

# Extraction of Proteins from Pulses

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**Abstract -This study helps to identify a plant-based cruelty free protein source in lower cost & expectable for all kind of customer pulse protein is the healthier alternative without compromising product quality<sup>1</sup>.**

**Pulses are rich in protein, carbohydrates, vitamins and minerals are low in fat. All though pea proteins experience greater integration into the plant protein ingredient market than others, lentil, chickpea, bean and beans are not far behind. Proteins must provide about 30-35% of calorie needs, 10-15% should come from fats of three types (saturated, mono saturated and poly unsaturated)<sup>2</sup>.**

**This review contains an overview of pulse proteins and the dry and wet fractionation methods used to produce high-protein ingredients. The influence of pulse type and processing on the techno-functional properties of ingredients is discussed.<sup>3</sup>**

**The advantages like decreased risk of disease, managing blood sugar, lowering cholesterol, weight loss.**

**Key words: Proteins, Process of Extraction, Functional properties, Benefits of pulses.**

## INTRODUCTION

In recent years the demand for alternative foods protein that could potential replace animal protein has been increased they are various courses behind the battery shift including substance and ethically consideration however animal based for such as milk meat and eggs are generally high in proteins with balanced amino acid profiles care must be taken when providing plant based substitute to insure adequate nutritional quality Milk substitute in a particular way widely in nutritional quality with the exception of soy beverages most product contain relatively little protein there for there are need for more plant base products with protein levels come variable to cow's milk.<sup>4</sup> Soy based products can provide a good source of high-quality proteins. Soy has negatively association consumers including Allergenicity and the prevalence of genetically modified varieties therefore there is need for alternative protein source various pulse crops

be used as alternative protein source such as per faba bean, lentil, Lupin, chickpea and common bean. However, soybeans most pulses are relatively high in starch and other carbohydrates. At present the only widely available pulse protein concentration or isolation are derived from pea. Pulse protein ingredients must possess good functional properties such as solubility and emulsive properties if they are to be applied in milk substitutes or other diary-type product.<sup>5</sup>

Pulses are widely used for food purpose because of they high protein content I nutrition and health beneficial properties appropriate function attributes and associated low production cost and abundance. The health benefits associated with pulse consumption include lowering of cholesterol levels, reducing the risk of various cardiovascular diseases and cancers and decreasing the risk of type 2 diabetes.<sup>6</sup>

Pulse also have an antioxidant and anti-carcinogenic effect because of the presence of Phyto chemicals saponins and tannins in them.<sup>7</sup>

The review attempts to provide an overview of pulse protein their chemistry structures manufacture of pulse concentrates and isolates functional properties and potential for application in milk alternative.<sup>8</sup>

## CHEMISTRY OF PROTEINS, PULSE PROTEINS, ANIMAL PROTEINS

### PROTEIN CHEMISTRY BASICS

- Proteins are polymer consisting of amino acids linked by peptide bonds Each amino acid consists of<sup>5</sup>
  - A Central carbon atom
  - An amino group NH<sub>2</sub>
  - A carboxyl group COOH
  - A side chain group (R group)
- Difference in sight chains distinguish different amino acids

### PULSE PROTEINS

Pulses naturally contain high levels of proteins making them a suitable starting material for production of plant based proteins. Pulses protein contain high levels of globulin which are storage protein in levels of 70-80%. Legumins and vicilin's represents the majority of these globulins. Protein or water-soluble albumin consisting of metabolic protein such as lectin, enzyme protease and amylase inhibitors. Pulse protein are generally limited in Sulphur containing amino acids methionine, cysteine or tryptophan.<sup>8</sup>



Figure 1 : pulse proteins

**ANIMAL PROTEIN**

Animal proteins are widely used for formation of protein particles in the food industry. Example-casins, whey protein, gelatin, egg protein and fibroin. Protein isolated from milk which is from animal source example casein and whey protein.



Figure 2: Animal protein

Whey protein is used in the food industry due to their high nutritional value. Example emulsification, gelling, foaming & thickening. Fibroin is used in biomedical applications because of its biocompatibility, biodegradability, and anti-microbial properties.<sup>9</sup>

**DIFFERENCE BETWEEN PLANT PROTEIN AND ANIMAL PROTEIN<sup>10</sup>**

ANIMAL PROTEIN	PLANT PROTEIN
<ul style="list-style-type: none"> <li>Sources such as meat, fish, poultry, eggs, and dairy, which are similar to the protein found in the body</li> </ul>	<ul style="list-style-type: none"> <li>The sources of vegetable whole grains, legumes, seeds and nuts</li> </ul>
<ul style="list-style-type: none"> <li>A complete protein, containing all essential amino acids</li> </ul>	<ul style="list-style-type: none"> <li>In complete protein providing only several essential amino acids to the diet</li> </ul>
<ul style="list-style-type: none"> <li>90% Absorbable</li> </ul>	<ul style="list-style-type: none"> <li>60-70% Absorbable</li> </ul>
<ul style="list-style-type: none"> <li>85% Digestible</li> </ul>	<ul style="list-style-type: none"> <li>95-100% Digestive</li> </ul>
<ul style="list-style-type: none"> <li>High In calories</li> </ul>	<ul style="list-style-type: none"> <li>Low in calories</li> </ul>
<ul style="list-style-type: none"> <li>Rich in saturated fat sodium calcium zinc phosphate and vitamin b12</li> </ul>	<ul style="list-style-type: none"> <li>Rich in unsaturated fat fibre potassium magnesium and folate</li> </ul>
<ul style="list-style-type: none"> <li>Contain heme iron which is highly bioavailable</li> </ul>	<ul style="list-style-type: none"> <li>Contain non-heme iron</li> </ul>
<ul style="list-style-type: none"> <li>Low in antioxidants</li> </ul>	<ul style="list-style-type: none"> <li>Highly in antioxidants</li> </ul>
<ul style="list-style-type: none"> <li>Has negative health effects</li> </ul>	<ul style="list-style-type: none"> <li>Has positive health effects</li> </ul>
<ul style="list-style-type: none"> <li>Contain a higher amount of uremic toxins and harbours proteolytic bacteria</li> </ul>	<ul style="list-style-type: none"> <li>Contain a low amount of uremic toxins and harbours saccharolytic bacteria</li> </ul>
<ul style="list-style-type: none"> <li>Little or no fiber</li> </ul>	<ul style="list-style-type: none"> <li>High fibre</li> </ul>
<ul style="list-style-type: none"> <li>High saturated fat</li> </ul>	<ul style="list-style-type: none"> <li>Low saturated fat</li> </ul>
<ul style="list-style-type: none"> <li>Unsustainable for global population</li> </ul>	<ul style="list-style-type: none"> <li>Far more sustainable</li> </ul>
<ul style="list-style-type: none"> <li>Large scale animal suffering</li> </ul>	<ul style="list-style-type: none"> <li>Minimal animal suffering</li> </ul>

Table 1: Difference between the animal protein and plant protein.

We can identify the plant proteins are more digested than animal protein

- Plant proteins having high fibre, low calories, and unsaturated fat which we can get by without animal suffering.<sup>9</sup>

- Plant protein are of rich source of potassium, magnesium, and folic acid. From table no.1

**EXTRACTION OF PROTEIN FROM PULSES  
PROTEIN EXTRACTION**

Protein extraction is dependent on many factors such as PH temperature particle size ionic strength type of salt used in and solvent to flavour ratio various extraction methods are being studied so as to maximize the protein heel without the protein functionality of the concentrate or isolated product the protein rich materials can be classified into dry and wet methods.<sup>8</sup>



Figure 3 pulses

**1.DRY FRACTIONATION<sup>10</sup>**

Dry process of pulses is typically done by air classification which involved separation of flavours on the basis of particle size and density using an air steam into protein and starch rich fraction

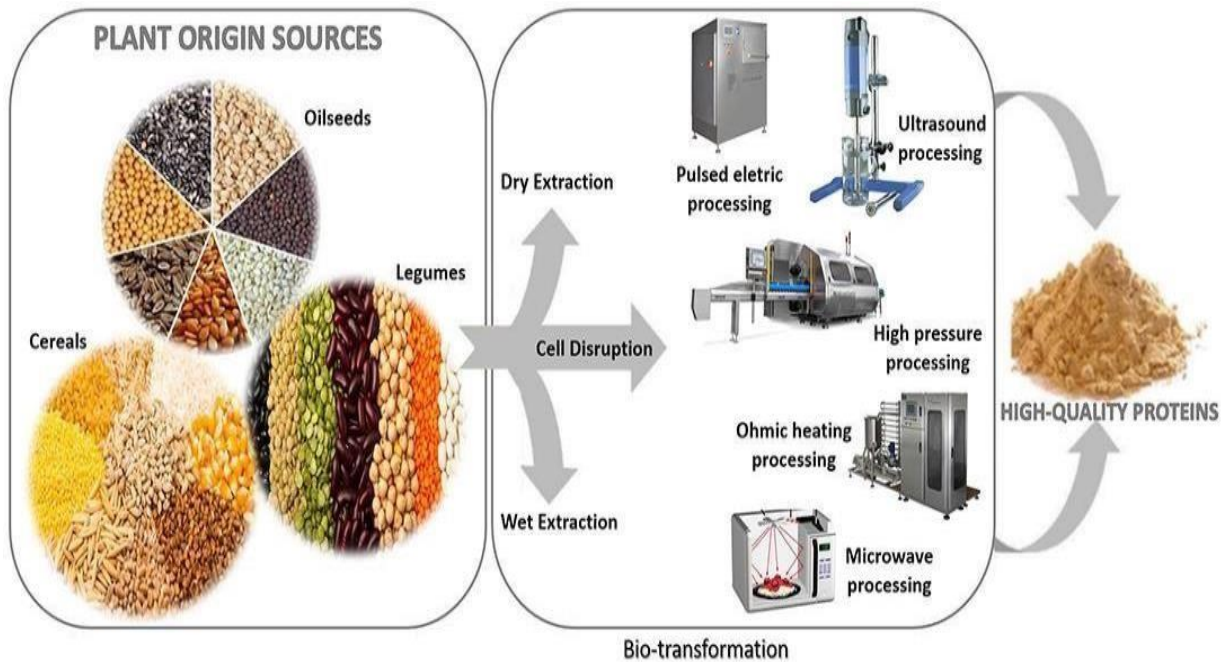


Figure 4:dry fractionation

**1.1AIR CLASSIFICATION**

Air classification has been used with a variety of different pulses including pea faba pea and Lupin. Advantages of this include reduction of Anti Nutritional Factors (ANF'S) removal of bitter astringent components and improve colour<sup>11</sup>

The principle behind a classification separation of particles in hair steam based on their size and density

pulse must be milled finely enough that cells are disrupted, allowing separation of starch granules from protein bodies starch granules should be liberated with minimal damage, while the protein matrix is ground smaller particles impact milling or jet milling may be used to ach.<sup>12</sup>

Macronutrient content of pulses and soyabean (g/100dry matter)<sup>12</sup>

S.NO	PROTIEN	FAT	CARBOHYDRATES	ASH	DIETARY FIBRE
kidney	17-27	1-5	63-74	3.2-5.2	18-30
Navy bean	19-27	2	67-75	4-4.9	14-25

Chickpea	19-27	1-3	52-71	1.8-3.5	6-15
Lentil	23-31	1-3	42-72	2.1-3.2	7-23
Pea	14-31	1-4	55-72	2.3-3.7	3-20
Lupin	32-55.5	5-15	4.5-47	2.6-5.09	14-55
Soybeans	32-43.6	8.1-24.7	31.7-35	4.5-6.4	19.7-31.9

Table 2:Macronutrient content of pulses and soyabean(g/100g dry matter)

We identify the lupin, lentil are the good source of dietary protein (approximately30%) and soyabean contain approximately 40%protien. from table no:2

NUTRITION VALUE OF DIFFERENT PULSES<sup>12</sup>

TYPES OF PULSES	COLOUR	NUTRITION	HEALTH & BENEFITS	PROTEIN %/g
Red lentils (masoor dal)	Brown, red & oragane	Rich in magnesium protein & calcium	Suitable for managing diabetes eating them may boost immunity	21.7%
Bengal gram (chana dal)	It is usually yellow	Rich in antioxidants, selenium, copper& zinc	It may reduce the risk of heart conditions. Good for skin health	21.5%
Black gram (urad dal)	Black, white, and green	Rich in proteins, phosphorous and isoflavones	Improves digestion good for skin diabetes boosts your energy	21.9%
Yellow pigeon peas (tur dal)	Varies from greenish brown to yellow	Rich in vitamins C, E, K, B, folic acid & phosphorous	May help in weight loss may help in healthy pregnancy.	20.4%
Green gram (moong dal)	Green (or) golden yellow	Rich in vitamins E, C, A& K, proteins.	Improve heart health control Bp	23.8%
Chickpeas (chole)	Smaller ones are black, while larger ones are white/ yellow	Rich in iron, fibre, proteins, and calcium	Eating them regularly may improves brain health. May help with iron deficiency.	19-27%
Lupin or lupini beans	yellow	Rich in protein, dictatory fibre, carbohydrates	Help lower cholesterol prevent heart disease	28-34%
Soya beans	Yellow, green, brown	Rich in fibre, protein.	Reduce risk of prostatic and breast cancer.	32-43.6%

Table 3: Nutrition value of different pulses

We can identify soyabean contain 30-40% and yellow bean contain approximately 34% protein which is highest source of protein among other lentils. from table no.03

METHODOLOGY

PROCESS OF EXTRACTION<sup>13</sup>

Rotar-type classifiers are generally used for Air classification of finely milled flavours. The flour is dispersed in an air stream and is then passed to a rotating classifier wheel, wait small and large particles are separated centrifugally the fine fraction is enriched in protein

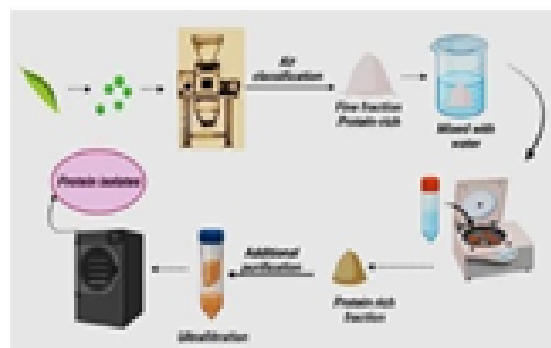


Figure6: process of extraction

1.2 Tribo – electric separation

Tribo electric separation is a relatively novel process for fractionation of flours. And has been explored for the fractionation of navy bean flour and gluten- starch mixtures. A similar technique was explored for protein enriched. of pea and lupin

flours, as well as there are classified fine and coarse fractions.<sup>14</sup>

The principle of this technique involves the environment of particles in a gas flow through a channel, where collision with the walls of the channel cause them to become charged. The application of an external electric field then allows the particles to be separated based on the difference in charge. The development of this method may be due to the lower protein content of the protein-rich fraction compared to air classification. Reported a enriched in starch. In some method the coarse fraction can also re-filled, and air classified again into coarse and fine fractionation in order to increase the yield and purity of each fraction. Air classification is considered more sustainable than aqueous fractionation due to far lower energy and water demands and doesn't require a dry process or the addition of chemicals which are necessary in some aqueous processes. Maximum protein content of 42% for the protein rich fraction of navybeanflour.<sup>14</sup>

## 2. Aqueous fractionation

Aqueous fractionation involve the extraction of protein from either flaked or milled pulses in an aqueous solvent. Followed by recovery/isolation of proteins. A de-fatting set may be carried out before extraction depending on the type of pulse used. Sometime an air classified high fraction is used as the starting material. The protein extract is usually dried to facilitate storage and transport. While the dry fractionated concentrates, the protein content is generally less than 70% of dry matter aqueous process generally result in higher protein content compared to dry fractionation. Often 80-90% although the protein content may be in lower in some cases. For simplicity all ingredients described in this section will be referred to as isolates.<sup>15</sup>

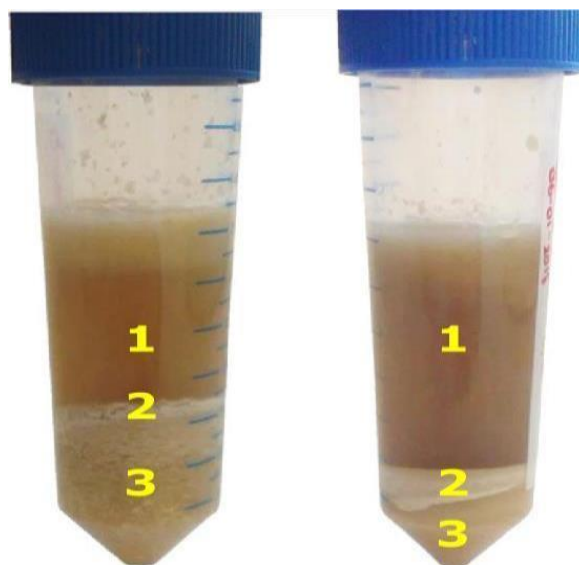
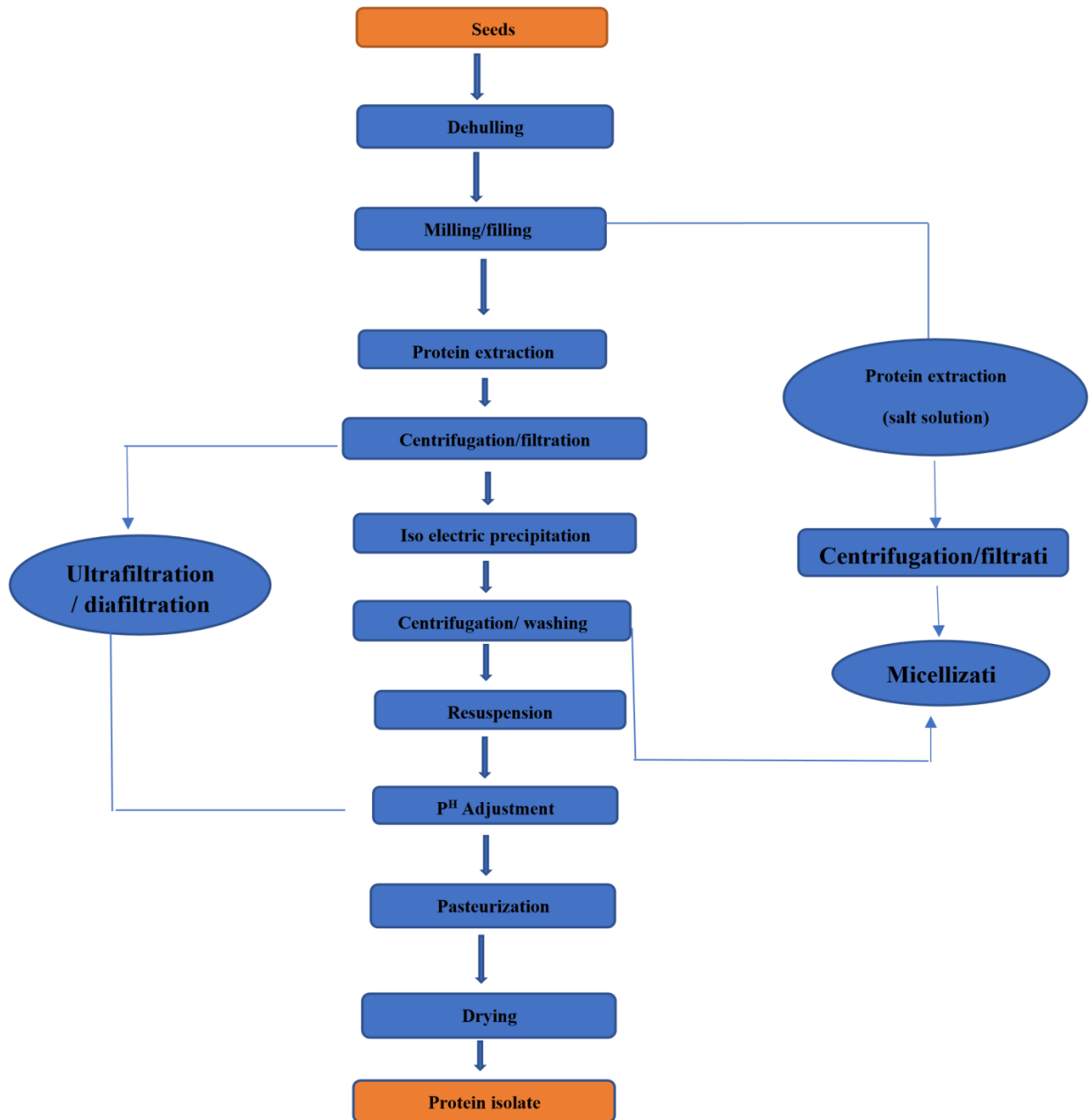


Figure 5: Aqueous fractionation

### 2.1 Isoelectric precipitation<sup>27</sup>

Isoelectric precipitation is the most used method for production of pulse protein isolate and has been used with a wide variety of different pulses. Advantages of pulse protein depend on P<sup>H</sup> environment. The lowest solubility of isoelectric point. P<sup>H</sup> ~ 4.5. most commonly pulse proteins are extracted in mild alkaline solution. The extraction P<sup>H</sup> usually with addition of NaOH (range 8 – 11) but May also be higher extraction PH temperature and time as well as protein content this mixture contains insoluble seed material including starch and insoluble fibres which must be including removed using filtration / sieving or centrifugation the protein is then precipitated with the addition of acid such as HCL (P<sup>H</sup>4-5).<sup>15</sup> The purity of the protein sediment may be increased by washing steps with water or acid solution the recovered protein is than resuspended and neutralised with alkali addition and heat treatment step carried out to improve microbial quality. The liquid protein isolate is dried to give a product which can be stored for later use. At laboratory scale freeze drying and drying and milling used. pilot or industrial scale-spray drying is used the remaining soluble protein processed separately, isolated using ultrafiltration or Dia-filtration to give an acid soluble protein isolate. While alkaline extraction is possible at neutral, or acid p<sup>H</sup> (at p<sup>H</sup> 7) neutral extraction has been used in the preparation of various protein isolate include Lupin soybean isolate (at P<sup>H</sup> 7.5) has been used for lentil protein isolate.<sup>16</sup>



Flow chart 1:Iso electric-precipitation

**2.2 Ultrafiltration [UF]<sup>25</sup>**

Ultrafiltration [UF] with Diafiltration is another technique which is used for pulse protein isolation. The protein extraction and fibre/ starch removal steps are similar to IEP. The protein extract is passed through UF membranes. Which are with a pore size such that protein is retained while smaller soluble component such as oligosaccharides are removed.

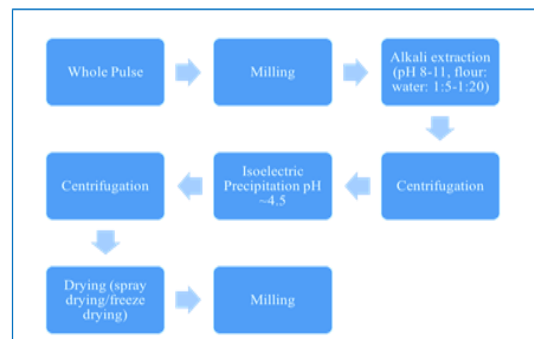


Figure 6:Ultra filtration

Salt extraction /micellization<sup>22</sup>

The isolate can be spray- dried or freeze- dried similar to IEP there are several potential advantages of UF compared to IEP Process. In addition, UF isolates tend to have lower ash and sodium content as, the neutralization step using alkali such as NaOH. Is not required. Ultrafiltration may result in higher protein content depending upon the process. UF Protein isolate compared to IEP protein isolates using various type of pea, lentil, and chickpea as the input material.<sup>16</sup> In this technique, proteins are extracted from seed material such as 0.5m NaCl at neutral PH. Following removal of starch / insoluble fibre, the protein extract is diluted with cold water the dilution cause the protein to precipitate due to the change in ionic strength. The term micellization is used as the proteins. Precipitate in the form of micelles the precipitation protein may be recovered by centrifugation washed resuspended protein may then be resulting isolated may differ from IEP isolates in terms of appearance and functionality similarity to UF the micellization process has the advantage of a milder process with less extreme PH changes and therefore potentially less protein denaturation during the process.<sup>17</sup>

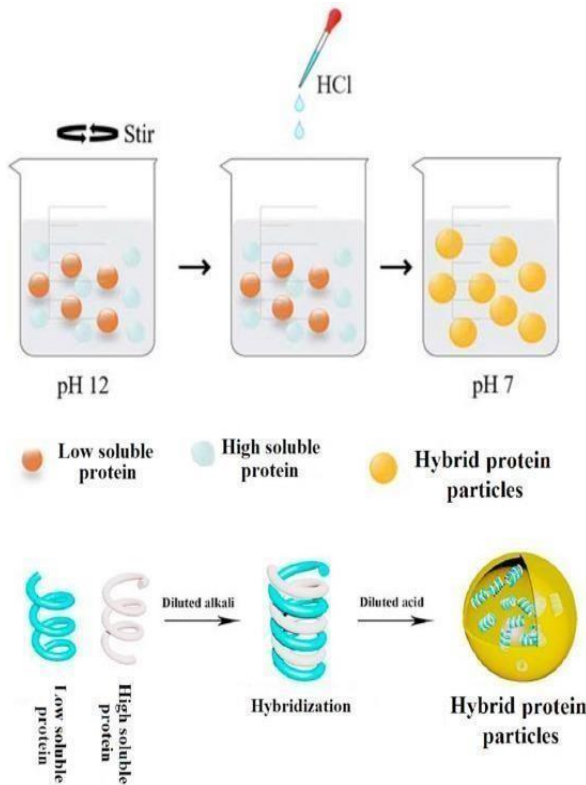


Figure 7:Salt extraction/Micellization

Functional properties<sup>20</sup>

- Solubility = solubility of most pulse protein is highest at low acidic and higher alkaline PH value.
- Water binding capacity = amount of water that can be absorbed per-gm of protein material.
- Emulsification = it is used to evaluate the emulsifying properties of protein flour.
- Fat absorption = also called oil absorption capacity is calculated as the weight of oil absorbed per weight of protein powder or legume flour.
- Foaming properties = used for measuring foaming properties are foam expansion, foam capacity and foam stability.
- Gelation = is important in the preparation of many foods [ example: puddi's, jellies, desert, and meat].
- Viscosity
- Cohesion /Adhesion
- Elasticity
- Flavour binding

Applications<sup>14</sup>:

- Baby food = use of whole pulses for the preparation of baby food but very little on the use of pulse extract.
- Imitation milk
- Bean curd
- Meat products
- Baked goods, glazes, frosting and pastes.
- Extrudate products
- Pastas and noodles
- Soups, gravies, cheese, desert
- Beverages and protein concentrates sausages.

Benefits of pulse protein<sup>19</sup>

- Sprouted lentils aid in muscle generation in body
- Aids in weight management
- Reduces risk in cancer
- Good source of vitamin and minerals
- Beneficial in preventing cell and gene damage
- Help in control diabetes
- Helps in relaxing cardiovascular muscle and lowering blood pressure
- Aid in digestion and boost metabolism
- Help in optimal brain functioning.

Disadvantages of animal protein<sup>18</sup>

- High cholesterol contributes to weight gain, high blood pressure, heart diseases
- Requires more energy to digest than plant proteins
- Becomes toxic animal protein stays in blood stream to 4 days and have toxic effects.
- Acidifying: cause acid levels to rise and leads to cancers, heart diseases.

CONCLUSION

Pulses are a promising protein class to fulfil in the growing needs for a plant-based proteins. They have shown promising functional properties though those properties are highly dependent on the processing methods used to produce them as the methods can have measurable effects on the protein composition and structure. The research in this area is limited, and more research is needed to conclusively decide which processes yield optimal protein isolates. Notably, the inconclusive effectiveness of enzymatic hydrolysis on improving pulse protein functionality suggest that methodologies should be re-evaluates to draw firm conclusions. Where trends are clear however protein manufacture can choose processing steps to produce protein with functionality that is tailor to a selected application.

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