at room temperature for 1 h, centrifuged at 3500g for 20 min. The supernatants will be collected and adjusted to pH 4.5 (the isoelectric pH) with 1.0 N HCl. The suspensions are then centrifuged at 3500g for 20 min. The supernatants will be discarded, and the precipitate dried using a LGJ-25 freeze dryer. The dry protein concentrate powder stored in a refrigerator for further processing (Wu, Wang, Ma, & Ren, 2009)

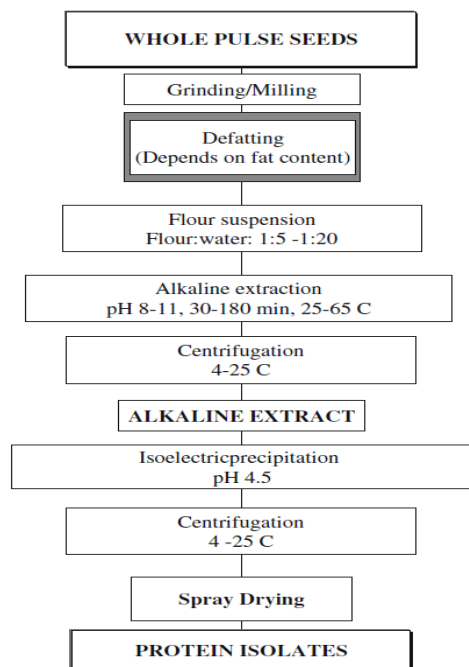


Figure 2: the schematic diagram for alkali solution and isoelectric precipitation process. Adopted from (Boye et al., 2010)

## (iii) Acid Extraction and Isoelectric Precipitation

According to Boye and co-workers, acid extraction is like alkaline extraction except

that the initial protein extraction is conducted in acidic condition of 0.4N there by adjusting the pH of the solution to 4. Most of works cited by Boye, et. al., 2010 shows that for acidic extraction the pH of 4.0 – 4.6 is maintained and mostly the protein isolate yield of 91 – 96% is achieved for different seeds. After the acid extraction, the isoelectric precipitation of performed as in (ii) above.

## (iv) Ultrafiltration, Diafiltration Methods and Extraction of Albumin Enriched Protein Fraction.

Ultrafiltration method involves grinding seeds using a household mill and the resulting flour will be double-treated for 40 min (25°C) with petroleum ether to remove the fat. The defatted flour would then be dispersed in deionized water (flour to water ratio: 1/10), the pH raised to 9.0 using 0.1% NaOH solution and the dispersion stirred for 45 min with the aid of a mechanical stirrer. The protein solution will be separated by centrifugation at 4000g, the slurry again treated for 45 min with deionized water (flour to water ratio: 1/5) at pH 9.0 for dialysis method, the alkaline protein solution should be diluted with one volume of deionized water and the pH lowered from 9.0 to the value of 7.0. After which the solution will then be subjected to ultrafiltration by employing Millipore membranes of regenerated cellulose with a molecular weight cut-off of 5000 Da and finally, freeze dry the resulting concentrated protein solution.

Finally, a chickpea protein isolate enriched in the albumin fraction (CPI-TF) can then be prepared by treating the defatted flour with

deionized water at pH 5.0, followed by centrifugation to remove the flour particles. The protein extract will then be diluted with deionized water, the pH increased to 7.0 and the diluted solution subjected for three times to ultrafiltration–diafiltration as described above (Papalamprou, Doxastakis, Biliaderis, & Kiosseoglou, 2009).

#### (v) **Enzymatic extraction**

Protein concentrate preparation using enzymatic extraction was reported by (Tirgar, Silcock, Carne, & Birch, 2017). DFM mixed with deionized water following the alkali Isoelectric precipitation method as described in (i) above, suspension will then be adjusted to pH 5.0 with 1 M HCl, followed by the addition of cellulase (2%, w/w) to DFM to enable hydrolysis of the fibre content. The suspension will be transferred to a 2 L conical flask and agitated at 37°C, and 150 rpm for 4 h in an orbital mixer incubator (Ratek, Victoria, Australia). The hydrolysed suspension cooled to 4 °C after which the alkali extraction will be performed as reported by (Tirgar et al., 2017). This result in a less viscous mucilage and finally the precipitated protein concentrate will be freeze dried, vacuum-packed and stored at 20 °C for further analysis (Tirgar et al., 2017).

#### (vi) **Enzymatic Solvent extraction**

The protein concentrate obtained from enzymatic extraction in (v) above, will then be mixed with 100 ml ethanol (95%) and the suspension kept overnight at 4°C. The precipitated protein will be separated by centrifugation at 15,300g, 4°C for 15 min, followed by washing, freeze-dried, vacuum-packed and stored at 20°C (Tirgar et al., 2017).

#### (vii) **Jet-cooking combined with enzyme-assisted ultra-filtration**

This method was developed to produce soy protein isolate (SPI), for using infant formulation. it is based on the use of jet cooking (JC) and enzyme assisted ultrafiltration (UF) to improve the quality and safety of the protein concentrate (Yang et al., 2014).

It involves extraction of protein from defatted flour in alkaline condition, followed by isoelectric precipitation to obtain the protein fraction to be used (Wang et al. (2012); Yang et. al., (2014)). The alcohol bleached soy protein concentrate (SPC) is treated with deionized water, then adjusted to pH 9.0 with 2 N NaOH. The initial volume of the solution is kept at 1000 mL. Stirring for 2 h at room temperature, the slurry was processed in a colloid mill with an angular speed of 1000 r/m, using the 0.15 mm grinding hole. The slurry then centrifuged at 3000g using High-Speed Refrigerated Centrifuge, at 25°C for 10 min to remove the insoluble matter. It will then be subjected to the Jet Cooking (JC) system (Nan-Liang Food Machine Company Co. Ltd., Guangzhou, China) at different temperatures (120, 130 and 140°C) for 90 s. The JC treatment involved injecting steam into the protein slurry using a hydro-heater to achieve transient heating and high shear. A tube is placed after the hydro-heater to provide a 90-s residence time for the slurry. The slurry will then be cooled to room temperature and adjusted to pH 5.0, which is the working pH for the subsequent enzymatic treatment. Phytase (1000 U/g protein) and acid phosphatase (0.5 U/g protein) are added and the slurry incubated

at 50 °C for 2 h. After the pH was adjusted to 8.0, it will then be subjected to Ultrafiltration system that composed of a peristaltic pump and JM-S0522W hollow fiber membranes (Guangzhou Jason Membrane Technology Co., Ltd., China). Two polyethersulfone (PES) membranes with the molecular weight cut-offs (MWCO) of 10 and 30 kDa are used in the Ultrafiltration system or polystyrene (PS) membrane with a MWCO of 80 kDa. The total surface area of the membranes is usually 0.4 m<sup>2</sup> and transmembrane pressure is adjusted to 0.2 MPa. The UF process is conducted at room temperature and monitored using the volume concentration ratio (VCR) of 4. The total permeation volume of the UF treatments was 2000 mL. The resulting supernatant will be adjusted to a pH of 4.5 and centrifuged at 3000g at 25 °C for 15 min. The precipitate obtained using the novel method will be wash twice and re-dispersed in deionized water (1:7, w/v), and neutralized to pH 7.0 with 2 N NaOH. It will then be dialyzed against deionized water at 4°C for 48 h through a dialysis membrane with an 8–14 kDa/mol molecular weight cut off and lyophilized. (Yang et. al., 2014)

#### 4.0 COMPARISON BETWEEN THE METHODS

According to Maure *et. al.* 2006, “there is no unique methodology or protocol for extracting protein from seeds or flours leading to optimal products. But certain physico-chemical, thermal, and enzymatic technologies can be applied to seeds, meals, concentrates or isolates to obtain products with desirable properties for food

applications”. Also, Wu . and co-workers 2009 shows that functional properties of oilseed proteins concentrate and isolate such as solubility, water holding and oil binding capacity, foaming capacity, emulsifying capacity and stability as well as rheological properties can be modulated by carefully selecting both original seed and the operational variables during extraction (pH, temperature, solvent, presence of salts, ionic strength) so as to obtain the production of desired quality for a particular application.

In their paper, titled Effect of extraction method on functional properties of flaxseed protein, Tirgar and co-workers reported that the protein concentrates obtained using enzymes – solvent extraction method has significantly higher protein content of  $86.80 \pm 0.91\%$ , compared to alkaline filtration and enzymatic extraction methods with,  $51.05 \pm 0.62\%$  and  $65.08 \pm 0.40\%$  respectively.

Peanut protein concentrate prepared by alkali solution and isoelectric precipitation (ASIP) possessed better solubility and foaming capacity compared to other modes of preparations and hence could be suitable for application in food products requiring foaming like cake, bread, ice cream and so on. Although protein concentrate produced from ASIP method possessed higher foaming capacity this is significantly lower than that of protein isolate with foaming capacity of up to 50% and hence described by Wu, et. al., 2009, as the best foaming agent. Water holding and oil binding capacity are properties of protein concentrate suitable for use in food formulations such as dry mixes, baked food, whipped toppings and salad dressing (Wu H, et. al., 2009). Previous researches by (Wu

H, et. al., 2009, (Mao & Hua, 2012) have shown that proteins concentrates produced through alcohol precipitation has better water and oil holding capacity.

The emulsion capacity (EC) is defined as the maximum quantity of oil that can be distributed within a solution of emulsifiers without the emulsion being separated (McClements, 2007): Tirgar et. al., (2017). Also, Tirgar et. al., 2017 pointed out that emulsifying properties such as emulsion capacity, emulsifying activity and stability of Alkali and enzymatic-extraction provide an avenue for use in production of emulsion-based foods needing good emulsifying properties whereas enzymatic-solvent extraction could generate a good source of protein for a food system. Yang et. al., (2014) introduces a novel enzyme assisted jet cooking procedure which is well suited for the large-scale production of soy protein isolate.

## 5.0 CONCLUSION

From for going, it can be concluded that, different methods of isolation and characterization of protein concentrate and isolates exist. These methods vary majorly on the medium of extraction (ie either acidic or alkaline), presence or absence of enzyme and use size exclusion principle.

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