



Isolation, Structural and Functional Characterization of Jackfruit (*Artocarpus heterophyllus* Lam) Seed Proteins

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Abstract

With increasing health awareness, the demand for concentrated proteins from plant sources has been rising for direct consumption or to use as an ingredient in food formulation. The characteristic information of any component is prerequisite for its efficient usage in food preparation. This study was undertaken to isolate the protein from jackfruit seeds and characterizing the physicochemical and functional properties of the isolated protein. The protein fraction from jackfruit seed flour was isolated using pH treatments and centrifugation process. The isolated protein was converted into powder form by a vacuum drying and grinding method. Total protein content in the isolate was determined by the Kjeldahl method. The functional properties such as solubility and gelling capacity and secondary structural elements of jackfruit seed protein isolate (JSPI) were studied. The crude JSPI contained 76.89% protein with 58.44% solubility in aquatic solvent. The conformational study by Fourier-transform infrared spectroscopy (FTIR) indicated that the β -sheet is the dominant secondary structure of JSPI that contained 50.28% β -sheet, 21.71% α -helix, 8.86% β -turn, and 19.15% unordered structure. The least gelation concentration of JSPI dissolved in 1.0 M NaCl solution was 12%. The pH of the solvent significantly affected the emulsifying and foaming properties ($p < 0.05$). Based on the observed structural and functional features, JSPI has prospects to be used as a supplementary ingredient in future food formulations.

Keywords: Protein isolate, functional properties, emulsion capacity, gelling ability, vacuum dryer and protein conformation

1. Introduction

Protein is one of the most abundant components, which take part in constituting every cell in the living being. About 65 % of the total demand for protein is being met from animal sources, whereas 35% is from plant sources (Pasiakos *et al.*, 2015). However, the demand for plant-derived proteins is continuously increasing due

to their health benefits, easy digestibility, and economic rationales (Houde *et al.*, 2018). Isolation and functional characterization of cereal and bean proteins such as wheat, flaxseed, and lentil protein have received worthwhile attention by the researchers (Idris *et al.*, 2003; Kaushik *et al.*, 2016; Joshi *et al.*, 2011). Nevertheless, in most of the cases, isolation of those proteins and their possible usage in new

food formulations can add a little nutritional benefit. Because the majority portion of those cereals and beans may be consumed as the original form with minimal processing. Therefore, searching for proteins from low-cost and underutilized sources, their isolation and characterization with a view to utilizing them in new food formulations able to harvest maximum benefits with respect to economic and environmental aspects. The jackfruit seed is a strong alternate for this purpose. Jackfruit seeds (JFSs) consist around 10 to 15% of the total fruit weight, having starch and protein content of 79.34% and 13.50% respectively, on a dry basis (Ocloo *et al.*, 2010).

This fruit grows abundantly in Southeast Asian countries such as Indonesia, Burma, and India as well as in some areas of Brazil, and Queensland of Australia. Jackfruit is the national fruit of Bangladesh that produces nearly one million tons in a year (BBS, 2015). A jackfruit may contain up to 500 juicy cells enclosing a seed each of them. The juicy flesh is the edible part of the fruit. A good portion of the seeds is discarded in the environment as social tradition, although a portion is consumed after minimal processing like roasting and boiling. These seeds can, however, be used as a good source of proteins, if processed suitably. Considering 14% of the total weight of jackfruit is made up of seeds, a share 140 thousand metric tons JFS are underutilized in Bangladesh. Moreover, a considerable amount of seeds is damaged due to the lack of proper storage costing a huge national loss every year. This loss can be overcome by using the ingredients of JFS in selected food products, such as cakes and biscuits (Hasan *et al.*, 2010).

Prior information regarding structural and functional properties of isolated proteins helps to use them with minimum capital investment in the new food formulation. For better application as an ingredient, the proteins should ideally possess several desirable functional properties such as solubility, water holding capacity, gelation, and foaming capacity. These functional properties influence the contribution of proteins

in food characters during processing, storage, and consumption (Kinsella, 1979). For example, the solubility of a protein determines the digestibility of the protein enriched foods. This characteristic property (solubility) not only enhances the digestive efficiency of protein but also positively influences other features such as foaming and emulsion ability (Mcwatters and Cherry, 1977). Knowledge of the emulsifying and foaming properties of proteins is necessary to evaluate their potential to use as food additives. Several molecular parameters such as pH, ionic strength, and viscosity are essential determinants in the formation, stability, and textural properties of protein-fat-water emulsions (Zayas and Lin, 1989). The secondary structures of a protein play an essential role in protein folding and unfolding processes. The 3D structure of the protein, which is vital for protein functionality, is attributed to compact and stable secondary structural elements such as α -helix, β -sheet, and β -turn. They are also crucial in determining evolution, size, and geometry of protein (Ji and Li, 2010). Fourier Transform Infrared (FTIR) spectroscopy provides information about the secondary structure of proteins by chemical composition and physical state of the whole sample (Haque *et al.*, 2014).

Although few researchers already studied some functional properties of jackfruit seed protein (Ulloa *et al.*, 2017; Reis *et al.*, 2016), these are not quite sufficient to suggest the pros and cons while intending to use this protein in food preparation. More investigations on the structural and functional properties of JSPI are required to incorporate this protein in future food formulation. Besides, such information of protein from Bangladeshi varieties of jackfruit seed is nearly zero in the literature.

Therefore, the current study was designed, firstly, to find an appropriate method for isolation of JFS proteins. Secondly, to analyze the isolated protein for different functional attributes. The protein was also analyzed by infrared spectroscopy to determine its secondary structural properties.

2. Materials and Methods

2.1. Raw materials

Jackfruit seeds were collected from the local market of Narsingdi, Bangladesh. They were sun-dried and the white outer layers of the seeds were separated. After cutting approximately 3×4 mm sized pieces, they were dried by Cabinet dryer at temperature 40 °C to bring the moisture content 10 - 12%. Finally, the seeds were ground to make JFS flour and passed through a sieve (mesh no 75). The obtained powder was packed airtight into containers for further uses.

2.2. Preparation of jackfruit Seed Protein Isolate (JSPI)

JSPI was prepared following the protocols of (Wang *et al.*, 1999; Idris *et al.*, 2003) with slight modification. The seed flour was suspended in distilled water (1:10 w/v). The pH of the slurry was adjusted to 9.0 using 1 M NaOH solution and kept in rest for one hour. The slurry was then centrifuged (at 12,600 g, for 15 min). The supernatant protein solution was separated and adjusted to pH 4.5 using 1 M HCl. The solution was then stirred for 30 min and left undisturbed for cold precipitation overnight (4 °C). The supernatant was carefully siphoned off, and the obtained protein precipitate was collected by centrifugation at 2217 g for 15 min. The accumulated protein was washed 3–4 times with distilled water to eliminate all soluble components. The final protein was poured into a clean tray and dried in vacuum dryer at 60 °C temperature and 85 kPa pressure for 48 h. The dried protein was collected and subsequently pulverized into a fine powder (JSPI) by using a grinder and sieved through sieve (mesh no 75). The resultant powder was vacuumed sealed in high-density polythene package for further use.

2.3. Determination of protein content

The protein content of JSPI was determined by the Kjeldahl method (AOAC, 2005). The value of 6.25 was used as a protein conversion factor. One (1) g of protein powder was used for digestion, distillation, and titration following the standard protocols for Kjeldahl method. The

average values from the replicated experiments were accepted.

2.4. Determination of functional properties of JSPI

2.4.1 Protein solubility

The solubility of JSPI was determined according to the method used by (Beuchat *et al.*, 1975) with slight modification. One (1) g of JSPI powder was diluted into 100 mL of distilled water. The suspension was mixed evenly through continuous stirring for 30 min. The suspension was then kept in the refrigerator (4 °C) for overnight and centrifuged (12,600 g / 20 min). The soluble protein content in the supernatant was determined by the Kjeldahl method (AOAC, 2005). The protein solubility (PS) was estimated from the amount of protein found in the supernatant and the total sample used to prepare the solution following the Equation Number 1.

$$PS (\%) = \left(\frac{\text{percent of protein in the supernatant}}{\text{total protein suspended in the solution}} \right) \times 100 \dots (1)$$

2.4.2 Water holding capacity

The water holding ability of JSPI was determined by the method stated by (Carcea, 1986) with a slight modification. One (1) g of protein sample was suspended into 10 mL of distilled water in a 15 mL graduated conical centrifuge tube. The suspension was stirred evenly and allowed to stand for 1 h at room temperature (27 °C). It was then centrifuged at 2217 g for 30 min, and measured the volume of the supernatant. The water-holding capacity was expressed from the amount of water held by 1.0 g of protein sample.

2.4.3 Oil holding capacity

The oil holding capacity of JSPI was determined following a similar method for water holding capacity, where corn oil was used as a suspension medium instead of water.

2.4.4 Bulk density

The JSPI powder was put into a 25 mL measuring cylinder. The initial weight and the initial volume of the sample were recorded. Then

JSPI was poured into the cylinder properly. The cylinder was tapped frequently during pouring of protein to avoid the void space. Again, the weight and the volume of the sample were recorded. The final weight and the final volume of the sample were recorded from these differences. The bulk density (g/mL) was calculated as weight of powder (g) divided by powder volume (mL) (Okaka and Potter, 1979) according to Equation Number 2.

Bulk density (g/mL) = (Final weight of sample / Final volume of sample) -----(2)

2.4.5 Gelation characteristics

The gelation capacity of JSPI was determined according to the method followed by (Coffmann and Gracia, 1977) with a slight modification. A range of sample suspensions of 2 to 14 percentage (w/v) was prepared in 5 mL distilled water and 1.0 M NaCl solution. The test tubes containing these suspensions were then heated for one 1 h in a boiling water bath followed by rapid cooling under running cold tap water. The tubes were further cooled for 2 h at 4 °C. The least gelation concentration (LGC) was determined as the minimum concentration required to form a self-supporting gel when the sample did not fall or slip from the inverted test tube.

2.4.6 Measurement of foam properties

Foaming capacity and stability of JSPI at different pH (1.5-11.5) were determined according to the method followed by (Aruna and Prakash, 1993). The protein samples (2 g) were taken into 250 mL beakers and diluted with 100 mL of distilled water. The pH values were adjusted from 1.5 to 11.5 by using HCl and NaOH. The suspensions were mixed thoroughly using magnetic stirrer, and finally, they were homogenized at 300 g for 5 min in a homogenizer (WiseMix™, HG-15 D). The volume of the produced foam in each beaker was measured by measuring cylinder within no later than 30 s. The increment of foam volume was estimated following the Equation Number 3 and expressed as percent foam capacity. The decrease of foam volume within 30 min was

measured, and the foam stability was calculated using Equation Number 4.

Foaming capacity (%) = (Foam volume / Initial sample volume) × 100 ----- (3)

Foaming stability (%) = (Foam volume after 30 minutes / Initial foam volume) × 100 ----- (4)

2.4.7 Measurement of emulsion properties

Emulsification capacity of JSPI at different pH (1.5-11.5) was determined according to the method stated by (Beuchat *et al.*, 1975) and expressed as percent of oil emulsified per g of protein. One (1) g of isolated protein powder was taken in a beaker and mixed with 25 mL distilled water. After complete dispersion by stirring using a blender at 8,000 rpm, 5 mL groundnut oil was added and blending was continued until phase separation was seen. The emulsion capacity was estimated based on separated cream following the Equation Number 5. Emulsion stability at different pH (1.5-11.5) was determined according to the method of (Pearce and Kinsella, 1978). The prepared emulsions were transferred into test tubes and held at 70 °C in a water bath for 45 min. Then the tubes were allowed to stand at room temperature for 3 h. Percent stability was calculated from the height of the remaining emulsified layer after experimental time to that of the original emulsified layer according to Equation Number 6.

Emulsification capacity (%) = (Cream volume / Initial sample volume) × 100 ----- (5)

Emulsion stability (%) = (Cream volume after 3 h / Initial cream volume) × 100 ----- (6)

2.4.8 Statistical analysis

The experiments were carried out in triplicate and the average values are reported in the ensuing sections. The statistical analyses were carried out by using data analysis tools, Microsoft Office Excel 2010. The significance difference between two mean values was determined using one way analysis of variance at 95% confidence level (p<0.05). The inbuilt program in Microsoft Excel™ was used for this purpose.

2.5. Secondary structural analysis

Fourier Transform Infrared (FTIR) spectrophotometer was used in the present study to observe the conformation and secondary structural motifs of the isolated protein. The IR spectra were acquired through Perkin Elmer FTIR (Spectrum-2) instrument operated by CPU32M software. The JSPI powder was scanned within 450 to 4000 cm^{-1} using a triglycine sulfate (TGS) detector. A total of 8 scans at 4 cm^{-1} resolution were accumulated at 0.2 cm/sec scanning speed. The JFS flour and the separated JFS starch were also scanned to observe the comparative spectra among those materials. The protein sample was diluted in deionized water at 1% concentration (w/v) for the secondary structural quantification. The spectrum of the aqueous protein solution was acquired in the same IR conditions described above. The blank spectrum of water was subtracted from the protein spectrum. The baseline subtracted protein spectra were analyzed by using Perkin Elmer's proprietary software (Version 10.05.03) and a peak fit software Peak Fit version 4.12 (Sea Solve Software Inc. Framingham, USA). The peak Fit Software was used for quantitative analysis of the secondary structure of the protein. The original spectra (of amide region-I, 1600-1700 cm^{-1}) without any smoothing were fitted with Gaussian shape and were analyzed by local least square (LLS) algorithm. Percentage of secondary structures (α -helix, β -sheets, β -turns, and random coils) was estimated using Equation Number 7 (Ngarize *et al.*, 2004; Haque *et al.*, 2015).

$$\text{Secondary structure (\%)} = A_{\text{ind}} / A_{\text{total}} \times 100 \text{ --- (7)}$$

Where, A_{ind} = Sum of the area of individual secondary structure within amide I band

A_{total} = Area of total amide I band

The positions or locations of bands for each secondary structural element (β -sheets, α -helix, and β turns) of tested proteins in H_2O were considered based on the information available in the literature (Kong and Yu, 2007; Haque *et al.*, 2014). As suggested in Table 1, the bands from 1620 to 1640 cm^{-1} and 1674 cm^{-1} to 1680 cm^{-1} were assigned to β -sheets. The bands from 1641 to 1647 cm^{-1} was assigned to random coil. The bands within 1648 and 1660 cm^{-1} were assigned to α -helix. Similarly, the bands appearing at and in the vicinity of 1663 cm^{-1} , 1671 cm^{-1} , 1683 cm^{-1} , 1688 cm^{-1} and 1694 cm^{-1} were assigned to β turns. The peaks between 1600 cm^{-1} - 1619 cm^{-1} were not considered while quantifying the secondary structural features, as they are known to be generated from aromatic side chains (Ngarize *et al.*, 2004).

3. Results and Discussion

3.1. Protein content

The protein content in the JSPI was 76.89%. This quantitative value is comparable with other research findings. For example, Sogi *et al.* (2002) prepared tomato seed protein isolate with 71.32% protein content whereas crude isolate of rice bran protein contained 60% protein (Chandi and Sogi, 2007). On the other hand, (Joshi *et al.*, 2011) estimated about 90% protein in lentil protein isolate. The variation of protein contents in the isolations might be due to the amount of protein fractions in the sources and intra-components bonds in the sources from where the proteins are isolated.

Table 1. Assigned peak locations for the secondary structural properties of JSPI in amide-I region

Peak locations	Secondary structural elements
≤ 1619	Aromatic side chain
1620-1640	β -sheet (low frequency)
1641-1647	Random coil
1648-1660	α -helix
1661-1673	β turn
1674-1680	β -sheet (high frequency)
1681 \geq	β turn

3.2. Functional properties of JSPI

3.2.1 Protein solubility

The solubility of JSPI was determined as 58.44%. This value is comparable with percent solubility of rice bran protein concentrate, which ranged from 47.69% to 73% based on varieties of rice (Chandi and Sogi, 2007). (Joshi *et al.*, 2011) found the percent solubility of vacuum dried lentil protein isolate was about 51%, although they reported higher solubility when the protein was dried using spray dryer and freeze dryer. Protein isolate with high solubility is preferred for direct consumption as well as utilization as a food ingredient. It is worthy to mention that the JSPI in the current study was prepared by using a vacuum dryer and laboratory grinder. However, the literature suggests that spray dried powder possess more amorphous particles, which provides increased solubility during rehydration (Chiou *et al.*, 2008; Haque *et al.*, 2015). Using of spray dryer for commercial production of JSPI is expected to produce protein isolate with higher solubility.

3.2.2 Water holding capacity

Water holding capacity of the JSPI was measured as 2.89 mL H₂O/g protein. High water holding capacity of proteins helps to reduce moisture loss from bakery goods. Also, it is required to maintain freshness and moist mouth feel of baked foods. In comparison, the water holding capacity of JSPI is lower than wheat bran protein, 4.20 mL H₂O /g (Idris *et al.*, 2003) but higher than that of cowpea protein 2.20 mL H₂O/ g (Ragab *et al.*, 2004). The obtained values indicate that a certain portion of JSPI needs to be

supplemented with wheat protein to have adequate swelling and water retention during food preparation.

3.2.3 Oil holding capacity

The oil-holding capacity of JSPI was 1.57 mL oil/g protein, which is slightly lower compared to wheat bran protein 1.70 mL/g (Idris *et al.*, 2003). High oil holding capacity is preferred in the formulation of food systems like sausages, cake batters, mayonnaise, and salad dressings. This property of protein also influences the taste of foods.

3.2.4 Bulk density

This important parameter signifies the behavior of a product in dry mixes and determines the packaging requirement of a product. The bulk density of JSPI was 0.67 g/mL. The obtained value is less compared to the bulk density of casein 0.89 g/mL (Sogi *et al.*, 2002). Low bulk density is advantageous for the formulation of weaning foods regarding packaging and transportation (Onimawo and Egbekun, 1998).

3.2.5 Gelation characteristics

The JSPI did not form a consistent gel in pure water. However, 12% (w/v) JSPI solution in 1.0 M NaCl made a firm gel (Table 2). The current protein isolate showed the lower least gelling concentration (12%) compared to lentil protein isolate (14%) (Joshi *et al.*, 2011). They used phosphate buffer at pH 7.0 instead of NaCl. The lower least gelation concentration implies the greater gelling capacity of the protein (Kaur and Singh, 2007).

Table 2. Gelation capacity (GC) of jackfruit seeds protein isolate in water and NaCl solution

Isolated proteins (%)	GC in H ₂ O	GC in 1.0 M NaCl solution
2	–	–
4	–	–
6	–	+
8	–	++
10	–	++
12	–	+++
14	–	+++

Note: Symbols: – no gel; + weak gel; ++ strong gel; +++ very strong gel

3.2.6 Foaming properties

The foaming capacity (FC) of isolated jackfruit seed protein was found to be pH dependent. As shown in Fig. 1, the alkaline pH provided a favorable condition for enhanced foam formation. The lowest FC (6%) was found at pH 5.5, near the isoelectric point of the protein. JSPI possessed foaming capacity of 42%, 25%, 46%, 58%, and 74% respectively, at pH 1.5, 3.5, 7.5, 9.5, and 11.5. The findings comply with the observations by (N-nadozie *et al.*, 2015) that the alkaline condition of the solution significantly increases the protein solubility and foaming capacity. This trend could be due to the variation of pH affects the net charge and electrostatic balance of the protein solution. In this way, the solubility of protein tends to be minimum near the isoelectric point where the net charge is zero. The higher FC at pH 11.5 was likely due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein (Idris *et*

al., 2003). These phenomena allowed the protein to diffuse more rapidly to the air-water interface to encapsulate air particles and then enhanced the foam formation.

The foaming stability (FS) requires the formation of a thick, cohesive, and viscoelastic film around each gas bubble (Damodaran, 1990; Halling, 1981). Isolated jackfruit seed protein showed poor foam stability at pH 5.5 (0.83%). On either side of pH 5.5, the foaming stability gradually increased and reached its maximum value at pH 11.5 (47%). About 26%, 39%, and 21% FS were observed at pH 7.5, 9.5, and 1.5, respectively (Fig. 1). (Sogi *et al.*, 2002) reported that tomato seed meals had negligible foam stability at pH 5.0, which improved as the pH was increased to 7.0. (Meuser *et al.*, 2001) also observed a considerable effect of pH on the stability of foams. The improvement of FS in the alkaline pH is likely due to increased solubility and surface activity of the soluble protein.

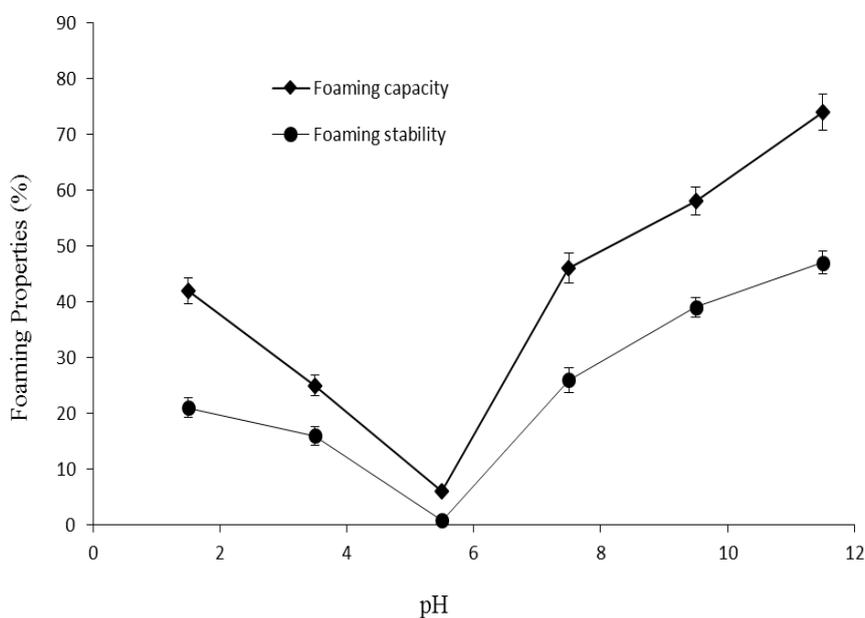


Fig. 1. Effect of pH on the foaming capacity and foaming stability of jackfruit seeds protein isolate (JSPI)

3.2.7 Emulsifying properties

Like foaming properties, the solution of pH has a significant effect on emulsion properties ($p < 0.05$). As shown in Fig. 2, JSPI had a minimum emulsion capacity (EC) (2%) at pH 5.5, which increased on either side of this pH. The pH 11.5 of the solution showed the highest EC (63%), which was followed by the pH 9.5 and 7.5 with EC values of 56% and 25%, respectively. The observed results suggest that the alkaline pH improved the emulsion capacity from the acidic one. Dependence of emulsion capacity on pH was expected, as the emulsion capacity of a protein is known to depend on the hydrophilic-lipophilic balance and electrostatic repulsion at the isoelectric point.

The pH of the solution affected the emulsion stability (ES) also. JSPI had minimum emulsion stability at pH 5.5 (0.43% after 3 h) and reached the maximum value of 52% at pH 11.5 (Fig. 2). Differences observed might be due to the variations of the hydrophilic-lipophilic balance of the protein along with the pH gradient. (Hung

and Zayas, 1991) suggested that various factors including pH, droplet size, net charge, interfacial tension, viscosity, and protein conformation could affect the values of ES.

3.3. Conformational properties of jackfruit seed (JFS) flour and JSPI

The IR spectra presented in Fig. 3 shows that the JFS flour is a mixture of a high amount of starch (band region 900-1200 cm^{-1}) and comparatively low amount of protein (band region 1200-1700 cm^{-1}). The spectral region for carbohydrate prominently erects by stretching vibration of C-C, C-O bonds and deformation of C-O-H, C-O-C bonds of various oligo- and polysaccharides (Naumann, 2000; Grube *et al.*, 2002). On the other hand, the polypeptide and protein repeat units give the characteristic IR bands through vibration by C=O and C-N stretching and N-H bending stresses (Susi and Byler, 1986; Surewicz and Mantsch, 1988). After isolation of protein, the spectrum of JSPI, in Fig. 4, presented the major amide regions of amide I-III (band region 1200-1700 cm^{-1}).

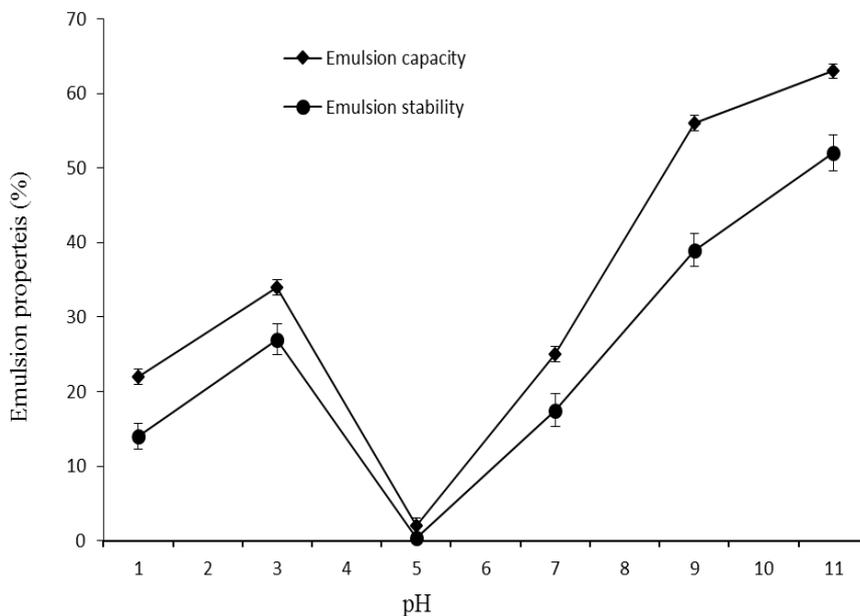


Fig. 2. Effect of pH on the emulsion capacity and foaming stability of jackfruit seeds protein isolate (JSPI)

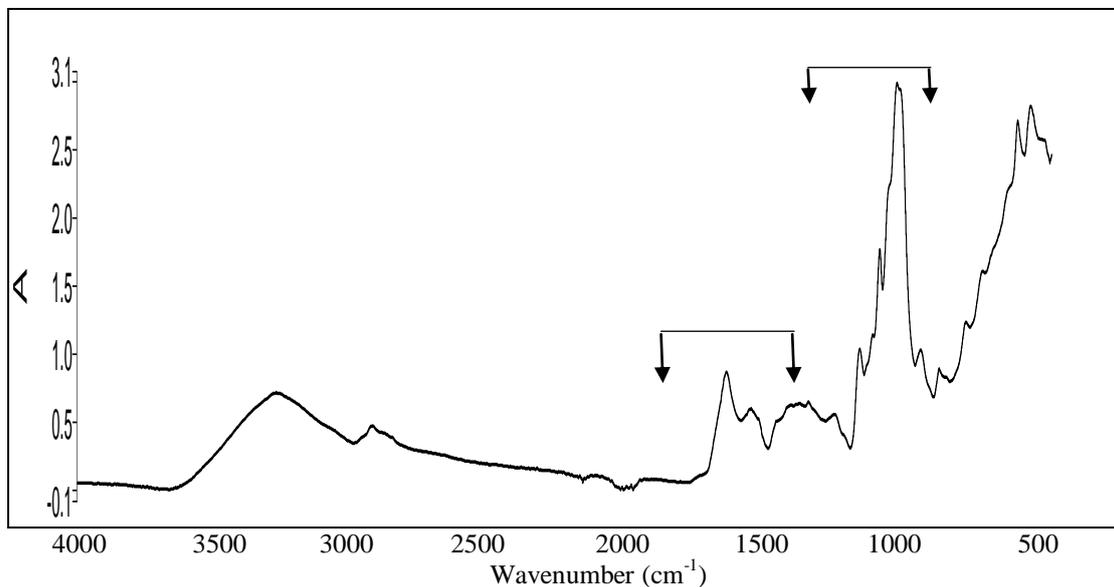


Fig. 3. FTIR absorbance spectra of jackfruit seed flour.

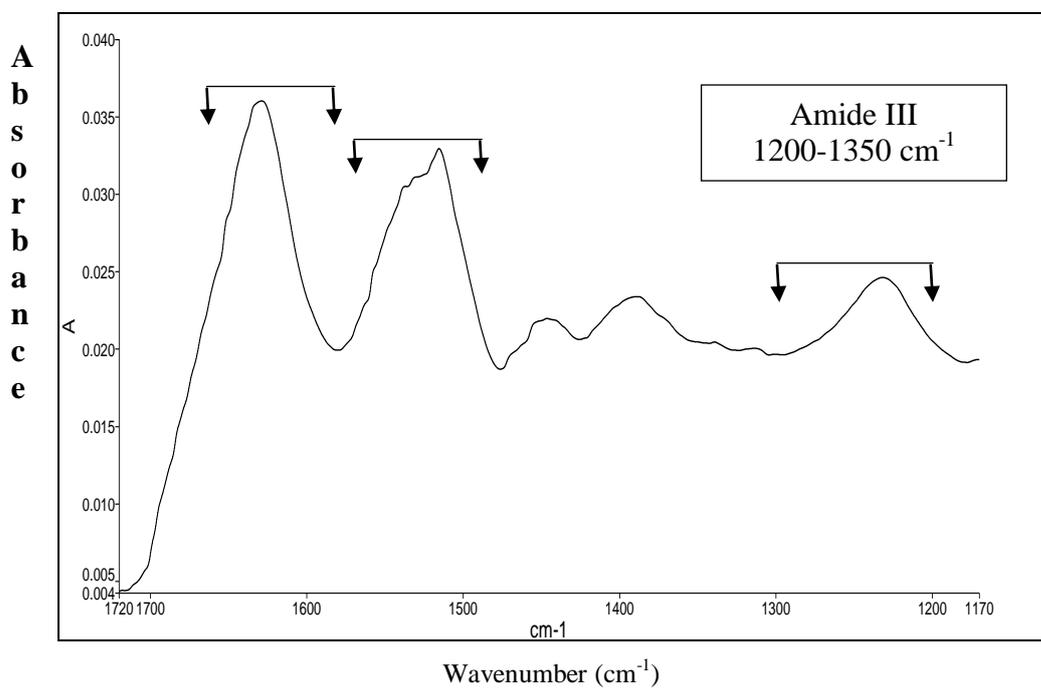


Fig. 4. FTIR spectrums of JFS protein isolate showing the important amide regions

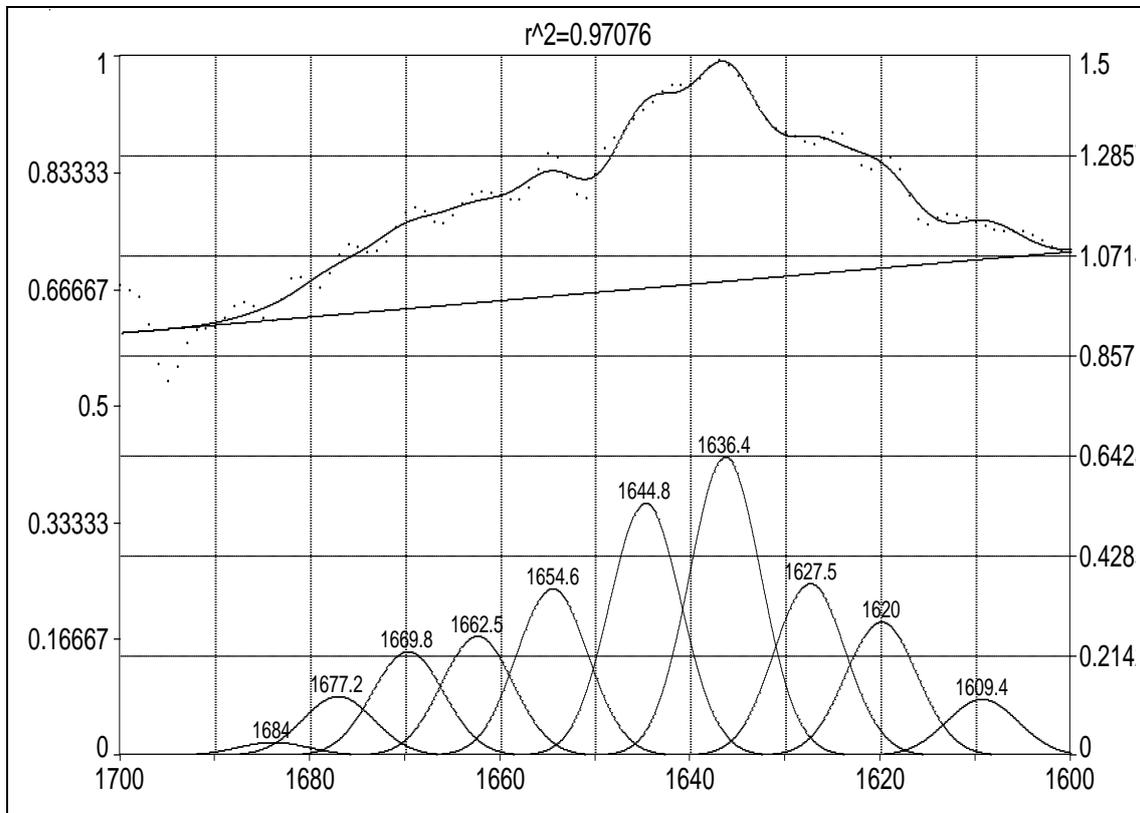


Fig. 5. Fitted spectra of the JFS protein isolate in the amide-I region.

The fitted spectrum for quantification of the secondary structural elements of the protein is presented in Fig. 5. There were nine bands, excluding side chain, produced for the best fitting of the amide-I region (1600-1700 cm^{-1}) with an r^2 value of 0.97. The quantification tools of the assigned bands estimated that the JFS protein contained 50.28% β -sheet, 21.71% α -helix, 8.86% β -turn, and 19.15% unordered structure. The results indicated that the β -sheet is the dominant secondary structure of JFS protein.

4. Conclusions

The protein fraction of the jackfruit seed was successfully isolated through pH treatment and centrifugation process. About 77% proteins were found to be present in the resultant JSPI. It

showed acceptable characteristics for the plan of using JSPI in food formulations. For example, the protein possesses attractive foam formation property, good solubility, and gel-forming ability. Variation of pH in the solution was found to have a significant effect on foaming and emulsion properties. Both the foaming capacity and emulsion capacity reached their maximum values (74% and 63%, respectively) at the pH of 11.5. The least gelling concentration of JSPI in NaCl solution was observed at 12%. Further, the protein isolate possessed adequate water holding capacity, oil holding capacity, and bulk density. The JFS protein contained 50.28% β -sheet, 21.71% α -helix, 8.86% β -turn, and 19.15% unordered properties in the secondary structure. The physicochemical and functional properties of JSPI claim that the jackfruit seed may be a good protein source for the food system.

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