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Production, physico-chemical and functional characterization of a protein isolate from jackfruit (Artocarpus heterophyllus) seeds

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ABSTRACT
Proteins from jackfruit seed defatted flour were fractionated, characterized and extracted using an alkaline solution and isoelectric precipitation, which was followed by an ultrasound treatment, for preparation and determination of the physicochemical and functional properties of a protein isolate. Glutelins were the dominant fraction, which are composed of 15–20 kDa polypeptides. The protein content, water and oil absorption capacity and least gelation concentration of the jackfruit seed protein isolate were 952.1 g/kg (dry basis), 6.42 ml water/g protein and 6.07 ml oil/g protein and 9% (at pH 6), respectively, whereas the greatest protein solubility, emulsifying activity, emulsion stability, foaming capacity and stability were 94.4%, 127%, 127%, 254% and 164%, respectively, and depended on the pH; the predicted PER was 2.36. In light of the functional and nutritive properties determined in this study, jackfruit seed protein isolate could be a novel protein source for use in food systems.

Introduction
The world produces approximately 1600 million tons of solid waste per year. The generation and inappropriate management of this waste is considered to be one of the primary environmental problems associated with the emission of methane and carbon dioxide, the emission of odors from landfill sites and damage to surface water and air quality (Angulo et al., 2012).

Some estimates indicate that up to 42% of food waste is produced by households, 39% of waste occurs in the food manufacturing industry, 14% occurs in the food sector (ready to eat food, catering and restaurants) and 5% is lost along the distribution chain (agricultural food loss is not included in this estimation). Additionally, food waste is expected to increase to approximately 126 Mt by 2020 if additional prevention policies or activities are not undertaken (Mirabella, Castellani, Sala, 2014). However, many of these wastes have the potential to be reused in other production systems.

Waste from the food industry, which has recently been considered for potential use in human food, is derived from the processing of various food groups, such as fruit and vegetables, milk, meat, root crops and oilseeds (Ribeiro da Silva et al., 2014). Of particular interest is the research focused on the recovery of waste from fruit industrialization in which waste may represent 10–60% by weight of the fruit
(O’Shea, Arendt, & Gallagher, 2012). Waste from fruits produced during processing includes the peel, rind, seeds and unusable pulp, which are generally not used and consequently discarded. The primary components present in the fruit waste are fiber, sugars, fat, proteins, pectins, organic acids, antioxidants, phenols, vitamins, minerals, flavors and other bioactive substances (Reis et al., 2012).

Conversely, jackfruit (Artocarpus heterophyllus L.) is a tree belonging to the family Moraceae and is widely distributed in tropical countries such as Brazil, Thailand, Indonesia, India, the Philippines and Malaysia (Madruga et al., 2014). Due to its spontaneous proliferation in warmer regions, jackfruit is now cultivated throughout the tropical coast of Mexico with the state of Nayarit being the main producer in the country. Jackfruit is composed of several berries having yellow pulp and brown seeds encased in a hard shell. Such fruits are oblong-cylindric in shape and typically 30–40 cm in length but can sometimes be up to 90 cm long. The fruit usually weighs 3.5–10 kg, although a weight of 25 kg has been reported. The residues after processing of jackfruit can constitute up to 70% of the total weight of the fruit. A portion of such residues is the seeds, which may constitute from 8% to 15% of the total weight of the fruit (Swami, Thakor, Haldankar, & Kalse, 2012).

Jackfruit seeds are a good source of starch (22%) and dietary fiber (3.19%). Additionally, jackfruit seed contains lignans, isoflavones and saponins, which are all phytonutrients that have health benefits that are wide-ranging from anticancer to antihypertensive, antiaging, antioxidant and antiulcer (Omale & Friday, 2010). Protein is another component that is present in jackfruit seeds with a composition that is 17.8–37% depending on the variety of jackfruit (Swami et al., 2012).

Therefore, jackfruit seeds may serve as a potential source of a protein isolate, and the characterization of properties such as water/oil absorption, solubility, jellification, emulsifying capacity, and foaming capacity is technologically important because they determine the potential application of protein isolate food ingredients (Bernardino-Nicanor et al., 2014). To our knowledge, there are no scientific information available on the preparation of protein isolate from jackfruit seeds or its functional properties.

The objective of this study was to prepare a protein isolate from jackfruit seeds and to characterize its physicochemical and functional properties.

Materials and methods

**Chemical and materials**

All chemicals were purchased from either Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA) if not stated otherwise. The jackfruit seeds were provided by Mexican Tropical Organics S. de R.L. de C.V. located at Carretera Los Cocos-Aticama s/n San Blas, Nayarit (Mexico). The jackfruit seeds were washed with tap water, sliced to 3 mm using a domestic food processor (Moulinex, Grupo Seb Mexico, S.A. de C.V., Mexico, D.F.), dried on a steel tray at 30°C (Mod. 27, Precision Scientific Group, Chicago, USA) for 8 h and then pulverized in a mill (Cyclotec Mod. 1093, Foss Tecator, Slangerupgade, Denmark). This jackfruit seed flour was defatted using a Soxhlet extractor with ethyllic ether for 16 h. The defatted flour was air-dried at room temperature.

**Protein fractionation**

Proteins were extracted from jackfruit seed defatted flour based on their solubility according to the Osborne fractionation procedure as described by Amza, Amadou, Balla and Zhou (2015). Total protein content of jackfruit seed defatted flour protein fractions (albumin, globulin, glutelin and prolammin) was measured using the Bradford method (1976) and bovine serum albumin as standard.

**Determination of molecular weight distribution**

A study for determination of the molecular weight distribution of the protein fractions from jackfruit seed defatted flour was conducted according to the method reported by Laemmli (1970). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) test was carried out under reducing and nonreducing conditions using buffer solution with and without 2-mercaptoethanol, respectively, on a gel slab comprised 14% separating gel and 4% stacking gel. Supernatant protein fractions (aliquots of 20 μL in buffer [20 μL] containing 65.8 mM Tris–HCl, 26.3% glycerol, 2.1% SDS and 0.01% bromophenol blue) were heated at 95°C for 5 min. Aliquots (20 μL) of the prepared samples were loaded onto the gels. A wide-range molecular weight marker 161-0317 (Bio-Rad Hercules, USA) ranging from 6.5 to 200 kDa was used to prepare a standard curve for molecular weight estimation. The molecular weights of the samples were obtained by Quantity One 1-D analysis software (Bio-Rad, Hercules, CA, USA).

**Preparation of the protein isolate**

First, a study was conducted to determine the minimal and maximal pH (in the range of 3–11) of protein extraction from the defatted flour according to the method reported by Bernardino-Nicanor et al. (2014). The defatted flour from the jackfruit seeds was mixed with distilled water at a ratio of 1:20 and adjusted to the pH of the maximal protein extraction using 1.0 M NaOH. The slurry was stirred for 30 min at 25°C and then centrifuged at 4500 rpm. The pH of the supernatant was adjusted to the pH of the minimal protein extraction (isoelectric point) with 1.0 M HCl, and the slurry was stirred for 20 min at 25°C. The precipitate was separated by centrifugation at 4500 rpm for 20 min at 25°C. Then, the protein precipitate was subjected to an alcoholic extraction with 96% ethanol, using a ratio of 1:10 (protein precipitate:ethanol), stirred for 20 min, separated by centrifugation at 4500 rpm for 20 min at 25°C and finally freeze dried.

To estimate the effect of ultrasound on the protein concentration of the protein in the isolate previously obtained, a suspension (ratio 1:10, protein isolate:water) of protein isolate was prepared and sonicated in an ultrasound bath (Bransons Mod. 3510R-MT, 100 W–42.5 kHz, Richmond, VA 23238) for 1 h. Subsequently, the protein isolate suspension was treated according to the same procedure previously applied to obtain the protein isolate from the defatted jackfruit seed flour, and this isolate will be hereinafter referred to as the jackfruit seed protein isolate treated with ultrasound (JPIU). This isolate was used for the physicochemical and functional analyses.
Physicochemical analyses

Physicochemical characterization was determined using proximate, bulk density and color analyses. For the proximate analysis, moisture, crude protein (N × 6.25), crude fiber and ash contents were determined in triplicate according to AOAC (1990) methods. The bulk density was determined using the method described by Monteiro and Prakash (1994). After a calibrated plastic centrifuge tube had been weighed, it was filled with a protein sample up to 25 ml and tapped to eliminate the spaces between particles; the volume was then recorded as the volume of the sample. After the tube had been weighed again, the bulk density of the protein sample was calculated from the difference in weight and expressed as g/ml. The color was determined using a Minolta CR-400 color meter (Minolta Ltd., Co., Tokyo, Japan). The measured values were expressed according to the CIELAB color scale where \( L^* \) = lightness, \( +a^* \) = redness, \( -a^* \) = greenness, \( +b^* \) = yellowness and \( -b^* \) = blueness. The \( L^* \), \( a^* \) and \( b^* \) values of the white standard tile used as reference were 97.14, 0.19 and 1.84, respectively.

Amino acid analyses and protein quality

The hydrolysis and quantification of amino acids of protein isolate from jackfruit seed defatted flour were performed according to the methods reported by Erkan, Selcuk and Ozden (2010), using a Waters high-performance liquid chromatographic system (Milford, MA, USA) consisting of a system controller, auto injector, liquid chromatographic pump, fluorescence detector and degasser. The different amino acids recovered were presented as g/100 g protein isolate. The protein quality of protein isolate was evaluated by mean of the amino acid score and the predicted protein efficiency ratio (PER) value.

The amino acid score was calculated by the following equation:

\[
\text{Amino acid score (\%)} = \frac{\text{g of amino acid per g test protein isolate}}{\text{g of amino acid per g standard pattern}} \times 100
\]

where the standard pattern used was the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO, 1991) reference for adults.

The predicted PER was calculated from the amino acid composition based on the equation developed by Alsmeyer, Cunningham and Happich (1974), as given below.

\[
\text{PER} = -0.684 + 0.456(\text{Leu}) - 0.047(\text{Pro})
\]

where Leu and Pro are the mass fractions of leucine and tyrosine, respectively, expressed in g per 16 g of N.

Functional properties

The water absorption capacity was measured according to the method outlined by Sosulski (1962). First, 0.5 g of protein isolate was placed in a previously weighed 25-ml centrifuge tube. Then, 10 ml of distilled water was added and the mixture was stirred to homogeneity with a glass rod and centrifuged at 3050 × g for 10 min at room temperature (25°C). The supernatant was decanted, and the residue was weighed together with the centrifuge tube. The water absorption capacity was expressed as g water absorbed/g protein. A similar method was used to measure corn oil absorption capacity; a 1-g sample was used for this purpose.

The emulsifying capacity was determined according to the method outlined by Beuchat (1977). A 2-g sample of protein isolate was blended with 100 ml of distilled water in an Osterizer blender (Tlalnepantla, Edo. de Mexico) on ‘high’ speed for 30 s. Corn oil was gradually added from a graduated burette while the mixture was being homogenized. The decrease in consistency (from a maximum), which was judged by a decrease in the resistance to blending, was considered to be the point of oil addition discontinuation. The amount of oil added up to this point was interpreted as the emulsifying capacity of the sample. The result was expressed as ml oil required to break the emulsion per g protein.

To determine the protein solubility, samples of protein isolate were suspended in water at different pH values (2–12) using the method described by Wang and Kinsella (1976). These solutions were then centrifuged at 3050 × g for 10 min, and the supernatants were analyzed for protein using the Kjeldahl method (AOAC, 1990).

The least gelation concentration was determined using the method described by Abbey and Ibeh (1988). Protein isolate samples were mixed with 5 ml of distilled water in centrifuge tubes to obtain 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20% concentrations; the pH of the suspensions was adjusted to 2, 4, 6, 8 or 10 using 0.1 M HCl or NaOH. The centrifuge tubes were heated for 1 h in a boiling water bath, cooled rapidly under running tap water and further cooled for 2 h in a refrigerator at 4°C. The least gelation concentration was considered to be the lowest concentration at which the sample in the inverted tube did not fall or slip.

A modified version of the method described by Yasamatsu et al. (1972) was used to determine the emulsifying activity (EA) and the emulsion stability of the isolate. Five suspensions were prepared by dissolving 1 g of protein isolate in 30 ml of water, and the pH of the suspensions was adjusted to 2, 4, 6, 8 or 10 with 0.1 M HCl or NaOH. Then, 30 ml of corn oil was added to each suspension. Each mixture was stirred in an Osterizer blender at ‘medium’ speed for 1 min and centrifuged at 1190 × g for 5 min. The volume of the emulsion layer was recorded. The EA was calculated as follows:

\[
\text{EA (\%)} = \left(\frac{\text{volume of emulsified layer}}{\text{volume of total content in tube}}\right) \times 100
\]

Additionally, to determine the emulsion stability, the samples were heated at 80°C for 30 min in a water bath, cooled to 25°C in running water and centrifuged as above. The emulsion stability was expressed as the percentage of EA remaining after heating.

To determine the foaming properties, five suspensions were prepared by dissolving 2 g of protein isolate in 100 ml of distilled water; the pH of the suspensions was adjusted to 2, 4, 6, 8 or 10 using 0.1 M HCl or NaOH. The suspensions were then whipped in an Osterizer blender at ‘low’ speed for 1 min at room temperature (25°C). The resulting foam was poured into a 250-ml cylinder. The total foam volume was recorded, and foaming capacity was expressed as the percentage increase in volume. The foam stability (FS) was determined according to the method...
proposed by Kabirullah and Wills (1982). The foam volume was recorded 30 min after whipping, and the FS was calculated as follows:

\[
FS(\%) = \left( \frac{\text{foam volume after 30 min}}{\text{initial foam volume}} \right) \times 100
\]

Statistical analysis
Data from all experiments were collected in triplicate and subjected to an analysis of variance and a multiple comparison using Duncan’s test through the use of Statgraphics Plus Version 4.0 (Manugistics, Inc., Rockville, MD, USA). The level of significance was set at \( p < 0.05 \).

Results and discussion
Protein fractionation
The Osborne solubility-based protein fractionation data indicated that NaOH-soluble (glutelin) and water-soluble (albumin) proteins were the predominant fractions in jackfruit seed, 683.6 ± 34.2 g/kg protein and 183.6 ± 8.2 g/kg protein, respectively, while, only 113.0 ± 5.6 g/kg protein of NaCl-soluble fraction (globulin) and 19.8 ± 0.8 g/kg protein of ethanol-soluble fraction (prolamin) was determined. Namely, the vast majority of storage proteins in jackfruit seed were of the glutelin and albumin form. This protein fraction distribution is similar to that found in other fruit seeds as seinat (Cucumis melo var. tibish) seeds which comprises mostly glutelins (386.5 g/kg), followed by albumins (345.3 g/kg), globulins (240.7 g/kg) and prolamins (27.5 g/kg) (Siddeeg, Xu, Jiang, & Xia, 2015), while that in the proteins of gingerbread plum seeds, the glutelin, albumin, globulin and prolammin fractions were 406 g/kg protein, 276 g/kg protein, 258 g/kg protein and 64.8 g/kg protein, respectively (Amza et al., 2015). Moreover, as determined in this study, the glutelins was the main fraction of the protein extracted from rice (Sing & Matta, 2008) and guava (Bernardino-Nicanor, Scilingo, Añon, & Davila, 2006) seeds.

Distribution of molecular weight
SDS-PAGE analysis results for the four protein fractions from jackfruit seed defatted flour at nonreducing and reducing conditions are shown in Figure 1. As observed in the nonreducing patterns, the albumin and globulin fractions have slightly different polypeptide composition profiles. The glutelin fraction appears as smears of two bands around the 20 and 15 kDa, in contrast with bands of high molecular mass (higher than 94 kDa) observed in glutelins from guava seed (Bernardino-Nicanor et al., 2006), while the globulin fraction had six bands at 32, 29, 21, 19, 15 and 10 kDa with the 21–10-kDa in the highest proportion, in comparison with the globulins from grape seed endosperm which showed many bands in the 25–65 kDa range, in particular, three major polypeptides of about 35, 40 and 60 kDa, as well as an approximately 8 kDa band (Gazzola et al., 2014). Moreover, the albumin fraction had five bands at 32, 29, 23, 19 and 15 kDa, while the albumin from Akebia trifoliata var. australis seed displayed eight distinct subunits with major bands at about 49.0, 46.5, 35.6, 30.3, 19.0, 16.5, 13.9 and 12.3 kDa, but the most concentrated bands were at 30.3 and 49.0 kDa (Du et al., 2012). Under reducing conditions, albumin, globulin and glutelin fractions had similar peptide compositions as the nonreducing gel, which suggests minimal concentrations on disulfide bonds in the proteins (Ajibola, Malomo, Fagbemi, Rotimi, & Aluko, 2016). The albumin and globulin fractions had six bands at 31, 27, 23, 20, 15 and 12 kDa. The prolamin fraction was not detected in reducing and without reducing conditions, suggesting that the protein in the extraction fractions was not very soluble in the reagent buffer and was possibly denatured by the organic solvent used for their extraction, such as has been observed in canola proteins (Tan et al., 2011).
Protein extraction from defatted jackfruit seed flour

Figure 2 shows the effect of the extraction pH on the protein solubility from the defatted jackfruit seed flour. According to the results, the maximal protein solubility was 80% at pH 12 and the minimal protein solubility was 19.4% at pH 4, which was considered to be the isoelectric point. The results of the maximal protein extraction at pH 12 for the defatted jackfruit seed flour from this study were similar to the results obtained from physic nut seed cake, which had an extraction of 81.7% at pH 12, whereas the lowest protein extraction was at pH 4.0 (Saetae, Kleekayai, Jayasena, & Suntornsuk, 2011). Therefore, a pH of 12.0 was selected as the pH with the highest extraction yield and was used for preparation of the protein isolates.

Table 1 shows the proximal composition of the defatted jackfruit seed flour and the protein isolates obtained in this study. Adetolu (2008) reported the following proximal composition (g/kg as dry basis) for jackfruit seed flour: total carbohydrates 606.1, protein 207.7, ash 69.1 and fat 117.1. The proximal composition reported for jackfruit seed flour by the author mentioned above shows significant differences with respect to the results of this study; these differences were likely because the seeds come from different jackfruit varieties or because the jackfruit flour analyzed in this study was defatted. Conversely, the protein composition of jackfruit seed protein isolate (JSPI) and of JPIU was 844.3 and 952.1 g/kg (as dry basis), respectively, which indicates the beneficial effect of ultrasound treatment on the protein concentration: an increases of 12.77%. Preece, Hooshayr, Krijgsman, Fryer, and Zuidamb (2016) reported that the effect of ultrasound on separation and extraction of soy protein intensifies the extraction of valuable components from soybeans, leading to improved yields of protein, oil and solids of ca. 10% after 1 min treatment. According to the microstructural analysis undertaken in the study mentioned above, the improved solubility was the main cause of the improved yields upon ultrasound treatment, and not cell disruption as is frequently stated in the literature. In other study, the protein yield (%) and content (%) of duck liver protein isolate from conventional extraction and ultrasound assisted extraction were increased from 42.6 to 74.5 and from 76.5 to 80.2, respectively (Zou et al., 2017).

The protein content of JPIU in this study was greater than the protein content of other protein isolates from fruit seed. Wani, Sogi, Singh, and Shivhare (2011) reported that the protein contents as dry basis were 837. and 790.5 g/kg for protein isolates from watermelon seeds of the Mateera and Sugar baby varieties, respectively, whereas the protein contents of the protein isolates from wild apricot kernel press cake (Sharma, Tilakratne, & Gupta, 2010) and tomato seeds (Savadkoohi & Farahnaky, 2012) were 765.8 and 821.5 g/kg, respectively.

Color and apparent density

The color values L*, a* and b* of JPIU are shown in Table 2. The JPIU in this study was darker in color (L* = 65.14,
Table 2. Color and apparent density of the jackfruit seed protein isolate treated with ultrasound (JPIU).

Table 2. Color y densidad aparente del aislado proteínico de semilla de jaca tratado con ultrasonido (APJU).

| Property                        | Value                  |
|--------------------------------|------------------------|
| \( L^* \) (Lightness)          | 65.14 ± 0.38           |
| \( a^* \) (Redness-greenness)  | −0.63 ± 0.01           |
| \( b^* \) (Yellowness-blue ness) | 23.17 ± 0.69          |
| Apparent density (g/ml)        | 0.36 ± 0.01            |

*Each value is expressed as the mean ± standard deviation (n = 3).
*Cada valor se expresa como promedio ± desviación estándar (n = 3).

\( a^* = 5.16, b^* = 23.17 \) than the safflower protein isolate obtained by ultrafiltration \( (L^* = 78.12, a^* = −0.63, b^* = 20.01) \) (Ulloa, Rosas-Ulloa, & Ulloa-Rangel, 2011) but lighter than the physic nut seed protein isolate \( (L^* = 36.05, a^* = 3.60, b^* = 5.95) \) (Saetae et al., 2011). The apparent density of JPIU was 0.46 g/ml (Table 2). The apparent density depends on the combined effects of interrelated factors, such as the attractive forces between particles, the particle size and the number of contact points between the particles. The apparent densities of the protein isolates from tomato seeds (Liadakis, Tzia, Oreopoulou, & Thomopoulos, 1998) and passion fruit seeds (Martínez, Medina, & Zambrano, 2011) were 0.33 and 0.43 g/mm, respectively, which are less than the apparent density of JPIU in this study.

**Amino acid composition and protein quality**

The results of the amino acid profile of JPIU are presented in Table 3. Total essential amino acid content of JPIU is slightly lower than the total nonessential amino acid content, which implies that it may be considered as a moderately good source of essential nutrients for human (Azeez, Lasekan, Jinap, & Sulaiman, 2015). From total content of essential amino acids of JPIU, total content of aromatic amino acids (phenylalanine + tyrosine, 15.96 g/16 g N) was highest followed of total content of sulfur amino acids (methionine + cysteine, 7.06 g/16 g N), threonine (5.79 g/16 g N) and lysine (5.72 g/16 g N), but such values are higher than the requirement of FAO/WHO. Food and Agriculture Organization of the United Nations/World Health Organization (1991) for adults. On the other hand, and considering the lower values of amino acid scores (Table 3), the first, second and third limiting amino acids for JPIU were valine, isoleucine and leucine, respectively, being 76.00, 80.25 and 98.43. Therefore, considering the amino acids requirements of FAO/WHO. Food and Agriculture Organization of the United Nations/World Health Organization (1991) standard for adults, the proteins of JPIU have a good balance of essential amino acids. With respect to the predicted PER, the value obtained for the JPIU of this study was 2.36, which is higher than the values 2.29, 2.14 and 2.04 for the protein isolates of kabuli chickpea, desi chickpea and soy, respectively (Wang et al., 2010).

**Water and oil absorption capacity and emulsifying capacity**

The factors affecting the water binding capacity of the protein include the amino acid composition, the protein conformation, the surface polarity and the surface hydrophobicity. The JPIU in this study had a water absorption capacity of 6.46 ml water/g protein (Table 4), which is greater than the values of 3.22 ml water/g protein, 3.57 ml water/g protein and 3.13 ml water/g protein of physic nut protein isolate (Saetae et al., 2011) and watermelon seed protein isolates from Sugar baby and Mateera varieties, respectively (Wani et al., 2011). This result indicates that JPIU had good water binding capacity possibly due to the interactions between polar amino acid residues of the protein and molecules of water. The water absorption capacity is a critical property of proteins in viscous foods such as soups, doughs, custards and baked products because these foods are supposed to absorb water without protein.

Table 3. Amino acid composition and nutritive quality of jackfruit seed protein isolate treated with ultrasound (JPIU).

Table 3. Composición de aminoácidos y calidad nutritiva del aislado proteínico de semilla de jaca tratado con ultrasonido (APJU).

| Amino acid                        | Jackfruit protein isolate (g/16 g N) | Amino acid score | FAO/WHO (1991) reference for adults |
|-----------------------------------|-------------------------------------|------------------|-------------------------------------|
| Essential amino acids             |                                      |                  |                                     |
| Lysine                            | 5.72 ± 0.10                         | 104.00 ± 4.45    | 5.5                                 |
| Methionine + cysteine             | 7.06 ± 0.07                         | 201.71 ± 7.83    | 3.5                                 |
| Cystine                           | 6.48 ± 0.05                         | 144.75 ± 6.39    | 4.0                                 |
| Threonine                         | 5.79 ± 0.08                         | 80.25 ± 3.85     | 4.0                                 |
| Isoleucine                        | 3.21 ± 0.07                         | Not determined   | Not determined                      |
| Tryptophan                        | Not determined                      | Not determined   | 1.0                                 |
| Valine                            | 3.80 ± 0.07                         | 76.00 ± 3.79     | 5.0                                 |
| Leucine                           | 6.89 ± 0.05                         | 98.43 ± 2.79     | 7.0                                 |
| Phenylalanine + tyrosine          | 15.96 ± 0.08                        | 266.00 ± 7.24    | 6.0                                 |
| Total essential amino acids       | 48.43 ± 0.12                        |                  |                                     |
| Nonsental amino acids             |                                      |                  |                                     |
| Arginine                          | 12.24 ± 0.06                        |                  |                                     |
| Aspartic acid                     | 7.58 ± 0.05                         |                  |                                     |
| Serine                            | 9.99 ± 0.09                         |                  |                                     |
| Glutamic acid                     | 8.23 ± 0.06                         |                  |                                     |
| Proline                           | 2.13 ± 0.07                         |                  |                                     |
| Glycine                           | 5.27 ± 0.09                         |                  |                                     |
| Alanine                           | 4.06 ± 0.02                         |                  |                                     |
| Histidine                         | 2.07 ± 0.04                         |                  |                                     |
| Total nonessential amino acids    | 51.57 ± 0.14                        |                  |                                     |
| Predicted PER                     | 2.36 ± 0.07                         |                  |                                     |
dissolution, which thereby provides body, thickening and viscosity (Seena & Sridhar, 2005).

The oil binding capacity is another important functional property of proteins in food systems. Nonpolar amino acid side chains of proteins can form hydrophobic interactions with lipid hydrocarbon chains that affect the oil binding capacity. The oil absorption capacity of JPIU was 6.07 ml oil/g protein (Table 4), which is greater than the values of 1.86 ml oil/g protein for physic nut protein isolate (Saetae et al., 2011), 2.37 ml oil/g protein and 2.49 ml oil/g protein for watermelon seed protein isolates from Sugar baby and Mateera varieties (Seena & Sridhar, 2005), 3.2 ml oil/g protein for guava seed protein isolate (Bernardino Nicanor et al., 2001) and 4.04 ml oil/g protein for tomato seed protein isolate (Liadakis et al., 1998). This result indicated that JPIU had a high content of nonpolar amino acids that can bind with lipid hydrocarbon chains. The mechanism of fat/oil absorption can be explained as the physical entrapment of oil. The fat/oil absorption capacity is a critical determinant of flavor retention. A high oil absorption capacity of proteins is required in the formulation of ground meat and of replacements and extenders for use in products such as doughnuts, baked goods, frankfurters, sausages and soups (Kaur & Singh, 2007; Singh, Kumar, & Bawa, 2008).

JPIU had an emulsifying capacity of 32.36 ml oil/g protein (Table 4). Previous reports have indicated that the emulsifying capacity is 115 ml oil/g protein, 164–169 ml oil/g protein and 130 ml oil/g protein for tomato seed (Liadakis et al., 1998), lupin (El-Adawy, Rahma, El-Bedawey, & Gafar, 2001) and sesame (Khalid, Babiker, & El Tinay, 2003) protein isolates, respectively. Therefore, the JPIU exhibited a lower emulsifying capacity than these other plant proteins.

Protein solubility of the protein isolate

Protein solubility relates to the surface hydrophobic (protein–protein) and hydrophilic (protein–solvent) interactions with water. It is affected by several factors, for example, the composition of the amino acids and the non-amino acids of the protein, the native or denatured state of the proteins and environmental factors (Saetae et al., 2011). The pH is an important environmental factor that has a significant effect on the solubility of proteins. The protein solubility of JPIU as a function of pH is shown in Figure 3. The data show two regions of protein solubility: at acidic pH, which includes the isoelectric point, and at alkaline pH. The minimum protein solubility was observed at pH 4 (5.2%), which indicates the isoelectric point of the protein; at pH 7 and 11, 44.0% and 94.4% of the protein were soluble. These results suggest that JPIU has good solubility under basic conditions, which agrees with the results for the protein isolate obtained from physic nut seed cake (Saetae et al., 2011). The protein solubility at different pH values may serve as a useful indicator of the performance of protein isolates in food systems in addition to the extent of protein denaturation as a result of heat or chemical treatment. Most plant proteins have isoelectric pH values between 4 and 5. At the isoelectric point, there is no net charge on the protein; as a result, there are no repulsive interactions or protein–protein interactions disfavoring solubility. At a low pH, large net charges are induced and the repulsive forces increase, which results in protein unfolding. At a pH greater than 6.5, all plant proteins have solubilities of >70% (Bora, 2002); however, in this study, we observed lower values.

Table 4. Some functional properties of the jackfruit seed protein isolate treated with ultrasound (JPIU).*

| Property                     | Value          |
|------------------------------|----------------|
| Water absorption capacity (ml water/g protein) | 6.46 ± 0.65 |
| Oil absorption capacity (ml oil/g protein)      | 6.07 ± 0.36   |
| Emulsifying capacity (ml oil/g protein)         | 32.26 ± 0.70   |

*Each value is expressed as the mean ± standard deviation (n = 3).

Figure 3. Effect of pH on the protein solubility of the jackfruit seed protein isolate. Data are the mean ± standard deviation of three replicates. Mean values labeled with different letters are significantly different (p < 0.05).

Figura 3. Efecto del pH en la solubilidad del aislado proteínico de semilla de jaca. Los datos son el promedio ± desviación estándar de tres réplicas. Los valores promedio etiquetados con diferentes letras son significativamente distintos (p < 0.05).
**Least gelation concentration**

The least gelation concentration indicates the gelation capacity; thus, a lower least gelation concentration improves the gelling ability of proteins. The gel formation of a protein is the result of the partial denaturation of proteins as well as protein aggregation. Protein denaturation allows for the exposure of reactive groups inside protein molecules, and aggregation improves water retention into the three-dimensional network structure of the protein’s reactive sides (Withana-Gamage, Wanasundara, Pietrasik, & Shand, 2010). The least or lowest gelation concentration of proteins from JPIU was 9% at pH 6 (Figure 4). The least gelation concentrations of chickpea protein, northern bean protein concentrate, cowpea, mung bean protein isolate, lupin seed protein (Kaur & Singh, 2007) and safflower protein isolate (Ulloa et al., 2011) are 14–18%, 8%, 12%, 10%, 14% and 2%, respectively.

**EA and emulsion stability**

Protein molecules are generally composed of nonpolar amino acids, charged amino acids and non-charged polar amino acids. These types of amino acid cause hydrophobic and hydrophilic properties such that proteins can interact with both oil and water molecules and act as emulsifiers. The emulsifying properties of JPIU are shown in Figure 5. In our study, the lowest values of the EA and the emulsion stability occurred at pH 4 and 2, respectively, which can be attributed to the low solubility of proteins at these pH values. Conversely, the EA (127%) and emulsion stability (127%) for JPIU were greater at pH 10 and 4–10, respectively, whereas the higher EA and emulsion stability for tomato seed protein isolate were 36.4% and 34.9%, respectively (Liadakis et al., 1998). The emulsifying properties of JPIU exhibit a similar pH-dependent behavior to the flaxseed protein concentrate (Martínez et al., 2006), the safflower protein isolate (Ulloa et al., 2011) and the physic nut seed protein isolate (Saetae et al., 2011).

**Foaming capacity and FS**

In food systems, foams are often very complex and include several phases, such as a mixture of gases, subdivided solids and liquids and multicomponent solutions of water, polymers and surfactants. The foam capacity of JPIU was pH dependent, as shown in Figure 6. The foam capacity was found to be low in the range of pH 4.0–6.0 with values of 13–42%, and the lowest value was at pH 4. The foam capacity of the protein isolate increased when subject to basic pH conditions and reached a maximum at pH 10 (254%). Greater values appear to be due to increasing solubility and increased net charges of JPIU where the hydrophobic interactions are weak and the flexibility of protein is increased. This caused increasing protein diffusion to the air–water interface for encapsulating air particles and enhancing foam formation. The maximum foaming capacity (254%) and FS (164%) were observed at pH 10, whereas the minimum foaming capacity (13%) and FS (68%) occurred at pH 4 and 8, respectively. In contrast to the results obtained in this study, the foaming capacity and stability of lupin protein concentrate were greatest at acidic pH values (Sathe, Deshpande, & Salunkhe, 1982). The maximum foaming capacity and FS for guava seed (Bernardino-Nicanor et al., 2001) and tomato seed (Liadakis et al., 1998) isolates were 50% and 40% and 64.9% and 66.3%, respectively. The major protein molecules of jackfruit seed may be globular proteins, which are difficult to surface denature and cause low foam capacity (Saetae et al., 2011). The foam capacity is usually an important factor in food products such as bread, cakes, toppings, whipped cream, ice cream, chiffon desserts and some confectionery products (Singh et al., 2008). Viewed as a whole, JPIU could be considered to act as a great foaming ingredient in different food products.

![Figure 4](image-url)
Conclusions

Jackfruit seed, a by-product of the fruit industry, is a potential raw material for the production of a protein isolate when subjected to ultrasound treatment. The JPIU showed good functional properties in terms of its water and oil binding capacity, protein solubility, gelation, emulsifying and foaming, in addition a good balance of essential amino acids which place it as of regular nutritive quality. Therefore, JPIU could be a novel protein source applied in food systems and may be suitable to add to breads, cakes, toppings, beverages, whipped cream, ice cream, chiffon desserts, salad dressing, sausage and meat products.

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Seena, S., & Sridhar, K.R. (2005). Physicochemical, functional and cooking properties of under explored legumes, Canavalia of the southwest coast of India. Food Research International, 38, 803–814. doi:10.1016/j.foodres.2005.02.007

Sharma, P.C., Tilakratne, B.M., & Gupta, A. (2010). Utilization of wild apricot kernel press cake for extraction of protein isolate. Journal of Food Science and Technology, 47, 682–685. doi:10.1007/s13197-010-0096-z

Siddeeg, A., Xu, Y., Jiang, Q., & Xia, W. (2015). In vitro antioxidant activity of protein fractions extracted from seinat (Cucumis melo var. tibish) seeds. Cyta - Journal of Food, 13, 472–481. doi:10.1080/19476337.2014.1003199

Sing, A., & Matta, N.K. (2008). Variation in protein fractions and their correlation studies in rice. Indian Journal of Crop Science, 3, 83–86.

Singh, P., Kumar, S.N., & Bawa, A.S. (2008). Functional and edible uses of soy proteins products. Comprehensive Reviews in Food Science and Food Safety, 7, 14–28. doi:10.1111/j.1541-4337.2007.00025.x

Sosulski, F.W. (1962). The centrifuge method for determination of water absorption in hard red spring wheats. Cereal Chemistry, 39, 344–351.

Tan, S.H., Mailer, R.J., Blanchard, C.L., & Agboola, S.O. (2011). Extraction and characterization of protein fractions from Australian canola meals. Food Research International, 44, 1075–1082. doi:10.1016/j.foodres.2011.03.023

Ulloa, J.A., Rosas-Ulloa, P., & Ulloa-Rangel, B.E. (2011). Physicochemical and functional properties of a protein isolate produced from safflower (Carthamus tinctorius L.) meal by ultrafiltration. Journal of the Science of Food and Agriculture, 91, 572–577. doi:10.1002/jsfa.4227

Withana-Gamge, T.S., Wanasundara, J.P.D., Pietrasik, Z., & Shand, P. (2010). Physicochemical, thermal and functional characterisation of protein isolates from Kabuli and Desi chickpea (Cicer arietinum L.): A comparative study with soy (Glycine max) and pea (Pisum sativum L.). Journal of the Science of Food and Agriculture, 91, 1022–1031. doi:10.1002/jsfa.4277

Yasamatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., & Wada, T. (1972). Whipping and emulsifying properties of the soy products. Agricultural and Biological Chemistry, 39, 719–728. doi:10.1080/00021369.1972.10860321

Zou, Y., Wang, L., Li, P., Cai, P., Zhang, M., Sun, Z., & Wang, D. (2017). Effects of ultrasound assisted extraction on the physicochemical, structural and functional characteristics of duck liver protein isolate. Process Biochemistry, 52, 174–182. doi:10.1016/j.procbio.2016.09.027