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# Development of a process technology for production of pea peel protein isolate from green pea peel powder using isolation technique

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#### Abstract

This project was undertaken to standardize the process parameters involved in the preparation a of production of pea peel protein isolate from green pea peel powder using isolation technique. The study also included nutritional and functional properties of the product. Pea peel protein isolate were successfully prepared from hot air dried peel powder by alkaline extraction method. The optimized parameters for hot air dried peel powder were hot air temperature of 40 °C and corresponding functional properties were: solubility (9.7%), foam capacity (24.5%) foam stability (19.5%) and water absorption capacity (4.89 %) at pH 9, whereas for pea peel protein isolate were: solubility (51.3%), foam capacity (56.8%), foam stability (43.1%) and water absorption capacity (3.43%) at pH 9.

Keywords: Pea peel, protein isolate, protein solubility, foaming capacity and stability

#### Introduction

Pea (*Pisum sativum* L.) is a winter season crop grown in many parts of the world. It is third most popular rabi pulse of India after chick pea and lentil. India is second largest producer of green peas after China. In the world's total produce, its production in India contributes to around 7% with the production figures of 7.8 lakh tonnes (http://www.commoditiescontrol.com) [4]. Besides Uttar Pradesh, Madhya Pradesh, Bihar and Maharashtra are the major pea producing states. Fresh peas are good sources of protein, vitamins, and soluble as well as insoluble fiber. It has a valuable and low cost source of high quality protein products which play significant roles in human nutrition. Plant proteins are used as additives in food to enhance the nutritional value and sensory properties of the final food product.

Due to seasonal and perishable nature of peas, its availability is limited only to some part of the year, which creates the need for its preservation. Its mature seeds are used as whole or split into dhal and put to use in various ways for human consumption. Peas are obtained about 65 % from fresh pea's pods, i.e. pea peels are about 35% on the basis of fresh weight. Thus based on India's yearly production of pea, more than 1 million ton of pea peel waste (by-product of pea) is generated annually alone in India, of which sizeable extent is discarded as waste during the processing of pea.

Various pea processing industries produce very large amount of pea peel wastes as by-product. They want efforts to preserve and marketing of pea peels, so that pea peels may be utilized. Until now, it is undervalued but it can be used by production of protein isolate and other product as a potential source for human and animal feed. It can be used for the following practical utility: (a) As an alternatives protein source for use in various foods products such as ice cream, cake icings, mixes, desserts, confectioneries, dry mixes, dairy products and beverages, (b) as a functional food ingredients to improve the nutritional quality of the product in commercial food production. Therefore, the aim of the study was to prepare the protein isolate from green pea peels and to evaluate nutritional and functional properties of the product

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# **Materials and Methods**

#### Raw materials

Fresh pea peels were collected from Bhanu Farm pea industry (Jabalpur, MP). They were

sorted, washed and allowed to dry at room temperature for further experiment. They were dried at  $40^\circ$  C in hot air tray dryer then ground to obtain a fine powder for extraction of protein isolate. The ground powder was passed through a sieve of 350  $\mu m$  size.

#### Preparation of protein isolate

Protein isolates were produced by wet processing in which low molecular weight water soluble components and the salt soluble proteins were extracted from the powder and then the globular proteins were subsequently isolated by a selective precipitation step at the isoelectric point, neutralized and dried. The precipitates were dried by optimizing parameter of hot air temperature (40, 42 and 44  $^{0}$ C). The schematic diagram of the most frequently used method described by Wolf [12] with minor modifications based on aqueous alkaline extraction followed by isoelectric precipitation is presented by following flow diagram:

Take appropriate amount of flour and add water to it (1:10 ratio)

Adjust the pH (9) and stir it for 1 h

Centrifuge the content at 8000 rpm for 15 min

Collect the supernatant

Adjust the pH of the supernatant to 4.5

Leave it for 2-4 h for precipitation

Centrifuge it after 2-4 h

Collect the precipitate

Wash the precipitate with water to remove traces of acid in precipitate

Neutralize the precipitate

Dry the precipitate/protein isolate

Fig 1: Flow diagram for the preparation of pea peel protein isolates

#### **Proximate Analysis**

Moisture, fat, ash and fibre contents were determined according to the methods of AOAC <sup>[2]</sup>. The carbohydrate contents were determined by a subtracting the sum of percentage of moisture, protein, fat, ash and fibre from 100%. The protein content of sample was determined by the micro-

Kjeldhal method <sup>[1]</sup>. Each analysis was done in triplicate, and data were reported as means.

#### **Water Protein Solubility**

200 mg sample were dissolved in 20 mL of distilled water and pH of the mixture was adjusted to 4, 5, 6, 7, 8, 9 with 1 N HCl and 1 N NaOH. The mixture was stirred at room temperature for 30 min and centrifuged at 8000 g for 20 min. Protein contents in the supernatant were determined using the following formula <sup>[9]</sup>. Protein solubility was then calculated by:

Solubility 
$$\% = \frac{\text{protein content in supernatant}}{\text{total protein content in sample}} \times 100$$

#### **Foaming Capacity and Foam Stability**

500 mg samples were dissolved in 50 mL of distilled water. The pH of the protein solution was adjusted to 4, 5, 6, 7, 8, 9 with either 0.1 M HCl or 0.1 M NaOH. At a speed of 10,000 rpm, the solutions were stirred for 2 min. Then it was immediately transferred into a 100 mL graduated cylinder. The volume was noted before and after stirring. Foaming capacity was expressed as the volume (%) increased due to stirring. For the measurement of foam stability, foam volume changes in the graduated cylinder were noted after 30 min. All analysis was performed in triplicate [10].

Foam capacity% = 
$$\frac{\text{(volume after whipping - volume before whipping) ml}}{\text{(volume before whipping) ml}} \times 100$$
Foam stability % =  $\frac{\text{(volume after standing - volume before whipping) ml}}{\text{(volume before whipping) ml}} \times 100$ 

# Water Absorption Capacity (WAC)

One gm of sample was weighed into 15 mL pre-weighed centrifuge tube and it was mixed in 10 mL of distilled water under continuous stirring with a glass rod. After 30 min, the tube was centrifuged at 2000 g for 20 min. The amount of supernatant liquid in the test tube was recorded. All analysis was performed in triplicate. WAC expressed as grams of water per gram of sample, was calculated by <sup>[9]</sup>:

$$WAC = \frac{W2 - W1}{W0} \times 100$$

Where, W0 is the weight of the dry sample (gm), W1 is the weight of the tube plus the dry sample (gm), and W2 is the weight of the tube plus the sediment (gm).



Plate 2: Green pea peel powder



Plate 2: Pea peel protein isolate

#### **Results and Discussion**

# Proximate chemical composition of fresh pea peel powder and pea peel protein isolate

The proximate composition of fresh pea peel powder and pea peel protein isolate is shown in figure 2 and 3. Fresh pea peel powder was used as starting material for the preparation of pea peel protein isolate. Protein content of fresh pea peel powder and pea peel protein isolate was different with value of 24.3% and 75.1% respectively. The yield of pea peel powder from fresh pea peel was 11.2%, whereas the yield of

pea peel protein isolate of 9.4% was obtained from pea peel powder. Results indicated that pea peel protein isolate could

be considered as an additional source of plant protein in food products.

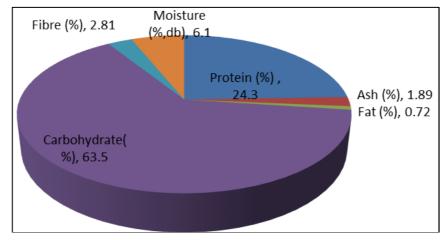


Fig 2: Chemical Composition of Fresh Pea Peel Powder

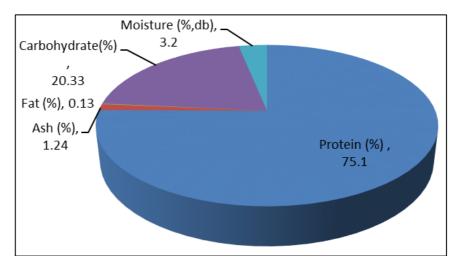


Fig 3: Chemical Composition of Pea Peel Protein Isolate

## **Protein Solubility**

The protein pH-solubility profiles of fresh pea peel powder and pea peel protein isolate were shown in Figure 4. Solubility of protein is variable and is influenced by the no. of polar and non-polar groups and their arrangement along with molecules solubility of protein depend on the pH and ionic strength. Fresh pea peel powder and pea peel protein isolate showed minimum proteins solubility at pH5 with values of 5.2 and 7.6 respectively, and maximum protein solubility at pH 9 with values of 9.7 and 51.3, respectively. It is cleared from figure 4 that solubility decreases as the pH increases

until it reaches the isoelectric point and then increases similar to <sup>[6,7]</sup>. Reduction of electrostatic repulsive forces between the positively charged proteins increases formation of protein aggregates in precipitation with large diameter and high bulk density then the protein solubility increases with further increase of pH. Hence it helps to keep them apart and increase protein-solvent interactions. Figure 5 showed that thermal treatments reduce the solubility of pea isolates. However, thermally treated pea peel protein isolate also showed similar trends.

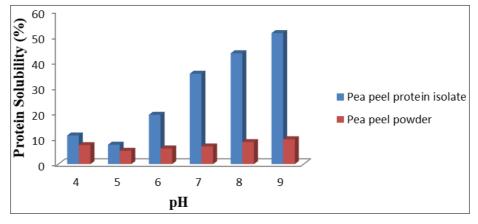


Fig 4: Effects of pH on protein solubility of pea peel powder and pea peel protein isolate

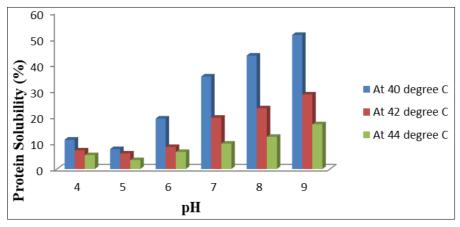


Fig 5: Effects of pH on protein solubility of thermally treated pea peel protein isolate

### **Foaming Properties**

The effects of pH on the foam capacity (FC) and foam stability (FS) of fresh pea peel powder and pea peel protein isolate are shown in Figure 6 and 7. At pH 9, the foam capacity (FC) of pea peel protein isolate was significantly higher than that of fresh pea peel powder with values of 56.8 and 24.5 % respectively. The foam stability (FS) of pea peel protein isolate was significantly higher than that of fresh pea peel powder with values of 43.1 and 19.1% respectively. The results showed that an increase in the net charge of the protein

weakens hydrophobic interaction and increases protein solubility and flexibility, allowing the protein to spread to the air—water interface more quickly and thus increasing foam formation [3,5]. The profile of foaming properties against pH for the pea peel protein isolate was similar to that of its solubility against pH (Figure 1). Results revealed that the foaming property of pea peel protein isolate was pH-dependent. Foaming properties improvement of pea peel protein isolate at alkaline pH may be due to increased solubility and surface activity of the soluble protein.

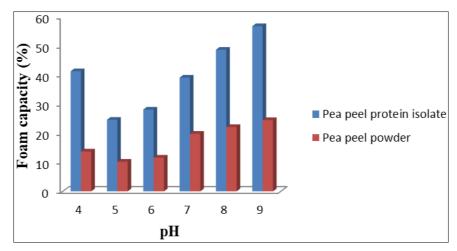


Fig 6: Effects of pH on foam capacity of pea peel powder and pea peel protein isolate

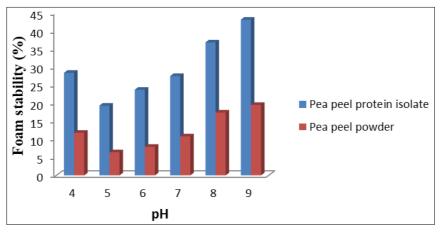


Fig 7: Effects of pH on foam stability of pea peel powder and pea peel protein isolate

# Water Absorption Capacity (WAC)

Water absorption capacity of fresh pea peel powder and pea peel protein isolate were obtained with values of 4.89 (g/g) and 3.43 (g/g) respectively at pH 9. However, the solubility of

pea peel protein isolate was significantly higher than that of fresh pea peel powder in Figure 2. It was suggested that there was no direct correlation between solubility and WAC of pea peel proteins as high protein solubility did not necessarily mean high WAC <sup>[8]</sup>. Water absorption capacity of proteins is a function of several parameters, including size, shape, hydrophilic-hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with proteins. Carbohydrates contain hydrophilic parts, which can enhance WAC. Water absorption capacity for fresh pea peel powder was increased, as carbohydrate content in the fresh pea peel powder was higher than that of pea peel protein isolate.

#### Conclusion

Pea peel protein isolate were successfully prepared from hot air dried peel powder by alkaline extraction method. The optimized parameters for hot air dried peel powder were hot air temperature of 40° C and corresponding functional properties were: solubility (9.7%), foam capacity (24.5%) foam stability (19.5%) and water absorption capacity (4.89 %) at pH 9, whereas for pea peel protein isolate were: solubility (51.3%), foam capacity (56.8%), foam stability (43.1%) and water absorption capacity (3.43 %) at pH 9. The chemical composition for pea peel powder were: protein (24.3%), ash (1.89%), fat (0.72%), carbohydrate (63.5%), fibre (2.81%), moisture content (6.1%) and yield (11.2 %), whereas for pea peel protein isolate were: protein (75.1%), ash (1.24%), fat (0.13%), carbohydrate (20.33%), moisture content (3.2%) and yield (9.4%). Hence, functional properties of protein products were improved when pea peel protein isolates were produced from fresh pea peel powder by isolation techniques. Pea peel protein isolates could be considered as a rich resource of vegetable proteins in food systems.

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**Conflict of interest Statement:** The authors declare that there is no conflict of interest.

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