

A Comprehensive Review on Protein Isolates from Legumes



S.Reginold Jebitta, Durga Devi P. R, Deva Dharshini L, Theerdham Naga Sai Harika, Vignesh K.

Abstract: Legumes play a vital function in human body due to dietary fiber, protein, minerals and vitamins and well-balanced essential amino acid. Legume proteins have gained increasing significance because of preferred functional properties, including gelling and emulsifying properties. Legumes contains anti nutritional compounds like Trypsin inhibitor(TIs), Phytic acid(PA), Tannin, Saponin, Lectins, They are not a major concern for most people, but may become a problem during periods of malnutrition, these can be easily removed by dehulled, cooking, thermal process, germination after soaking. Protein isolates are advanced form of protein containing the greater amount of protein with greater digestibility. There are different types of protein isolates like chickpea, whey protein Pea protein, cowpea protein isolates .The extraction methods of protein isolates are Iso electric extraction and alkaline extraction, citric acid extraction. Our aim of this paper is to optimize the protein isolate for diet people and innovate research in this field to produce some protein enriched food formulations.

Keywords: Legumes; Protein Isolates; Extraction Techniques; Protein Isolates.

I. INTRODUCTION

Legumes play a vital role in human nutrition diet there are about 16,000 types grown all over the world in different sizes, shapes, colors, and textures. As they provide a good source of protein, thiamine, folic acid, vitamin E, and fiber. The insoluble fiber in legumes helps to lower blood cholesterol (Baloch and Zubair, 2010). It belongs to the family of Leguminosae that is also called Fabaceae. Grain legumes also called pulses, which are grown primarily for their edible seeds. These seeds are harvested mature and marketed dry to be used as food or feed or processed into various products (Khan et al., 2009). Legumes contain relatively low quantities of the essential amino acid methionine (which is found in higher amounts in grains).

Grains, on the other hand, contain relatively low quantities of the essential amino acid lysine (Sai-Ut et al., 2009). Which legumes contain this is why some vegetarian cultures – in order to get a good balance of amino acids needed for growth and repair – combine their diet of legumes with cereal grains. Common examples of such combinations are dhal with rice in India,

beans with corn tortillas in Mexico, tofu with rice in Asia and peanut butter with bread in the USA and Australia. (Anjum et al., 2005). All major cultures grew some type of legume. In Asia, red adzuki beans are crushed into a paste to make sweets. Black beans are popular in Mexico and Brazil. And you'll find white cannellini beans in many Italian dishes. There are many different types of beans and legumes and they all vary nutritionally. Nutritional values for legumes depend on the type. For example, a half-cup (86 grams) of cooked black beans (boiled with no salt) has to be 114 calories. 7.6 grams of protein, 20 grams of carbohydrates, 0.5 grams of fat, 0 milligrams of cholesterol as (Khattab and Arntfield, 2009).

Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients. Antinutrients that may have practical importance occur mainly in plant foods, especially in the hulls of cereal grains (bran), legumes and tea. The Food processing, such as fermentation, germination, malting, soaking and cooking can greatly reduce the amount of antinutrients in foods. Inhibitors of digestive enzymes, which prevent the digestion and hence absorption of certain nutrients: Phytate from whole grain cereals and legumes can reduce digestibility of proteins and carbohydrates. Alpha-glycosidase inhibitors inhibit the digestion of carbohydrates; acarbose is used as a glucose-lowering drug in diabetes mellitus. Amylase inhibitors, such as white bean extract, inhibit the digestion of carbohydrates, so they may reduce the absorption of glucose, but their role in weight loss or diabetes is uncertain. Lectins in legumes (black, Lima and kidney beans, soybeans and lentils) can inhibit carbohydrate digestion. Protease inhibitors, for example trypsin inhibitors in soy, sweet potatoes or raw potatoes, inhibit the digestion of proteins. Lipase inhibitors, for example, orlistat—the anti-obesity drug—inhibit the digestion of fats. The chelating agents of the antinutrients in legumes are dietary fibers, Phytic acid, polyphenols and soy protein isolate, calcium and zinc is used to bind to nutrients in the intestine and prevent their absorption. The beneficial effects in legumes are nutritional quality have been shown on soaking, cooking, boiling, germination, roasting and dehulling and also there are various processing methods like hydration is used to eliminate the antinutrients through heat like treatment (Shimelis and Rakhshit, 2007).

Manuscript received on March 10, 2020.
Revised Manuscript received on March 22, 2021.
Manuscript published on March 30, 2021.

S.Reginold Jebitta, Department of Food Technology, Kalasalinagm Academy of Research and Education, Srivilliputtur (Tamil Nadu), India.

Durga Devi P. R., Department of Food Technology, Kalasalinagm Academy of Research and Education, Srivilliputtur (Tamil Nadu), India.

Deva Dharshini L., Department of Food Technology, Kalasalinagm Academy of Research and Education, Srivilliputtur (Tamil Nadu), India.

Theerdham Naga Sai Harika, Department of Food Technology, Kalasalinagm Academy of Research and Education, Srivilliputtur (Tamil Nadu), India.

Vignesh K., Department of Food Technology, Kalasalinagm Academy of Research and Education, Srivilliputtur (Tamil Nadu), India.

© The Authors. Published by Blue Eyes Intelligence Engineering and Sciences Publication (BEIESP). This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Mostly isolated protein used for plant based food which they enhance the nutritional value (Agbede et al., 2005). Rangel et al., (2004) recognized that cowpea protein isolates (CPI) was done by isoelectric precipitation from defatted cowpea meal. The Proteins content of isolated protein from chickpea flour by isoelectric precipitation technique ranged from 84.8- 87.8% (Paredes-Lopez et al., 2006).

In addition to the protein isolates with meek flavor are used in various uses in the field of food industry, medical industry (Davis, 2004). Heat treatment like boiling, slow cooking in white bean and chickpea is used to extract the protein (Arcan and Yemenicioğlu, 2007).

II. PROTEIN ISOLATES FROM LEGUMES:

Isolates are a most refined form of protein it contains the greatest amount of protein but it does not contain the dietary fiber. Isolates are originated around 1950 (Jay and Michel 2004). Protein isolate is the final form of protein containing a greater amount of protein with greater digestibility. The major source of protein was isolated protein is now a day's, especially for the body builder's vegetarian athletics. It is a very cheap protein with wide applications in the beverages and dairy industry. It is widely produced from the peanut, cowpea, soybean, by the isoelectric and alkaline method so the pure form of isolates of 90% protein produced.

2.1 WHEY PROTEIN ISOLATES(WPIs):

Whey is the liquid by-product of cheese products which can be processed into a spray dried products further like whey protein concentrates (WPC), whey protein isolate (WPI) or whey protein hydrolysates (WPH) (Brucic et al. 2009). During cheese manufacturing, the whey protein remains in the liquid phase which represents about 20 percent of the milk protein (Kimberlee 2012). Whey proteins are widely used as ingredients in different foods (dairy, meat and bakery products) due to their unique functional and nutritional properties (Brucic et al. 2009).

2.2 PEANUT PROTEIN ISOLATES(PPIs):

Peanut contains 26-29% of protein with smart organic process quality. Peanut proteins are used for useful properties like emulsification, forming and organic process properties in numerous Food merchandise. They're additionally used for human nutrition in developing countries to supplement cereals, beverages and skimmed milk. Peanut protein isolate is ready from the defatted peanut cake or pulverized by macerating with high salt phosphate buffer (20 Na₂HPO₄, a pair of millimeter KH₂PO₄, 5.4 mM KCl, 1M NaCl, pH 7.4), followed by natural process and supplementing the supernatant with (NH₄)₂SO₄ to 90% of saturation. Once natural process, the pellet is separate against water long at 40C and freeze-dried (Mouecoucou 2004).

2.3.CANOLA PROTEIN ISOLATES (CPIS):

Canola meal has been the second largest feed meal after soybean flour. It's an honest amino acid profile with a well balance amino acid composition though it's found solely marginal employed in the food business, thanks to the presence of anti-nutritional factors. The overwhelming majority of canola protein isolates are ready by alkaline extraction technique followed by isoelectric precipitation.

Although, this extraction technique generates high yield of protein isolates, however the isolate created by this technique are found to own poor solubility and digestively, this is often likely thanks to the character of proteins constituent of canola meal that comprises alkaline-soluble fraction which will be simply changed throughout the extraction method. (Alashi 2011).

2.4.APPLICATIONS OF PROTEIN ISOLATES:

Protein isolate used for emulsion stability and its color flavor are critical in the dairy industry.(Kudre 2013). Isolates up being used to fortify all types of pasta products such as macaroni spaghetti. They are used as a Proteinaceous ingredient in many food products. Whey protein isolate was used in beverage applications. (Kudre 2013).It was also used for vegetarian meat analogs example soya protein. Protein isolates were used in baking applications due to their water and fat absorption property type (Sipos 2013).

III. EXTRACTION TECHNIQUES FROM LEGUMES:

Presently, legume extraction and fractionation technologies have been developed mainly to extract proteins and, to a much lesser extent, other molecules (e.g., fibers or phenols) from legume seeds. These technologies have been demonstrated to also apply to legume by-products and wastes (Kamani M.H., 2019). Protein extraction can be carried out either through dry or wet processing. Dry fractionation seems to be the most promising technology for protein extraction from legume seeds and it represents a good alternative to water extraction because it preserves protein functional (Schutyser M., Ity; 2011). Wet processing technologies generally provide flours with higher protein purity than dry processing. Among these technologies, alkaline/acid, solvent, and enzymatic extractions and the use of ultra filtration membranes were the most applied processes to legumes seeds and fractions (Brummer Y., Kaviani M., 2015).

3.1 ALKALINE/ACID ISOELECTRIC PRECIPITATION:

Proteins are first dissolved in alkaline (alkaline extraction) or acidic (acid extraction) conditions, followed by a clarification step and then precipitation by adjusting the pH to the isoelectric point (pI) of the protein. In solutions pH < pI, assume that the proteins are net positive charge, whereas at PHS > pI s assume that the proteins are net negative charge. Near the pI value, proteins are likely to carry a neutral net charge, allowing neighboring proteins to aggregate via attractive van der Waals forces and hydrophobic interactions. Under these conditions, protein-protein interactions are favored over protein-water interactions, and thus protein is precipitated out of the solution. Studies were carried out by Kaur & Singh (2007), Ghribi et al. (2015), and Papalamprou et al. (2009).

Where chickpea flour was mixed with distilled water and pH was brought to 9 with 0.1 M NaOH. Proteins were precipitated out of solution at a pH level of 4.5 with 1 N HCl. Values of 90% to 94%, 91% to 92.7%, and 92.5% were reported for protein content by these groups of authors respectively. The strong bases and acids used in this combined process lead to the accumulation of salts and an increase in the ash content of the final pea isolate (Karaca, Low, and Nickerson, 2011). Reinkensmeier et al. (2015) precipitated yellow pea proteins out of solution after acid extraction of pea flour at a pH of 1.5 followed by acid precipitation and produced an isolate with a protein content of 81.2%.

3.2 ACID EXTRACTION:

Acid extraction (in principle kind of like base-forming extraction) involves the preliminary extraction of proteins beneath acidic conditions.

This method may lead to high solubilization of supermolecules before protein recovery (IEP, Ultrafiltration (UF)), as proteins tend to be additional soluble beneath acidic conditions (pH below 4).

At hydrogen ion concentration <4 (acidic condition), the solubility of bean proteins inflated, creating it attainable to solubilize proteins before their recovery by isoelectric precipitation, cryo-precipitation, or membrane separation (Boye et al., 2010).

3.3 ULTRAFILTRATION/DIAFILTRATION METHODS:

Membrane separation ways were shown to supply supermolecule isolates with higher practicality and were effective in reducing levels of anti-nutritional parts that embody peptidase and enzyme inhibitors, lectins, and polyphenols. UF and microfiltration square measure membrane-based fractionation ways mistreatment pressure because the driving. For the preparation of supermolecule materials mistreatment immoderate filtration, the supernatant when base-forming or acidic extraction is processed mistreatment either UF or diafiltration (DF) along to isolate the supermolecule material. UF is commonly combined with DF to enhance supermolecule recovery, wherever water is adsorptitious to the retentate for dilution functions, followed by re-ultra filtration force for separation. immoderate filtration removes similar particles within the vary of zero.001–0.02 μm. Boye et al. evaluated the supermolecule content of isolates obtained from completely different pulses (pea, chickpea, and lentil) mistreatment base-forming extraction-IEP and UF/DF extraction ways. The supermolecule content in concentrates obtained by the UF/DF methodology was found to be above in those obtained by IEP.

3.4 SALT EXTRACTION:

Salt extraction is followed by the suitable supermolecule concentration and desalting methodology. h the foremost common salts being ammonia sulphate and salt (Singhal et al., 2016). the bulk of hydrophobic moieties square measure buried within the quaternary or tertiary structure thanks to a hydrophobic result, and also the majority of deliquescent moieties square measure on the surface, liberated to participate in protein-water interactions. 'Salting-in' of proteins usually happens at low salt levels, wherever the ions act to extend the order of the protein's association

layers and promote protein-water interactions. However, at high levels of salt, association layers will be noncontinuous as ion-water interactions become favored over protein-water interactions in a very 'salting-out' method. Salts shaped between cations and anions with higher precipitation tend to decrease the solubility of non-polar amino acids, pro hydrophobic interactions to 'salt-out' proteins. Salts shaped between cations and anions with lower precipitation; tend to higher the solubility of non polar amino acids, pro hydrophobic interactions to salt in proteins. Alsohaimy et al. (2007) prepared supermolecule isolates from chickpea, lupin, and lentils mistreatment IEP and ammonia sulphate precipitation. For all of those legumes, the latter methodology resulted in higher supermolecule content (chickpea – ninety.6%, lupin – 92.6%, and lentil – ninety three.0%) as compared to the previous methodology (chickpea – eighty one.4%, lupin – 87.3%, and lentil – eighty.0%).

3.5 WATER EXTRACTION:

Proteins will be extracted directly with water at a neutral hydrogen ion concentration as a result of they're soluble in water. The pure water extraction method isn't a standard technique, and this could be attributed to its inability to solubilize heaps of globulins and thus as several proteins because the alternative ways. Values of hour to sixty seven were conjointly reported for water-extracted chickpea and faba bean mistreatment CaSO₄ as a coagulator to isolate proteins out of resolution (Cai, Klamczynska, and Baik, 2001). In each of the ways utilized by these authors, the extraction method was done double to extend yield. These square measure the sole samples of water extractions performed in literature and extractions were performed with H₂O at temperature with vigorous agitation.

IV. SOY PROTEIN ISOLATES:

As a important components of soya bean Protein is rich in essential amino acids and have high nutritional value. Function properties of soybean have solubility, emulsification, gelation and foaming. Soya bean plays important role in animal diets in many Oriental countries due to its low cost and high nutritional value.. The soybean contains High in fiber, high in protein, low in saturated fat, cholesterol free, lactose free, a good source of omega 3 fatty acids, a source of antioxidants, high in phyto estrogens. In additional soybean have lipids 20 percentage, water 10 percentage, minerals such as iron, copper, magnesium calcium, manganese, zinc, cobalt and potassium, Vitamin such as thiamin and riboflavin.

4.1 METHODS OF ISOLATION:

Soaking of the soya bean, grinding in cold water, filtering, cooking at the 93 to 100 degree Celsius for 30 minutes. This method would be modified by grinding in hot water which has the advantage of LOX inactivation. (Imran et al., 2003). Mechanical extraction can also be employed however compare to solvent extraction, the oil yield is most as lucrative. Defatted soy flour refers to the same material as defatted soybean flakes, but with a finer particle size. It can be used as a feed, yet more value can be created when the protein are extracted from it. To produce soy protein concentration, defatted soybean flakes are added to alcohol or water to remove carbohydrates.



Soybean protein isolates containing 19-2.36% of protein 0.51 % of at 1.49 % of ash and the 4.52 % of moisture was provided by have been high tech limited. 3.Soy protein hydrolysates was prepared from local ingredients. To be specific, the soybeans, following a specified water /material ratio ranging from 1:2 to 1:5, were cooked in pressure cookery (120 degree, 1.2 atm), and main tained at this temperature for 10, 15, 20, 25, and 30 min. The ratio between beans and water, as well as the time of thermal pre-treatment giving the highest protein recovery efficiency, were chosen for the optimization study. During the boiling, the pot was covered in order to prevent water loss. The total protein concentration was determined by Lowry assay, based on the color change of the sample solution in proportion to the protein concentration, which can be measured by using a UV-Vis Spectrophotometer.

4.2. Flow Chart

Method 1:

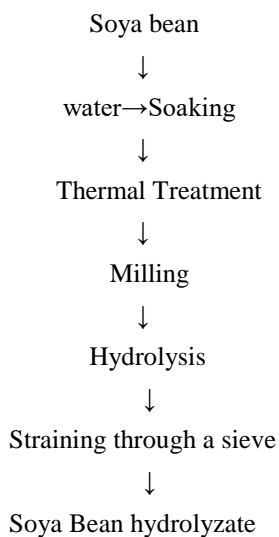


Figure 1.1 Soy Protein Isolation Method One

Method 2:

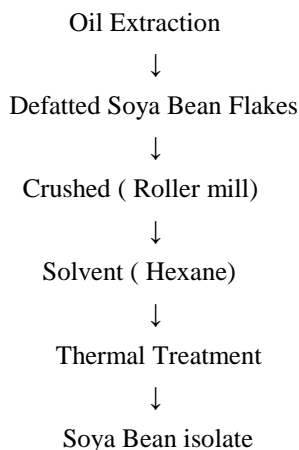


Figure 1.2 Soy Protein Isolation Method Two

5.1.PEA MACROMOLECULES PROTEIN:

Whole peas were dehulled employing a stake Grain Testing Mill and clean of loose Hull by aspiration. Dehulled peas were then polished by a cyclone sample Mill fitted with a one millimetre screen. before macromolecule extraction, all flours were defatted exploitation dissolver. In brief, meal was mixed with hexane(1:3.w/v) for forty min employing a magnetic stir plate at five hundred rate followed by

decanting of the dissolver. This method was then continual 2 extra times. once the ultimate defatting, the mixture was filtered through whatman paper then air dried for ~18 h in a very fumehood. The defatted flour was hold on at 4°C.(Andrea K. Stone, 2014). Whole dry peas were ground into a sixty mesh flour employing a Cyclone Sample Mill. macromolecule isolates were ready. Dispersions of 100 percent (w/v) meal in water were adjusted to pH scale eight.5 with 1N NaOH, and mixed with a magnetic stirrer at close temperature (25°C) for thirty min at 10°C. so as to get magnified yields, the extraction and natural process procedures were continual on the residue. The extracts were combined and also the pH scale adjusted to four.5 with 1N HCl to precipitate the macromolecule. The macromolecule was recovered by natural process at 1570 X g for ten min at 10°C followed by removal of the supernatant by decantation. The surface of the ensuing macromolecule curd was washed with H2O and also the curd was re-dispersed in H2O. the typical yield of macromolecule isolated from the meal was a pair of.75 g protein/20 g flour.(E. A. JOHNSON, 2012)

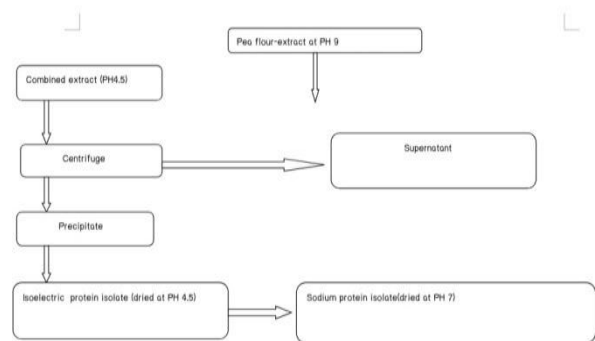


Figure 1.3 Pea Protein Isolates Flow Chat

V. CHICKPEA MACROMOLECULE ISOLATES:

The assembly of legume macromolecule concentrates or isolates is of growing interest to food business thanks to their practical properties and skill to boost the nutritional quality of food.

6.1 METHODS OF ISOLATION:

Dispersions of defatted chickpea flours(5% weight/volume) in H2O with pH scale vary nine with zero.1N NaoH at temperature (30°C),then agitated for one hour and centrifuge at 8000g for fifteen min. so as to get magnified yields, the extraction and {centrifugation|natural method|natural action|action|activity} process were continual. and also the extracts were combined and also the pH scale adjusted to four.5 with 1N HCL to precipitate the macromolecule (M. Kaur, 2007). For the Micellization procedure, Na chloride(0.5m and pH scale seven.0) was accustomed extract proteins from defatted flour (10% weight /volume). This extract was targeted to [*fr1] volume by ultrafiltration in a very pelicon equipment. Proteins were flocculated by adding water at 4°C at pH scale seven.0 that is that the usual pH scale for Micelization. Cold water has been reportable to boost potency of macromolecule natural action.



The isolate were recovered by natural process at ten000*g for 10 min and freeze dried then particle macromolecule isolates. (o. Paredes-Lopez, c. Ordorica-falomir, 2008). Dehulled water-soaked chickpeas were polished with 2 volumes (w/v) of deionised water in a very Waring mixer at medium speed for thirty s then at high speed for an additional thirty s. The ensuing suspension was mixed with an oversized quantity of deionised water and wet sieved by passing it through 50-, 100- and 200-mesh Tyler screens to separate the fibrous residues. The collected filtrate suspension containing starch and macromolecule was then centrifuged at 3000 × g for ten min at four four. The fibrous residues were re-extracted 2 additional times with one weight unit weight unit NaoH answer (pH 8) underneath similar conditions. Then all alkali extracts and supernatant remaining from starch separation were pooled, the pH scale was adjusted to four.3 (pI for chickpea5) with one weight unit weight unit HCL and also the mixture was maintained at four four for one h. once proteins had been precipitated, the mixture was centrifuged at 4000 × g for twenty min. The sediment was washed doubly with 2 volumes (w/v) of deionised water. The precipitated macromolecule was redispersed in deionised water and also the pH scale was adjusted to seven with one weight unit weight unit NaOH before desiccation. The freeze-dried macromolecule isolates were hold on in airtight instrumentality.

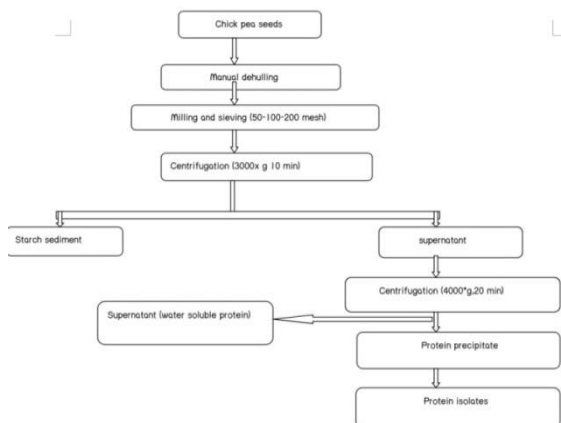


Figure 1.4 Chickpea Protein Isolates

VI. COWPEA PROTEIN ISOLATES:

Cowpea (*Vigncentrifuga unguiculata*) is also called Black pea. It is a tropical legume widely consumed by the population of Africa and the northeast. It is low in anti-nutritional factors. over the last 30 years, the usage of the concentrated and isolated protein from the plants has been increased gradually. Cowpea has an annual Global production of 12.5 million tons (Huang et al, 2012) grow well in the diverse range of condition and environmental and contains only moderate level of bioactive and anti-nutritional factors. It contains a high amount of protein which was nearly 22.9%-77% and low-fat content like 1.4% and high carbohydrate 61% (Rukmana, oesman).

7.1. METHODS FOR PROTEIN ISOLATES EXTRACTION FROM COWPEA

7.1.1.OKEZIE AND BELLO DESCRIBED METHOD:

70 grams of flour was mixed in 1400 ml of water is mixed to form a 1: 2 ratio of slurry. The solution pH is 6.7 and was allowed to settle for 3 hours for segmentation. The spend Residue was separated from dissolved protein extract by centrifuge. The pH was adjusted with HCl at 4.2-4.3. The

supernatant was collected by centrifugation and freeze-dried.

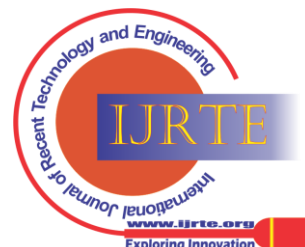
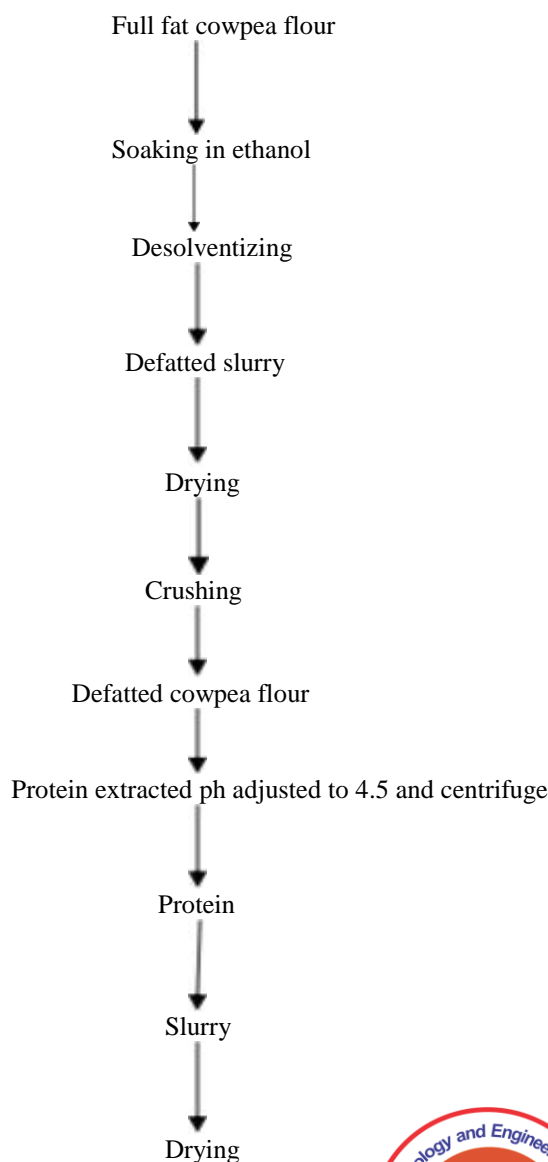
7.1.2. ISOELECTRIC ISOLATION METHOD USING ETHANOL (Lopez and Ordorica-Falomir (2012)

Cowpea was soaked for 5 minutes then dehulled the skin and blended the cowpea with ethanol of 6:2 to ratio then add 1N of NaOH and incubated at 55 degree Celsius at 30 minutes. Centrifuge these materials at the 200 RPM at 10 minutes then collect supernatant and at 1N of HCL and again centrifuged at a speed of 200 RPM at 10 minutes then the supernatant was freeze-dried and used as an isolator of protein.

7.1.3. JOHNSON AND BREKKE METHOD:

Cowpea flour was mixed with 20 ml of distilled water and makes a suspension with PH 8 and 0.1N of NaOH was added. This slurry was mixed with a magnetic stirrer for 1 hour and 25 degrees Celsius and at 0.1 NAOH with continuously stirring. Then the slurry was centrifuged at 2000× g for 30 minutes and then the supernatant was filtered by Whitman Paper .the pH was adjusted to 4.5 with 0.1 ml of HCL. then the mixture was isolated by centrifuge 2000 ×g for 30 minutes and was distilled and centrifuged again these will be freeze-dried and used as protein isolate

7.1.4. FLOW CHART:



PHYSICAL PROCESSES	HOW IT'S DONE
BLANCHING	Mild boiling (75 °C – 95 °C) to inactivate endogenous enzymes and avoid cooking.
AUTOCLAVING	Heating at ultrahigh temperatures (>100 °C). Performance is dependent on temperature, moisture, pressure relations.
ORDINARY COOKING	Usually preceded by soaking or another domestic processing, de-hulling, germination fermentation, and so on.
EXTRUSION	A form of high-temperature short time (HTST) processing involving a combination of high temperature, pressure, and sheer processing.
SOAKING	Exposure to water and salt solutions with or without additives to encourage ANF loss.
ROASTING	Dry heating @120°C-250°C
PROCESSING USING CHEMICAL METHODS	Treatment with thiols, calcium salts, sulfites

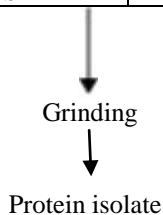


Figure 1.5. Cowpea protein isolation

VII. ANTINUTRIENTS FROM LEGUMES:

Legume grains contain fascinating levels of ingredients that may enhance organic process quality like high supermolecule concentration, potassium, fiber, and low glycemic index.

Consumption of legume seeds is believed to possess a robust impact on pressure level reduction whereas conferring inhibitor edges (Polak et al. 2015; Vaz Patto et al. 2015). the most drawback related to legumes has the presence of antinutrient factors like enzyme matter, antiviral, Phytic Acid, Saponin, Tannins,

PolyPhenol. many Antinutrients have shown to a useful Properties[Anticancer, Antimicrobial]. this kind of attention-grabbing compounds could increase the chance within the field of medication, materia medica, and nutrition. In Antinutrients, area unit they're completely different process techniques are needed to get rid of or eliminate the antinutritional factors. The physical and chemical ways embody Soaking, Cooking, Germination, Fermentation, Selective Extrusion, Irradiation, and protein Treatment. These food process techniques scale back anti-nutritional factors, increase supermolecule edibility and improve the biological worth of cereal crops (Handa et al. 2017; Jaybhaye and Srivastav 2015). In industrial process, the antinutrient has been reduced by the strategy of canning, toasting, and fractionation. Webersax et. al(2003) instructed that the process techniques of soaking and heating have improved the edibility characteristics of legumes in 2 ways(1) Denaturation is employed to create the supermolecule is to be a lot of vulnerable to the mode of

protein action.

(2) By applying thermal treatment, enzyme matter was destroyed, and conjointly it enhances the organic process worth of food. the supply of essential amino acid and different amino acids conjointly will increase.

Figure 1.6: physical processing method from legume.

8.1 ANTINUTRIENT FACTOR FROM LEGUMES:

8.1.1.PHYTATES/PHYTIC ACID:

Phytate is generally seen in roots, tubers. Phytate is mostly referred to as myoinositol-1,2,3,4,5,6-hexakis dihydrogen phosphate, that should be gift in foods at numerous levels starting from zero.1 to 6.0% (Gupta et al. 2015). The phytic acid content ranges in cereals and legumes square measure zero.14%-2.05%.(N.R.Reddy et, al;2005). it's the principal storage variety of phosphorus in plant structure, particularly in bran and seeds. catabolites of phytic acid are known as B polyphosphates. Generally, it had been found in legumes at the stage of Harvest. By removing the bran layer or aleurone(PA is gift during this layer) has been permitting the straightforward separation or edge methodology. In dicotyledons like legumes, oilseeds, and nuts, phytates square measure found in shut association with proteins, that reduces the convenience of separation by a straightforward process methodology like edge.Therefore, to avoid the negative consequences of phytic acid is very important to grasp the toxicity nature of food and therefore the degree of toxicity in food.

8.1.2. TANNIN:

Tannic acid is one in every of the many antinutritional factors gift in dry beans. it's chiefly situated within the episperm. The tannic acid content of dry bean having the vary from zero.0-2.0%.it was strictly reckoning on the kind, species, and color of the legumes. The mass from 500-3000 Da(Dalton).one of the vital property of those compounds was precipitate the supermolecule. Proteins, that usually type complexes with tannins square measure comparatively massive and hydrophobic, additionally to Associate in Nursing open and versatile structure that's enriched with aminoalkanoic acid (Frutos et al. 2004). In tannin, there square measure 2 sorts of tannic acid groups: reaction (e.g. gallotannins and ellagitannins) and condensed (e.g. proanthocyanidins). In ruminants, the reaction tannic acid was belonged to the family of glycosides and conjointly known as tannins. it's a gaggle of polyester, carbohydrates, and acid, which derivatives like digallic acid. This tannic acid was without delay de-escalated throughout the digestion method.The non-hydrolyzable tannic acid was conjointly known as condensed tannic acid, it doesn't contain sugar compounds and it's turned to polymerize and insoluble product. tannic acid chiefly focuses on the bran section of legumes. once eaten, tannins type complexes with proteins, that cause the inactivation of the many biological process enzymes and reduce supermolecule edibility (Joye 2019). Mechanical dehulling nearly quantitatively take away the condensed tannic acid (Deshpande;2005) was according the isolated, characterised, and pure the condensed tannic acid from faba bean.



8.1.3. SAPONIN:

Glucoside could be a present compound in legumes. the power was to create stable, soap like foams in binary compound solutions. once glucoside encompasses a high concentration, it had been bitter. the matter related to glucoside was to degrade the organic process absorption capability. By the character of this compounds were simply bind with the minerals (eg) Iron, zinc, and atomic number 20.

All glucoside has not noxious, seldom a couple of saponins were smart for health(steroids, triterpene). principally glucoside cluster was able to act with the steroid alcohol cluster of the red blood cell membrane, that ends up in hemolysis(Fleck et. al;2019). it's been shown the repressive activities of biological process enzymes like enzyme, glycosidase, trypsin, chymotrypsin therefore this protein causes symptom connected health disorders(Alie et al;2006); The glucoside content encompasses a low level in legumes suggests that it couldn't be injurious to health, however it gave some adverse effects.

8.1.4. TRYPSIN INHIBITOR:

Legumes (TIs) square measure classified into two families in keeping with their molecular size: Kunitz (KTIs), with molecular weights around twenty kDa, and Bowman-Birk (BBTIs) of roughly eight kDa. transient ischemic attack is focused principally within the seed leaf (>90%), for different legumes like chickpea they're distributed within the three characteristic anatomical elements, seed leaf (77.2% to 75.8%), embryonic axis (11.9% to 15.5%), and episperm (10.9% to 8.7%) (Sreerama et al 2010; city et al et al et al 2015). Bowman-Brik(BBTIs) and Kunitz inhibitor(KTIs) square measure sorts of TIs inhibitors. The KTIs have inhibited the antiviral and it's been isolated and characterised. KTIs shaped the 2 disulfide bridges. Compare to the quality methodology of KTIs, there was no differentiation between BBTIs and KTIs.The development of Enzyme-linked immune assay(ELISA). This has been accustomed denaturation of supermolecule by heat or pH scale against the molecules with no cross-reactivity. In BBTIs, consists of an oversized quantity of aminoalkanoic acid, therefore it shaped the Seven disulfide bridge. The inactivation ways of enzyme matter (TIs) have 3 varieties they're physical processes, chemical processes, and biological process.

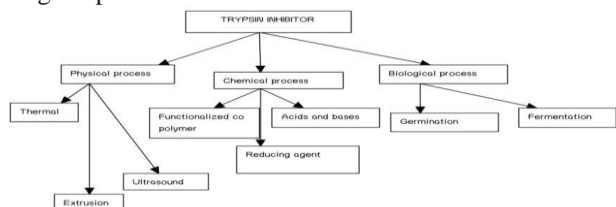


Figure 1.7 Types of Trypsin Inhibitor

8.1.5. LECTINS :

Lectins were the first toxic factor in the legume. It has easily bound with certain carbohydrates or Sugar. It has been determined in more than 800 varieties including 600 genera of legumes. The total protein content of legume seeds contains 2-10% lectins or Hemagglutinin. It has been found in plant species such as "Wheat, Bran, quinoa and peas" etc. Most of the plant lectins are more toxic. In that case of the source has poison rich (eg) a Lectin from the castor oil plant which could be lethal. Lectins belong to the nightshade

family. The Researchers noted that the reduction of protein digestibility and affect the growth inhibitory Hormone, So it depressing the appetite. When we use Purified Lectins from various legumes that added the source to animal diet. If we used heat processing techniques, It has been shown some adverse effects could be overcome. In the beginning period, the antinutritional and toxic content was removed partially or fully eliminated by heat treatments and it has shown some conventional cooking methods such as Pressure Cooking, Boiling, and Simmering. Raw beans have higher amounts of lectins, consumption of raw beans thus may cause abdominal cramps (El-Adawy 2002; Mubarak 2005).

VIII. CONCLUSION:

From a technical point of view, these studies have demonstrated that n legume seeds such as peas, chick pea and cowpea can be used as protein sources in the form of isolate and concentrate. Protein isolates extraction method demonstrates that the isolates have higher content of protein then the normal protein. Wet processes, including acid precipitation as well as ultra filtration, have also been processes shown to be technically easy to develop. The isolates of all legumes have justified that it does not antinutritional factors after physical processing method. Pea and cowpea isolate functionality behaviour and protein content also seems to be partially different from that of soybean isolates. Further work has to be done in this area to determine the most useful specific applications of these new isolates compared to those of soybean. Studies will also be necessary to evaluate the marketability of these new products made of the legumes In conclusion, the feasibility of producing protein isolates from legume is related to the particular functional properties of the isolates compared to soybean isolates and to the marketability of the by-products. For these reasons, in addition to the studies carried out on the isolate processing and on the physiochemical and functional properties of proteins.

REFERENCES:

1. Peter H.G. and Carroll P. V. (2003) Legumes: Importance and Constraints to greater use.Plant Physiology.
2. Allen O. N. and Allen E. K. (1981) In leguminosae. A source book of characteristics, uses and Nodulation.
3. Duranti M. (2006) Grain legumes proteins and nutraceutical properties.Fitoterapia
4. Pitchford P. (1993) Healing with whole foods. 3rd edition, North Atlantic Books, California.
5. Khalil, I. A., & Durani, F. R. (1989). Nutritional evaluation of tropical legume and cereal forages grown in Pakistan.
6. Tropical Agriculture (Trinidad), 67,. Khalil, I. A., & Durani, F. R. (1990). Haulm and Hull of peas as a protein source in animal feed. Sarhad Journal of Agriculture, 6,. Khalil, I. A., & Manan, F. (1990). Chemistry-one (Bio-analytical chemistry) (2nd ed.). Peshawar: Taj kutab Khana. Khalil, I. A. (1994).
7. Nutritional yield and protein quality of lentil (Lens culinaris Med.) cultivars. Microbiologie Aliments Nutrition., NRC (1980).
8. Recommended Dietary Allowance (9th ed.). Food and Nutrition Board NRC. Washington, DC, USA: National Academy of Sciences. Raghuvanshi, R. S., Shukla, P., & Sharma, S. (1994).
9. Nutritional quality and cooking time tests of lentil. Indian Journal of Pulses Research, Zarkdas, C. G., Yu, Z., Voldeng, H. K., & Minero-Amador, A. (1993). Assessment of the protein quality of new high protein Soybean Cultivar by amino acid analysis. Journal of Agricultural and Food Chemistry.



10. M.S.Butt, R.Batool; Nutritional and functional properties of some promising legumes protein isolates. *Pakistan Journal of Nutrition*, 9(4), 373ñ379 (2010).
11. N.J.Enwere; Foods of plant origin. Afro-Orbis Publishers, Nsukka 124ñ145 (1998).
12. N.J.Enwere, P.O.Ngoddy; Effect of heat treatment on selected functional properties of cowpea flour. *Trop.Sci.* 26, (1986).
13. Canella M, Castriotta G, Bernardi A (1979) Functional and physicochemical properties of succinylated and acetylated sunflower proteins. *Lebens Wiss. University u-Technology* 12: 95.
14. Horax R, Hettiarachchy NS, Chen P, Jalaluddin M (2004) Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). *J Food Sci* 69: fct114-fct118.
15. Abbey BW, Ibeh GO (1988) Functional properties of raw and heat processed cowpea (*Vigna unguiculata*, walp) flour. *J Food Sci.*
16. Aluko RE & Yada RY (1993): Relationship of hydrophobicity and solubility with some functional properties of cowpea (*Kgna unguiculata*) protein isolate. *J. Sci. Food Agric.*
17. Aluko RE & Yada RY (1995): Structure-function relationships of cowpea (*Kgna unguiculata*) globulin isolate. Influence of pH and NaCl on physicochemical and functional properties. *Food Chem.*
18. Demchenko AP (1986): *Ultraviolet Spectroscopy of Proteins*. Heidelberg: Springer.
19. Gibrat R & Grignon C (1982): Measurement of the quantum yield of 8-anilino-1-naphthalene sulphonate bonds on plant microsomes. Critical application of the method of Weber and Young. *Biochim Biophys Acta* 691.
20. S.S. Jha, D Ohri. [2002] Comparative study of seed protein profiles in genus *Pisum*. *Biologia Plantarum*.
21. Sammour R. H., [1991] Using electrophoresis techniques in varietal identification, biosystematic analysis, phylogenetic relations and genetic resources management. *Journal of Islamic Academy of Sciences*.
22. Arulsekhar, S. and D. E. Parfitt, [1986] Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. *Hortscience*.
23. Bradford, M.M. [1976] A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann Biochem*.
24. Laemmli, U.K. [1970] Cleavage of structure proteins assembly of the head of bacteriophage T4. *Nature*.
25. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY.
26. Blum, H., Beier, H. and Gross, H.J. [1987] Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis*.
27. Lecomte N, Zayas J, Kastner C. Soya proteins functional and sensory characteristics improved in comminuted meats. *Journal of Food Science*. 1993.
28. Can Karaca A, Low N, Nickerson M. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*. 2011.
29. Gupta R, Dhillon S. Characterization of seed storage proteins of Lentil (*Lens culinaris* M.). *Annals of Biology*. 1993.
30. Saharan K, Khetarpaul N. Protein quality traits of vegetable and field peas: Varietal differences. *Plant Foods for Human Nutrition*. 1994.
31. Osborne TB. The vegetable proteins. *Monographs in Biochemistry*. London: Longmans, Green and Co.; 1924.
32. Oomah BD, Patras A, Rawson A, Singh N, Compos-Vega R. Chemistry of Pulses. In: Tiwari BK, Gowen A, Mckenna B, editors. *Pulse Foods Processing: Quality & Nutritional Applications*.
33. Hu HY, Pereira J, Xing LJ, Zhou GH and Zhang WG, Thermal gelation and microstructural properties of myofibrillar protein gel with the incorporation of regenerated cellulose. *LWT - Food Sci Technology* (2017).
34. Lin DQ, Zhang LT, Li RJ, Zheng BD, Rea MC and Miao S, Effect of plant protein mixtures on the microstructure and rheological properties of myofibrillar protein gel derived from red sea bream (*Pagrosomus major*). *Food Hydrocolloids* (2019).
35. Pan LH, Feng MQ, Sun J, Chen X and Xu XL, Thermal gelling properties and mechanism of porcine myofibrillar protein containing flaxseed gum at various pH values. *CyTA - J Food* (2016).
36. Zhao YY, Zhou GH and Zhang WG, Effects of regenerated cellulose fiber on the characteristics of myofibrillar protein gels. *Carbohydrate Polym* 209:276-281. (2019).
37. Siddiq M and Uebersax MA. Dry beans and pulses production and consumption—An overview. *Dry Beans and Pulses Production, Processing and Nutrition*(2012)
38. Paglarini CDS, Martini S and Pollonio MAR, Using emulsion gels made with sonicated soy protein isolate dispersions to replace fat in frankfurters. *LWT -Food Sci Technology* (2019).
39. Gao XQ, Zhang WG and Zhou GH, Emulsion stability, thermorheology and quality characteristics of ground pork patties prepared with soy protein isolate and carrageenan. *J Sci Food Agriculture* (2015).
40. Lee HC, Jang HS, Kang I and Chin KB, Effect of red bean protein isolate and salt levels on pork myofibrillar protein gels mediated by microbial transglutaminase. *LWT - Food Sci Technology* (2016).
41. Kaur M and Singh N, Studies on functional, thermal and pasting properties of flours from different chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry*
42. chicken meatballs during frozen storage. *J Food Science Technology*(2015).
43. Verma AK, Banerjee R and Sharma BD, Quality of low fat chicken nuggets: effect of sodium chloride replacement and added chickpea (*Cicer arietinum* L.) hull flour. *Asian Australasian J Animal Science* (2012).
44. Siddhuraju, P., Vijayakumari, K., & Janardhanan, K. (1996). Chemical composition and nutritional evaluation of an underexploited legume, *Acacia nilotica* (L.) Del. *Food Chemistry*, 57(3), 385–391.
45. Association of Official Analytical Chemists (1980). *Official Methods of Analysis*, 13th edn. Washing, DC.
46. Association of Vitamin Chemists (1966). *Methods of Vitamin Assay*, 3rd edn. InterScience Publishers, New York.
47. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). *Anal Chem*.
48. Hussain, M. A., Akinyele, I. O. & Omololu, A. (1984). Perceptions of mothers on medical problems associated with cowpea consumption. *Pro. And Abstract Worm Cowpea Research Conf. HTA*, pp.
49. Kitson, R. E. & Mellon, M. G. (1944). Colorimetric determination of phosphorus as molybdivanadiphosphoric acid. *Ind. and Eng. Chem.*, 16
50. Mehta, V., Jood, S. & Bhat, C. M. (1985). Effect of processing on flatus producing factors in legumes. *J. Agric. Food Chem*.
51. Vose, J. R. (1980). Production and functionality of starches and protein isolates from legume seeds (field peas and horse beans). *Cereal Chemistry*.

AUTHORS PROFILE



Dr. S. Reginold Jebitta, Assistant professor, Department of Food technology, completed my Doctor degree in Food processing and Engineering published 13 paper and 5 conferences



P. R. Durga Devi, I am undergraduate student in kalasalingam university with CGPA 8.5. I published 2 papers in a conference. Currently I am doing research on Alternative protein (Plant based)



L. Deva Dharshini, I am undergraduate student in kalasalingam university with CGPA 7.8. I published 1 paper in a conference. Currently working on a meat alternative.



T. Nagasai Harika. I am undergraduate student in kalasalingam university with CGPA 7.7. I published 1 paper in a conference. Currently working on a meat alternatives project.



K. Vignesh. I am undergraduate student in kalasalingam university with CGPA 6.8. Currently I am working on a meat alternative project.

