ORIGINAL RESEARCH ARTICLE

Isolation of protein isolated from defatted *Carica papaya* linn. seeds: influence of pH and NaCl on its solubility and functional properties

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ABSTRACT.

This work reports the isolation of protein from defatted *Carica papaya* Linn. Seed (CPS), with the crude protein product containing 88.08% protein. Under its natural conditions, the solubility of this protein isolate is comparable to sweet lupin protein isolate (98.79%). The solubility of the protein decreases with decreasing pH, with the minimum solubility observed at its isoelectric point (pH 4). The water holding capacity, oil holding capacity, foaming capacity, foam stability, emulsifying capacity and emulsion stability were found to be 3.65 g/g, 3.60 g/g, 26.14%, 13.06%, 74.05m^2/g, 100.80%. The profiles of functional properties were determined with varying pH values and NaCl concentration, and improved properties were observed in the alkaline pH range and in the presence of NaCl.

Keyword: *Carica papaya seed; functional properties; foaming properties; Protein isolate; proximate composition.*

Introduction

Production of food products with high protein content from under-exploited sources is a recent development in response to the rising protein malnutrition challenges in developing countries. Several new protein sources have been identified, such as cashew nut protein isolate (Deng *et al.*, 2011; Ogunwolu, Henshaw, Mock, Santros, & Awonorin, 2009), milkweed seed protein isolate (Hojilla-Evangelista *et al.*, 2009), soybean protein (Idouraine *et al.*, 1991), hyacinth bean protein isolate (2.54g/g) and Bambara protein isolate (Eltayeb *et al.*, 2011). Pawpaw is the fruit of *Carica papaya* which belongs to the genus Carica. It is an invaluable plant that is prevalent throughout tropical Africa, North American and Asia (FAO, 2007). The seeds of the papaya fruits are made up of a sarcotesta and endosperm and account for about 18 % of the fresh fruit weight (Kadiri & Olawoye, 2016). *Carica papaya* seed (CPS) is a major by-product of pawpaw fruit juice production. After removal of lipid from the seed, the residual flour has protein content as high as 32.40% (Kadiri *et al.*, 2016). The uses of CPS as a protein source will not only help to reduce waste from fruit juice production utilising this fruit but will present a low-cost protein source for human consumption.

Plant protein should ideally possess numerous desirable physicochemical and functional characteristics such as solubility, foaming and emulsification, water and oil binding capacity and gelation (Wang & Kinsella, 1976). These intrinsic properties affect the behaviour of proteins in foods during processing, manufacturing and storage (Kinsella, 1979). Therefore, it is imperative to study the relationships between functional properties and the environment of the protein such as pH and ionic strength to enable the effective utilisation of low-cost proteins (Aluko *et al.*, 2005; Myers, 1988).

The isolation and characterization of proteins from defatted CPS have not previously been studied. This study aimed to isolate protein from defatted CPS, characterise the protein isolate by studying its chemical composition, and monitor the effects of environmental conditions such as pH and NaCl concentration on its functional properties.

Materials and methods

Preparation of defatted CPS powder

The defatted CPS powder was produced according to a cold-extraction method at 4^{o}C as suggested by Gbadamosi *et al.* (2012) for the extraction of walnut protein. Dried CPS was milled using Kenwood grinder and sieved through a 300 μm sieve (Endecott's sieve, United Kingdom). The flakes were then extracted with acetone, using a flake to a solvent ratio of 1:10 (w/v), with continuous magnetic stirring (1-kamag reo magnetic stirrer model, Drehzahl electronics, USA) for 4 h. The defatted flakes were then spread on a stainless tray and placed inside a fume cupboard at room temperature for 6 h to dry and to remove the traces of the solvent used. The flakes were then ground to obtain an average particle size of 150 μm powder, packed in plastic tubes and stored at 10^{o}C in a refrigerator.

Protein isolation from defatted CNS

The protein isolate was prepared from the defatted flour by a method described by Chavan *et al.* (2001) as modified by Gbadamosi *et al.* (2012). A known weight (100 g) of the defatted flour was dispersed in distilled water (1,000 ml) to give final flour to liquid ratio of 1:10. The suspension was gently stirred on a magnetic stirrer for 10 min. The pH of the resultant slurry was then adjusted with 0.1 M NaOH to the point at which the protein was most soluble (pH 10). The extraction proceeded with gentle stirring for 4 h keeping the pH constant. Non-solubilized materials were removed by centrifugation at 3500 × g for 10 min. The protein in the extract was precipitated by drop-wise addition of 0.1 M HCl with constant stirring until the pH was adjusted to 4.0. The mixture was centrifuged (Bosch, TDL-5, United Kingdom) at 3500 × g for 10 min to recover the protein. After separation of proteins by centrifugation, the precipitate was washed twice with distilled water. The precipitated protein was re-suspended in distilled water and the pH was adjusted to 7.0 with 0.1 M NaOH, centrifuged and freeze-dried (Labconco Free Zone 2.5 Benchtop freeze dryer model 7670520, Kansas City, MO). The dried protein was ground and stored at 20^{o}C.

Chemical composition of protein isolate

Protein (method 920.87), ash (method 923.03), fat (method 922.06) and includes moisture (method 925.10) was determined following AOAC (2005) methods. The carbohydrate content of the samples was calculated by subtracting the sum of grammes of moisture, protein, fat and ash from 100 g of the samples.

Carbohydrates = [100- (%Proteins+ %Lipid+ %Ash)]
All data were reported on a dry weight basis (DWB).

Protein Solubility

Protein solubility was analysed according to the method of Cepeda *et al.* (1998). The protein sample (125 mg) was dissolved in NaOH solution (20 cm^3, 0.1 mol dm^{-3}) and stirred (Cenco, Netherland) at 30^oC for 1h. The pH of the mixture (10 cm^3) was adjusted using 1.0 mol dm^{-3} HCl solution or 1.0 mol dm^{-3} NaOH solution. The mixture was then centrifuged at 4,500 × g for 30 min. The supernatant was collected and the soluble protein was determined by the procedure of Lowry, Rosenbrough, Fair, and Randall (1951).

Functional properties of the protein isolate

Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) were determined by the method described by Sze-Tao and Sathe (2000). The WHC and OHC were expressed as grammes of water and oil held per gramme of flour sample, respectively.

Effect of pH and NaCl concentration on emulsifying activity index and emulsion stability

The effect of pH and salt concentration on emulsifying activity index (EAI) of the protein isolate was studied using the method described by Wanasundara & Shahidi (1997) with slight modifications. The protein isolate (50 mg) was dispersed in 0, 2.0 or 4.0 mL of a 10.24% (w/v) NaCl solution to make a final NaCl concentration of 0.0, 0.50 or 1.0 M. The pH was then adjusted with 1 m HCl or 1 M NaOH, followed by addition of distilled water to reach a 10 mL volume. The protein solution was mixed with 5 mL of pure gino oil, and the mixture was homogenised using Polytron PT 3100 homogenizer (Kinematica AG) at 4026 g for 60 s. Fifty microliters of an aliquot of the emulsion were transferred from the bottom of the centrifuge tube after homogenisation and mixed with 5 mL of 0.01% sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (722-2000 Spectronic 20D, England). The EAI was expressed as interfacial area per unit weight of protein (m^2 g^{-1}). The emulsions were allowed to stand for 10 min at room temperature and the emulsion stability index (ESI) was determined as described above for EAI and expressed as a percentage of the initial EAI (Aluko & Yada, 1993).

Effect of pH and NaCl concentration on foaming capacity and stability

Foaming capacity and stability as influenced by pH and salt concentration were determined by a modification of the method described by Chavan *et al.* (2001). Approximately 2 g of protein isolate was dispersed in 100 ml of NaCl solutions at concentrations 0.0, 0.5 and 1.0 M, respectively. The pH of the protein solution was adjusted separately to pH 2, 4, 6, 8 and 10 with either 1 M HCl or 1 M NaOH. The solution was then homogenised for 2 min using a blender set (O'Qlink, China) at high speed and then transferred into 250 ml measuring cylinder. The percentage of the ratio of the volume increase to that of the original volume of protein solution in the measuring cylinder was calculated and expressed as foaming capacity or whippability (Ogunwolu *et al.*, 2009). Foam stability was expressed as a percentage of the volume of foam remaining in the measuring cylinder to that of the original volume after 30 min of the quiescent period.

$$\boldsymbol{Foaming\ capacity}\ (\%) = \frac{\boldsymbol{V_2 - V_1}}{\boldsymbol{V_1}} \times \mathbf{100}$$

$$\boldsymbol{Foaming\ stability}\ (\%) = \frac{\boldsymbol{V_3 - V_1}}{\boldsymbol{V_1}} \times \mathbf{100}$$

$$\boldsymbol{V_1 = volume\ before\ whipping}\ (\boldsymbol{ml})$$

$$V_2 = volume\ after\ whipping\ (ml)$$
$$\mathbf{V_3 = volume\ after\ standing\ for\ 30\ min\ (ml)}$$

Bulk Density

The method described by Kaur and Singh (2007) was used in the determination of the bulk density of the samples. Samples were gently filled with 10 mL graduated cylinder (with least count 0.5 mL) and the bottom of the cylinder gently tapped 5 times until there was no further diminution of the sample level after filling to the 10 mL mark. Bulk density was calculated as the mass of sample per unit volume of sample (g/ml).

In vitro Protein Digestibility Determination

In vitro protein digestibility of the protein isolate was evaluated using the method of Saunders *et al.* (1973) and as modified by Chavan *et al.* (2001). Two hundred and fifty milligrammes of the sample was suspended in 15 ml of 0.1 M HCl containing 1.5 mg pepsin, followed by gentle shaking for 1 h at room temperature. The resultant suspension was neutralised with 0.5 M NaOH and treated with 4.0 mg pancreatin in 7.5 ml of phosphate buffer (0.2 M, pH 8.0). The mixture was shaken using a shaker (Innova 40/40R, New Brunswick Scientific, Canada) for 24 h at room temperature. The mixture was then filtered using Whatman No 1 filter paper and the residue washed with distilled water, air-dried and used for protein determination using Kjedhal procedure (AOAC, 2005) as described earlier. Protein digestibility was obtained by using the equation below;

$$In\ vitro\ protein\ digestibility\ (\%) = \left(\frac{I - F}{I}\right) \times 100$$

$$where,\ \ I = protein\ content\ of\ sample\ before\ digestion$$
$$F = protein\ content\ of\ sample\ after\ digestion$$

Discussion and results

Chemical composition of the protein isolate

Three extraction agents (water, 0.1M NaOH and 0.1M HCl) were used to separate protein from the deffated CPS. Acid precipitation at the isoelectric point of the protein was used to separate the protein from other components such as carbohydrates and fibre. Approximately 72g of crude protein was recovered from 100g of defatted CSP. The isolated crude protein product contained 88.08% protein, 0.14% fat, 0.15% ash and 0.96% carbohydrate. Fibre was not detected in the isolate. The high protein content of the protein isolate was as a result of the production process which increased the content of protein in the finished product. The protein content of PI compared well with those of Bambara protein isolates (85.97 %), heat-processed peanut isolates (84.20%) and conophor nut protein isolates (80.00 %) as reported by Eltayeb *et al.* (2011), Kain *et al.* (2007) and Gbadamosi *et al.* (2012), respectively. PI protein was, however, lower than safflower protein isolates (90.1 %) and heat coagulated mustard protein isolates (95%) reported by Ulloa *et al.* (2011) and Sadeghi & Bhagya (2009), respectively. The significance of this finding is that CPS protein isolates might find usefulness in the supplementation of foods produced with high carbohydrate to protein ratio

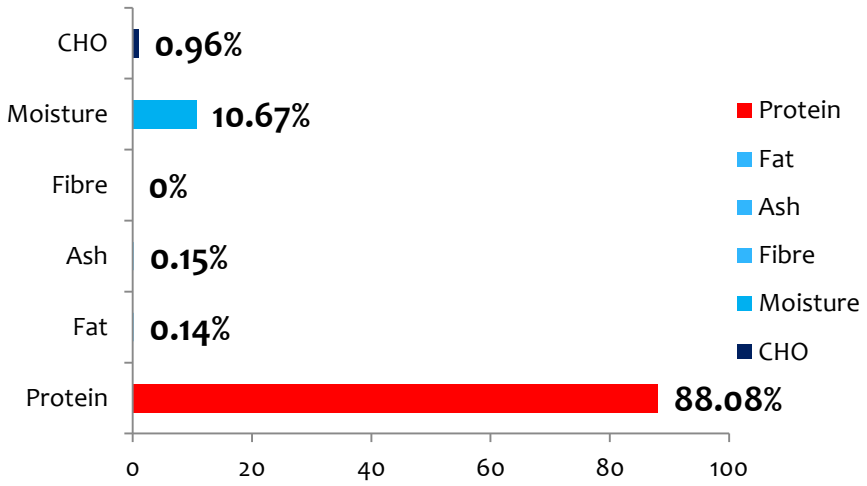

Figure 1: Chemical composition of *Carica papaya* seed protein isolates

Effect of pH on the solubility and functional properties of the protein

The effect of pH on the solubility and functional properties of isolated protein is shown in Table 1.

A minimum solubility of 33.96% was observed at pH 4, and the solubility increased rapidly with increasing pH after this point (pH 4) which is comparable to the value (30%, pH 4.5) obtained for cashew nut protein isolate reported by Ogunwolu *et al.* (2009). At pH 2 and 10, 56.58% and 99.25% protein were soluble, respectively. In general, the dependence of the solubility of this protein product on pH agrees with the observations of previous studies (Gbadamosi *et al.*, 2012; Ogundele *et al.*, 2013). Damodaran (1997) stated that the minimal protein solubility occurs at its pI and that the majority of food proteins are acidic, with minimum solubility at pH 4-5 and maximum solubilities at alkaline pH. At low or high pH, proteins have either net positive or net negative charges, leading to an electrostatic repulsive force that helps to keep protein molecules apart, disrupting the native protein structure, shifting the equilibrium toward the unfolded form and subsequently exposing the buried functional groups in protein molecules, thus leading to an increase in protein solubility (Yuliana *et al.*, 2014). In contrast, the occurrence of minimum solubility near the isoelectric point is due primarily to both net charges of the protein peptides, which increases as pH moves away from the isoelectric point, and surface hydrophobicity that promotes the aggregation and precipitation via hydrophobic interactions, resulting in less interaction with water and thus reducing protein solubility.

The minimum FC occurred at pH4 (21.92%) which happens to be the isoelectric pH of the protein and increases with pH above the pI, with values ranging from 35.93% to 59.13% (Table 1). The increase in FC with pH is likely due to increased solubility and surface activity of the soluble protein, which has an important influence on the foaming behaviour of proteins as reported by L'Hocine *et al.* (2006). Yuliana *et al.*, 2014 reported that this might be due to the increased net charges on the proteins, which weakened the hydrophobic interactions and increased the flexibility of the protein. This allowed the protein to diffuse more rapidly to the air-water interface to encapsulate air particles, enhancing foaming. Deng *et al.* (2011) and Gbadamosi *et al.* (2012) also mentioned that high protein solubility is required to increase FC and FS.

A similar trend was observed in the dependence of FS on pH. FS was lowest (5.4%) at the pI (pH4) while increasing the pH to 10 resulted in the highest FS. The high FS values at pH above the pI could be attributed to the increasing solubility index of the protein molecules which result in an increase in viscosity and formation of a multilayer cohesive protein film at the interface. The increase in FS at higher pH may have been due to a decreased tendency for foam particles to coalesce as a result of the higher net negative charge of the protein (Yuliana *et al.,* 2014). Makri and Doxastaskis (2006) added that pH alters the structure of protein molecules, leading to differences in FC and FS.

Table 1 shows that pH has similar effects on the emulsifying properties of the protein isolate as it does on the foaming properties. The lowest EAI value (10.50 m^2/g) was found at pH 4 while the highest (78.20 m^2/g) was observed at pH 10. The relationship between EAI and pH is similar to that between protein solubility and pH. Several studies have shown that the pH-emulsifying properties profile of various proteins including conophor proteins (Gbadamosi *et al.,* 2012) and cashew protein (Ogunwolu *et al.,* 2009) resembles the pH-solubility profile. This agrees with the reports of Gbadamosi *et al.* (2012), Chavan *et al.* (2001), Ragab *et al.* (2004), Osman *et al.* (2005), Aremu *et al.* (2008) and Ogungbenle *et al.* (2009). The pH affects EAI primarily by altering the charge distribution of protein molecules (Deng *et al.,* 2011). At pH above 10, hydrophobic forces decrease as a result of the increased protein net charge and the increased flexibility (Yuliana *et al.,* 2014). This facilitates the diffusion of proteins to the air-water interface thus resulting in poor the EAI observed.

The lowest ES value (55.20%) was also observed at the pI as was the EAI and was observed to increase with increasing alkalinity (pH). The low stability of the emulsion under acidic conditions may be attributed to the increased interaction between emulsified droplets, thus facilitating protein aggregation and reducing ES as suggested by (Yuliana *et al.,* 2014). Increased pH toward alkaline values increases coulombic repulsion between neighbouring droplets and the hydration of the charged protein molecules. These factors reduced the interfacial energy and lead to the coalescence of emulsion droplets (Chavan *et al.,* 2001).

Table 1
Effect of pH on the solubility and functional properties of the protein (FC, FS, ESI and EAI)[a]

Protein properties	pH				
	2	4	6	8	10
Solubility (%)	56.58	33.96	67.40	87.64	99.25
FC (%)	35.93	21.92	25.81	31.04	59.13
FS (%)	6.62	5.40	17.23	19.45	21.41
EAI (m^2/g)	15.92	16.50	54.88	67.94	78.20
ESI (%)	100.70	55.20	97.68	106.78	122.56

[a]These results represent the means of three determinations. The SD values range from 0 to less than 5% and thus are regarded as insignificant.

Effect of NaCl concentration on protein solubility and functional properties

Table 2 shows the effect of NaCl concentration on protein solubility and functional properties. Protein solubility increased from 38.34% to 65.27% when the NaCl concentration was increased from 0 to 0.5 mol dm^{-3}. A slight increase in the molar concentration of NaCl to 0.5 mol dm^{-3} induced salting-in which had a positive effect on protein solubility. Nevertheless, further increases in NaCl concentration resulted in the interaction of negatively charged chloride ions with positively charged protein molecules, leading to a decrease in electrostatic repulsion, thus enhancing hydrophobic interactions (Osman *et al.,* 2005; Yuliana *et al.,* 2014).

The effects of NaCl concentration on FC and FS are similar to the effects on other functional properties. The results show that FC, FS and protein solubility increased with increasing NaCl concentration up to 0.5moldm^{-3}. This was proposed to be due to the weakening of hydrophobic interactions between protein molecules and the formation of interfacial protein layer with cross-linking bonds among peptide. A further increase in NaCl concentration was however observed to have adverse effects on FC due to the salting-out effect. Lawal *et al.* (2005) explained that the ion screening effect at high salt concentrations improves the hydrophobic interactions of proteins and destroys protein films, promoting flocculation, aggregation and precipitation. On the other hand, further addition of NaCl improves the FS of the protein. This phenomenon is likely due to the increased solubility and surface activity of the soluble protein as reported by Yuliana *et al.* (2014).

As shown in Table 2, EAI and ESI increase rapidly with increasing NaCl concentration up to 0.5 mol dm^{-3} after which a reduction was observed with increasing concentration to 1.0 mol dm^{-3}. The sudden increase in these properties might be due to improved hydrophobic protein-protein interactions condition which may well favour emulsion by improving the rheological properties of the interfacial protein films that encapsulate the oil droplets while the salting-out effect is believed to have set in at 1.0 mol dm^{-3} which has the reverse effect on this interaction (protein-protein interactions) thus the decrease in EAI and ESI observed.

Table 2
Effect of NaCl on solubility and functional properties of the protein[a]

Protein Properties	Salt concentration (mol dm^{-3})		
	0	**0.5**	**1**
Solubility (%)	38.34	65.27	63.03
FC (%)	15.93	21.92	25.81
FS (%)	6.62	5.40	17.23
EAI (m^2/g)	36.07	48.09	45.89
ESI (%)	43.55	82.78	54.00

[a]The results represent the means of three determinations. The SD values range from 0 to less than 5% and thus are regarded as insignificant.

Oil absorption capacity (OAC)

At neutral pH and without NaCl addition, the OAC was found to be 3.60/g protein. This value is within the values reported for soy bean protein (3.29g/g) (Idouraine *et al.*, 1991) and cashew nut protein isolate (4.42g/g) (Ogunlowu *et al.*, 2009) but higher than the values reported for hyacinth bean protein isolate (2.54g/g) and Bambara protein isolate (1.02g/g) (Eltayeb *et al.*, 2011). Kinsella (1976) stated that the oil binding mechanism can be explained as the physical entrapment of oil by capillary attraction.

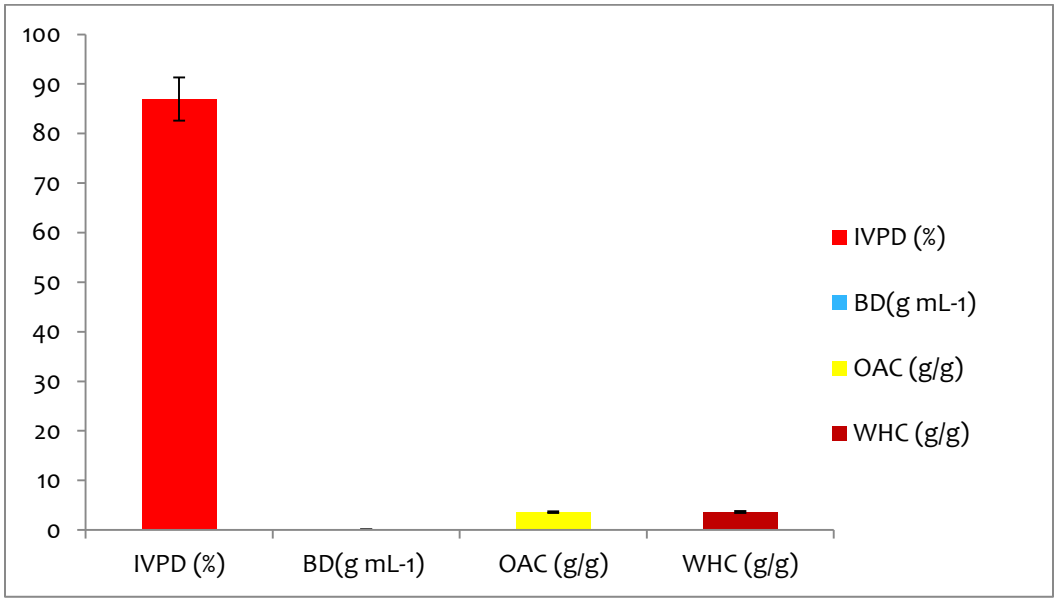

Figure 2: Physiochemical properties of Carica papaya seed protein isolate

Bulk Density

The bulk density of the protein isolate was found to be 0.150 g mL^{-1}. This is, however, lower than that of conophor protein isolate (0.66 g mL-1), cashew nut protein isolate (0.31 g mL-1), and sesame protein isolate (0.169 g mL^{-1}) reported by Gbadamosi *et al.* (2012), Ogunwolu *et al.* (2009) and Kanu *et al.* (2007), respectively but comparable to the protein isolate of cashew nut shell (0.148 g mL^{-1}) reported by Yuliana *et al.* (2014). Bulk density depends on interrelated factors such as the intensity of attractive inter-particle forces, particle size and the number of contact points (Peleg & Bagley, 1983). The lowest bulk density observed for the protein isolate might be as a result of the processing methods used for the recovery of the protein which eliminated food constituents such as carbohydrates and fibres which are denser in weight resulting in a food product with low bulk density.

In vitro protein digestibility (IVPD)

The IVPD was found to be 86.94%. The result is comparable with lentil protein isolate (88%) but higher than that of Conophor protein isolates (73.47%) as reported by Suliman *et al.* (2006) and Gbadamosi *et al.* (2012). This high protein digestibility with increased protein concentration is in agreement with the report of Gbadamosi *et al.* (2012) where increased digestibility correlates with increased protein concentration. This result suggests that CSP proteins could well be explored in food formulation.

Conclusion

A product with 88.08% protein content was successfully isolated from defatted CPS. The product also contains 0.14% fibre, 0.15% ash and 0.96% soluble sugar. The pI of the protein occurred at pH 4 and was observed to be the point where the protein was least soluble. Other properties were investigated at the pI includes; FC, FS, EAI and ESI. Adjusting the pH and NaCl concentration improves the characteristics of this protein product by affecting its solubility, foaming and emulsifying properties including FC, FS, EAI and ESI. This suitable property exhibited by the protein isolated from CPS deffated flours implies that it may find suitable applications as functional ingredients in food product development, supplementation or fortification.

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