Functional, thermal and structural properties of fractionated protein from waste banana peel

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ABSTRACT

Defatted banana peel powder was fractionated using the Osborne method to extract albumin, globulin, prolamin, and glutelin for physicochemical and functional characterization. The total recovery of protein was ~89.76%. Albumin was the dominant one in terms of yield (62.4%) and protein content (65.15%) among all the fractions. The mean diameter of albumin (635.05 μm) and glutelin (642.62 μm) were significantly smaller than globulin (726.81 μm) and prolamin (986.45 μm). The highest water (1.86 ± 0.12 g/g), oil (1.97 ± 0.12 g/g) holding capacity, and emulsion capacity (59.27 ± 1.25%) were found for the albumin fraction. In contrast, the glutelin fraction showed the highest foaming capacity (19.13 ± 0.41%) and dispersibility (951.55 ± 3.83 g/kg). The denaturation temperature of protein fractions was found in the range of 30.31–82.08 °C. FTIR confirmed low carbohydrates and protein richness of albumin fraction. XRD revealed the crystalline nature of albumin (65%) and the amorphous nature of other fractions (41–45%). The morphology of all fractions was different, which influenced the functional characteristics.

1. Introduction

Banana (*Musa sp.*) is the oldest and most significant tropical fruit devoured worldwide by individuals of all age groups (Vu, Scarlett, & Vuong, 2019). Banana is popular and its consumption is increasing because of its pleasant aroma, soft texture, and high content of flavonoids, phenolic compounds, and anti-oxidants (Mohapatra, Mishra, & Sutar, 2010; Vu et al., 2019). However, a significant issue related to huge consumption is the generation of a large quantity of squanders in the form of peel, which covers around 30–40% of the total fruit weight (Emaga, Andrianaivo, Wathelet, Tchango, & Paquot, 2007). A large quantity of banana peels produced from different enterprises and homegrown family units are thrown in the field or in the water body, which causes ecological issues. Therefore, to overcome these issues and with the motive of financial development, various countries have already started processing banana peels under the waste utilization segment (Rodriguez-Ambriz, Islas-Hernandez, Agama-Acevedo, Tovar, & Bello-Perez, 2008). The chemical composition of banana peel changes during the maturation period and are suitable to produce various products such as wine, flavoring oil, fertilizers, soap, and cellulose nanofibers (Faturoti, Emah, Isife, Tenkouano, & Lemchi, 2006; Khawas & Deka, 2016; Udosen & Enang, 2000). Banana peel powder with

banana fruit powder or other agricultural products may be used for new product development as the banana peel is a rich source of crude protein (6–9%), crude fat (3.8–11%), dietary fiber (43.2–49.7%), various other minerals and free sugar (Mohapatra et al., 2010). However, as banana starts to mature, the concentration of soluble sugar increases along with protein and lipid content, and hemicelluloses decreases (Mohapatra et al., 2010).

Plant proteins are gaining enormous attention and replacing meat proteins because of higher nutritional and environmental advantages. Studies show consuming a diet that is rich in plant-sourced protein improves kidney function (Bernier-Jean et al., 2021), prevents metabolic dysfunctions (Chalvon-Demersay et al., 2017), improves physical performance (Gazzani et al., 2019), and reduces the risk of developing numerous chronic diseases (Lynch, Johnston, & Wharton, 2018). In contrast, the production of plant-based protein is resource-effective and environmentally non-destructive with lower emission of greenhouse gases compared to raising animals (Baroni, Cenci, Tettamanti, & Berati, 2007; Pimentel & Pimentel, 2003). Recently, various plant proteins like protein isolate from quinoa; album seeds came up with a better nutritional profile and biological values and replacing meat proteins (Mir, Riar, & Singh, 2018). Similarly, banana peel which is treated as a waste can be a promising alternative because of its high nutritious value and

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protein occurrence (Salazar, Arancibia, Raza, López-Caballero, & Montero, 2021; Torres-León et al., 2018). Though the extraction of protein from various underutilized and agricultural waste products like winged bean seed (Makeri, Mohamed, Karim, Ramakrishnan, & Muhammad, 2017), prickly pear seed cake (Borchani et al., 2021), jackfruit seed (Ulloa et al., 2017) have been reported in recent years, the studies on physicochemical, structural, and thermal properties are still limited. In addition, the increase in popularity of protein supplements in form of powder, bar, and ready-to-drink is expected to increase the global protein demand. Hence, the recovery of protein from waste (banana peel, whey, etc.) and underutilized sources (pseudocereals) could reduce the burden on stable sources like seeds, and animal products.

To the best of our knowledge, the extraction, classification, and characterization of banana peel protein have not been explored to date. Therefore, the present work was designed to fill the gaps related to banana peel protein fractionation, followed by their physicochemical, functional, and structural characterization.

2. Materials and methods

2.1. Procurement of raw materials

Waste banana peels of locally available varieties (*Musa acuminata*) were collected from the local fruit juice vendors of Sant Longowal Institute of Engineering and Technology campus, Longowal, Punjab, India. 1–1.5 kg of raw banana peels was collected at a time and total of 17 numbers of such samplings were carried out. A total of 15 kg of banana peels were used for this study. Fully matured yellow-colored banana peels, free from any kind of black spots and bruises, were selected. The careful selection was performed to minimize the variation due to the sample. However, these carefully chosen peels may not necessarily reflect the conditions of those discarded in large amounts. This should be considered as a limitation of the study.

2.2. Preparation of banana peel powder

Banana peels were washed after collecting from the fruit juice vendors using 1.25% H_2SO_4 and 1.25% NaOH solution, followed by thorough washing using tap water. Banana peels were then cut into pieces. To stop enzymatic browning, peels were immersed in 0.5% (w/v) citric acid solution for 10 min followed by overnight oven drying at 60 °C. The dried peels were ground using a lab-scale grinder and sieved using a 60-mesh size to get a uniform particle size of 250 μm. The powders were stored in air-tight plastic packets at room temperature (25 °C) for further experiment.

2.3. Proximate analysis of banana peel powder

The moisture content of dried peel powder was 9.97 ± 0.85%, determined by the gravimetric method (method 925.09). Crude protein (method 979.09), crude lipid (method 920.39), and crude fiber (method 962.09) were examined using standard protocol (AOAC, 2000). The loss in the ignition method was used to determine ash content (method 923.03). Total carbohydrate was estimated by subtracting moisture, fat, fiber, protein, and ash from 100. All the proximate values of banana peel powder were reported on dry basis (g/100 g dry solid).

2.4. Osborne fractionation of banana peel protein

Protein fractions, i.e., albumin, globulin, prolamin, and glutelin, were extracted from defatted banana peel powder using the method described by Osborne and Voorhees (1894) with slight modification. Defatted banana peel powder was dissolved in deionized water (Elix 3 Serial No. F7CA82546B, Merk, India) at the ratio of 1:10 (w/v) (pH-7.0) and kept in a magnetic stirrer for overnight mixing. The solution was then centrifuged at 8000 rpm for 15 min. The supernatant was decanted

and saved as Albumin-1. Following the similar procedure, Albumin-2 and Albumin-3 were collected from the supernatant of Albumin-1 and Albumin-2, respectively. Three stage extractions were basically conducted to extract the maximum amount of protein. Similarly, Globulin-1, 2 and 3, Prolamin-1, 2, and 3, and Glutelin-1, 2, and 3 were extracted using 0.5 N NaCl, 70% aqueous ethanol, and 0.2% NaOH solution, respectively. The rest of the procedure for the extraction of globulin, prolamin, and glutelin was the same as for albumin. After extraction, all the protein solutions were kept in a refrigerator at 4 °C for further processing.

2.5. Preparation of protein powder

To prepare protein powder, supernatants collected from albumin, globulin, prolamin, and glutelin were separately freeze-dried (Allied Frost, Macflow Engineering Pvt Ltd, New Delhi, India) and kept under refrigerated condition in air-tight plastic packets for further analysis.

2.6. Determination of yield and concentration of proteins

The yield of each fraction of protein extracted from banana peel powder was determined according to Eq. (1) (Kaushik, Dowling, McKnight, Barrow, Wang, & Adhikari, 2016).

$$\text{Yield}(\%) = \frac{P}{S} \times 100 \qquad (1)$$

Where, P = Weight of particular protein fraction (g); S = Dry weight of banana peel powder taken for protein extraction (g).

The protein concentration of each fraction was estimated using the Kjeldahl apparatus (Kel Plus-Kes 12 L, Plus-Classic DX, Pelican, India).

2.7. Characterization of protein fractions

2.7.1. Particle size
Protein powder was examined for particle size distribution using a laser light particle size analyzer (Shimadzu SALD-2300, M/s Shimadzu Corporation, Japan). 0.1 g of albumin, globulin, and glutelin protein sample was individually dissolved in 9.9 mL of aqueous ethanol and 0.1 g of prolamin protein sample was dissolved in 9.9 mL of deionized water. After preparation of the sample, protein suspension was vortexed for 2 min to break aggregates and the prepared sample was added dropwise to a cuvette until the refractive index reached in between 20 and 40% range.

2.7.2. Color characteristics
Color characteristics of all the extracted protein fractions were measured in triplicates according to International Commission on Illumination Standard. L*, a*, and b* were calculated using a Hunter Calorimeter equipped with an optical sensor (Hunter Associates Laboratory Inc., Reston, VA, USA). For each protein fraction, three different samples (approx. 2 g) were scanned separately to take three readings of color values. The whiteness index (WI) and total color difference (ΔE) of protein fractions were determined using Eqs. (2) and (3), respectively.

$$\text{WI} = L^* - 3b^* \qquad (2)$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \qquad (3)$$

Where, ΔL^2, Δa^2, and Δb^2 represent the differences between the standard reading (white board) and the instantaneous individual reading of the samples.

2.7.3. Water and oil holding capacity
Water holding and oil holding capacity of protein fractions was determined by the method described by Tounkara et al. (2013) with some minor modifications. 0.5 g of each sample in triplicate was

dissolved with 10 mL of deionized water in a centrifuge tube and vortexed for 30 s. After vortexing solution was kept idle at room temperature (24 °C) for 30 min then centrifuged at 3000 rpm for 25 min. The supernatant was poured in a 47- mm GF/C filter paper (Whatman, UK) and the recovered sediments were precisely weighed, and gain in weight was recorded as water absorption (g/g). To measure oil holding capacity, 1 g of each sample in triplicate was dissolved in 10 mL pure refined soybean oil in a centrifuge tube and vortexing was done for 5 min. After mixing all the samples were kept idle for 30 min at room temperature (24 °C) followed by centrifuging at 3000 rpm for 20 min. The supernatant was immediately decanted and the weight gain of the sample was recorded as oil absorption (g/g). Oil holding capacity was determined according to Eq. (4).

$$\text{Water/Oil holding capacity}(g/g) = \frac{(W_2 - W_1)}{W_s} \qquad (4)$$

Where, W_1 = Weight of centrifuged tube (g); W_2 = Weight of centrifuged tube after draining the supernatant (g); W_s = Sample weight (g, db).

2.7.4. Emulsifying capacity

The emulsifying capacity of all the four protein samples was measured according to Tounkara et al. (2013). 1 g of each protein sample was dissolved in 50 mL of 0.5 N NaCl solution followed by the addition of 50 mL pure refined soybean oil. The mixture was then homogenized to prepare an emulsion. After homogenization, the mixture was kept in a water bath at 90 °C for 10 min followed by centrifuging at 3000 rpm for 20 min. Emulsion capacity was determined according to Eq. (5).

$$\text{Emulsifying capacity}(mL/g) = \frac{(V_1 - V_2)}{W_s} \times 100 \qquad (5)$$

Where, V_1 = Volume of oil mixed to prepare emulsion (mL); V_2 = Volume of oil released after centrifugation (mL); W_s = Sample weight (g, db).

2.7.5. Foaming capacity and foaming stability

The foaming capacity of protein fractions was measured in triplicate, according to Mir, Riar and Singh (2019). Foam stability was determined by the method described by Tounkara, Amza, Lagnika, Le, and Shi (2013) with little modification. In brief, 1 g of each protein sample was dissolved in 100 mL of deionized water and pH of 7.4 was adjusted using 1 N NaOH and 1 N HCl. After pH adjustment, the solution was blended for 3 min using a magnetic stirrer, and immediately after blending, the solution was transferred to a 250 mL measuring cylinder, and volume of foam was recorded. Foaming capacity was measured according to Eq. (6).

$$\text{Foam capacity}(\%) = \frac{V_2}{V_1} \times 100 \qquad (6)$$

Where, V_1 = volume of protein sample solution (mL) and V_2 = volume of foam (mL).

To determine the foam stability, the fall of foam was measured for 1 h at every 10 min interval and during that 1 h period, the cylinder was kept idle at room temperature without any external disturbance.

2.7.6. Dispersibility index

Dispersibility of all the protein samples in triplicate was measured according to Akpossan, Digbeu, Koffi, Kouadio, Dué, and Kouamé (2015) with little modifications. 1 g of each protein sample was dissolved in 25 mL deionized water and vortexing was done for 30 min using a magnetic stirrer followed by centrifuging at 3000 rpm for 20 min. After centrifugation, the supernatant was collected and kept for drying in a hot air oven at 110 °C for 12 h then weighed. The dispersibility index of protein was measured according to Eq. (7).

$$\text{Dispersibility index}(g/kg) = \frac{(W_2 - W_1)}{W_s} \times 1000 \qquad (7)$$

Where, W_1 = Weight of empty petri dish (g); W_2 = Weight of petri dish after 12 h drying (g); W_s = Sample weight (g, db).

2.7.7. Wettability

The wettability of albumin, globulin, prolamin, and glutelin extracted from banana peel powder was measured in triplicate, according to Akpossan et al. (2015) with little modifications. 1 g of each flour sample in triplicate was poured in a 10 mL measuring cylinder. The cylinder with the sample was inverted and held 10 cm above water (deionized water) contained in a 500 mL beaker. To stop the abnormal flow of the sample, the opening of the cylinder was sealed using fingers and gently sample was discharged onto the water surface. The wettability of the protein sample was measured by recording the time taken by the sample to get completely wet.

2.7.8. Thermal properties of protein fractions

Thermal properties of protein fractions such as onset denaturation temperature, peak denaturation temperature, and enthalpy of denaturation were measured using a differential scanning calorimeter (DSC-4000, Perkin Elmer, USA). Protein samples (10–15 mg) were poured in a hermetically sealed aluminum pan and scanning was done within the temperature range of −10 to 120 °C at a scan rate of 10 °C/min.

2.7.9. X-ray diffraction pattern (XRD)

XRD pattern of banana peel-derived protein fractions was measured using an X-ray diffraction meter (D8 ADVANCE, Bruker, Germany). X-ray diffractometer and CuKα < 1 radiation was used for the experiment purpose. Diffractograms were taken between 5° and 70° at a rate of 1.2°/min and with a step size of 0.02°.

2.7.10. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectra were recorded on Perkin Elmer Spectrum (RX-I, FTIR, USA) according to Kumar, Ganesan, Selvaraj, and Rao (2014). The sample was scanned in the spectral range of 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1}.

2.7.11. Scanning electron microscopy (SEM)

A surface electron microscope (JSM7610 F Plus, JEOL, Japan) was used to determine the surface morphology and check the microstructure of the protein fractions, according to Zhu et al. (2018) at 2500× magnification. In brief, Samples were evenly distributed on a conductive adhesive surface, and then covered with gold tape and photographed at an accelerator potential of 25 kV.

2.8. Statistical analysis

All the experiments were performed in triplicate. One-way analysis of variance (ANOVA) was performed to check the variation in properties. Simultaneously, Duncan's multiple range tests (SPSS version 16.0, IBM, USA) were applied to check the homogeneity among the protein fractions at a probability factor of 0.05.

3. Results and discussion

3.1. Proximate composition of banana peel powder

Proximate analysis of banana peel flour showed that total carbohydrate (42.88 ± 0.38%) was the major chemical component present in banana peel flour. However, banana peel flour was found a rich source of crude protein (10.16 ± 0.17%) and crude fat (10.35 ± 0.35%). Emaga, Robert, Ronkart, Wathelet, and Paquot (2008) and Mohapatra et al. (2010) also reported that ripened banana peel is a high source of

Table 1

Yield of protein fractions derived from banana peel powder.

Protein Fractions	Extract I (g)	Extract II (g)	Extract III (g)	Total yield (g)	Yield (%)
Albumin	2.40 ± 0.16^a	2.02 ± 0.24^b	1.92 ± 0.35^b	6.34 ± 0.25^x	62.40
Globulin	0.41 ± 0.12^a	0.37 ± 0.14^a	0.48 ± 0.08^b	1.26 ± 0.14^y	12.40
Prolamin	0.25 ± 0.02^a	0.24 ± 0.06^a	0.20 ± 0.04^b	0.70 ± 0.04^z	6.88
Glutelin	0.31 ± 0.11^a	0.27 ± 0.09^a	0.24 ± 0.06^a	0.82 ± 0.11^z	8.07
				Total 9.12 ± 0.14	89.76

Values are means $\pm$ standard deviation of triplicate. Values with different superscripts a, and b in a row are significantly ($p < 0.05$) different. Values with different superscripts x, y and z in a column are significantly ($p < 0.05$) different.

Table 2

Protein fractions derived from banana peel powder.

Protein Fractions	Extract I (%)	Extract II (%)	Extract III (%)	Total protein (%)
Albumin	27.23 ± 1.15^a	23.10 ± 1.34^a	14.82 ± 1.54^b	65.15 ± 1.36^x
Globulin	4.20 ± 0.43^a	3.35 ± 0.53^a	4.62 ± 0.34^a	12.17 ± 0.66^y
Prolamin	2.95 ± 0.35^a	2.65 ± 0.53^a	1.72 ± 0.12^b	7.32 ± 0.82^z
Glutelin	3.25 ± 0.42^a	2.85 ± 0.15^{ab}	2.12 ± 0.13^b	8.22 ± 0.95^z
			Total	92.86 ± 1.05

Values are means $\pm$ standard deviation of triplicate. Values with different superscripts a, and b in a row are significantly ($p < 0.05$) different. Values with different superscripts x, y and z in a column are significantly ($p < 0.05$) different.

crude protein (6–9%) and crude fat (3.8–11%). The presence of this high amount of protein content in banana peel flour indicates that banana peel flour or protein derived from banana peel flour can be used to develop new or value-added products. Crude fiber and ash content in banana peel flour were found as $11.04 \pm 0.46\%$ and $15.56 \pm 0.27\%$, respectively. Ash is an important factor and higher ash content indicates the availability of nutritionally important mineral elements in banana peel flour.

3.2. Yield and concentration of different protein fractions

The yield of individual proteins was expressed (Table 1) as the percentage of total banana peel crude protein. After adding all the four fractions, total protein content was found $92.6 \pm 2.05\%$, and total yield recovery was 9.12 ± 0.24 g/100 g, which was approximately 89.76% to crude protein (10.16 ± 0.17 g/100 g) of banana peel powder. Albumin showed the highest protein concentration ($65.15 \pm 1.36\%$) (Table 2) and yield (6.34 ± 0.25 g) compared to globulin, prolamin, and glutelin. During extraction of protein fractions, factors like concentration of solvents, time of mixing and centrifugation, and powder particle size might be the reason behind some portion of proteins (around 10% to crude protein) were left with the residues (Tounkara et al., 2013).

Albumin, a protein from the globular protein family, is derived from the plasma of humans and animals as well as from eggs which are extensively used in sectors like; biotechnology, food and beverages, chemicals and enzymes, aquaculture, and others. However, extraction of albumin from other sources is required to develop, to reduce the dependency on animal sources. In this study, albumin showed the highest protein concentration ($65.15 \pm 1.36\%$) and yield (6.34 ± 0.25 g) compared to globulin, prolamin, and glutelin. Ripen banana peel contained a very low quantity of starch (Mohapatra et al., 2010) which is likely to be the reason behind the highest yield and quantity of protein content in the albumin fraction (Agboola, Ng, & Mills, 2005). Apart from

the dominancy of albumin among other protein fractions derived from the banana peel, it has been also noticed that albumin quantity found in banana peels were much higher than others since various plant materials such as fruits, vegetables, and cannery waste exhibit different composition (Amza, Amadou, Balla, & Zhou, 2015; Siddeeg, & Xia, 2015; Ulloa et al., 2017).

The yield of globulin protein derived from banana peel powder was found to be 1.26 ± 0.14 g/100 g of the powder. In terms of protein content, globulin was in second position ($12.17 \pm 0.66\%$) after albumin. However, there was a huge difference between the protein concentration of albumin and globulin. A combination of albumin and globulin protein together showed that banana peel powder had the highest proportion of albumin and globulins. Solubility of albumin and globulin together derived from the banana powder was found to be lower at higher pH, and solubility increased at the pH range between 4.0 and 7.0 (Mowlah, Takano, Kamoi, & Obara, 1982). Therefore, a high quantity of albumin and globulin could indicate that banana peel proteins have the potential to be utilized to produce acidic and neutral beverages.

Yield and protein concentration in prolamin from banana peel

Table 3

Color, functional, thermal and FTIR characteristics of banana peel protein fractions.

Properties		Protein Fraction			
		Albumin	*Globulin*	*Prolamin*	*Glutelin*
Color value	L*	44.92 ± 0.54^c	64.29 ± 0.42^a	50.87 ± 1.12^b	44.12 ± 0.66^c
	a*	2.58 ± 0.29^b	-1.52 ± 0.18^c	5.30 ± 0.18^a	5.22 ± 0.06^a
	b*	15.31 ± 0.05^c	10.60 ± 0.07^d	20.21 ± 0.29^b	28.32 ± 0.07^a
	Whiteness Index	-1.01 ± 0.50^b	32.49 ± 0.62^a	-9.76 ± 0.87^c	-40.84 ± 0.58^d
	ΔE	4.52	7.52	11.24	7.3
Functional Properties	WHC (g/g)	1.86 ± 0.12^a	1.13 ± 0.96^b	0.36 ± 0.11^d	0.96 ± 0.14^c
	OHC (g/g)	1.97 ± 0.12^{ab}	1.51 ± 0.16^b	1.37 ± 0.09^c	1.73 ± 0.11^a
	EC (mL/g)	59.27 ± 1.25^a	49.96 ± 0.25^b	30.77 ± 0.75^c	59.16 ± 0.24^a
	FC (%)	14.33 ± 0.36^d	17.10 ± 0.31^b	15.23 ± 0.28^c	19.13 ± 0.41^a
	Dispersibility (g/kg)	701.38 ± 3.07^d	791.46 ± 2.94^c	924.49 ± 3.82^b	951.55 ± 3.83^a
	Wettability (min)	9.54 ± 0.06^b	9.62 ± 0.50^b	14.32 ± 0.25^a	9.80 ± 0.14^b
Thermal Properties	T_o (°C)	57.80 ± 2.45^a	44.30 ± 1.65^b	20.09 ± 1.55^c	26.95 ± 1.17^d
	T_p (°C)	82.08 ± 0.82^a	74.12 ± 0.35^b	30.31 ± 0.68^c	48.70 ± 0.78^d
	T_e (°C)	117.51 ± 3.15^a	112.93 ± 1.80^b	53.04 ± 1.70^c	102.43 ± 1.80^d
	ΔH (J/g)	78.41 ± 0.90^a	47.03 ± 0.75^b	6.45 ± 0.25^c	13.17 ± 0.62^d
FTIR Peak (cm^{-1})	Amide I	1627	1601	1596	–
	Amide III	–	–	–	1442
	Stretching vibration of C—H	2915	2887	2882	2883
	Stretching of –OH	3254	3261	3370	–
	Stretching vibration of C—O	1015	1015	1015	1015

Values are means $\pm$ standard deviation of triplicate. Values with different superscripts a, b, c and d in a row are significantly ($p < 0.05$) different. (WHC: Water holding capacity; OHC: Oil holding capacity; EC: Emulsion capacity; FC: Foaming capacity; T_o: Onset temperature; T_e: End temperature; T_p: Denaturation temperature; ΔH: Enthalpy of denaturation).

powder was found 0.70 ± 0.04 g and 7.32 ± 0.82%, respectively, which was the lowest among all fractions. Almost all the proteins derived from fruits, vegetables, and cannery wastes showed such a low quantity of prolamin content (Amza et al., 2015; Siddeeg and Xia, 2015; Ulloa et al., 2017). Prolamins extracted from various fruits and vegetable wastes were sometimes not even detected during SDS-PAGE analysis as prolamin extracted from fruits, vegetables, and cannery wastes were not very soluble and probably denatured by the ethanol or organic solvent used for extraction (Ulloa et al., 2017). Yield and protein concentration of glutelin from banana peel powder was found 0.82 ± 0.11 g and 8.22 ± 0.95%, respectively. Fractionation of banana peel proteins revealed that peel powder is an indigent source of glutelin and prolamin. Glutelin proteins come under the group of prolamin proteins and are mostly found in cereals (corn, wheat, barley, rice, etc.) in a large quantity.

3.3. Particle size

The particle size of protein fractions was significantly different, with no significant difference (p < 0.05) between albumin and glutelin fractions. The mean particle diameter of albumin and glutelin were found 635.05 ± 14.45 μm and 642.62 ± 10.39 μm, respectively. These diameters were much smaller than globulin (726.81 ± 6.80 μm) and prolamin (986.45 ± 9.45 μm). In addition, the particle size of albumin and glutelin makes them better protein fractions in terms of promising functional properties than prolamin and globulin. It has been reported that smaller particle size promotes the dissolution rate of protein in the aqueous phase, which results in better functional properties (Kumarakuru, Reddy, & Haripriya, 2018). However, as the quantity of glutelin was very low, the functional properties of albumin derived from banana peel were found significantly promising and advantageous among all the protein fractions. It has been reported that functional properties such as solubility, protein water interactions, emulsion capacity, foaming capacity, and surface hydrophobicity of various protein isolate increased due to smaller particle size (Mir et al., 2019). Previously, the size of protein isolates from chenopodium album seed (245.63 μm, Mir et al., 2019), and pea protein isolates (192.3 μm, Xiong, Xiong, Ge, Xia, Li, & Chen, 2018) were reported, which were smaller than the size of protein fraction in the present study.

3.4. Color characteristics

Color characteristics (L*, a*, b* values, ΔE, and whiteness index) of different protein fractions are presented in Table 3. Significant differences (p < 0.05) were observed between color parameters. The L* values for different protein fractions varied from 44.12 to 64.29. The L* values for albumin, globulin, prolamin, and glutelin were 44.92 ± 0.54, 64.29 ± 0.42, 50.87 ± 1.12, and 44.12 ± 0.66, respectively. The highest ΔE value was observed for prolamin and the lowest ΔE value was observed for albumin. a* and b* values for different protein fractions ranged between −1.52 to 5.30 and 10.60 to 28.32, respectively. The difference in color parameters of protein fractions arises due to the level of solubilization of color pigments of peel powder in the different solvents. Glutelin protein fraction was the darkest among all the protein fractions, which might be due to the use of NaOH during fractionation, which helped in solubilization of the pigments by weakening the intermolecular interaction such as van der Waals and hydrogen interactions (Grossmann, Ebert, Hinrichs, & Weiss, 2018).

3.5. Functional properties

3.5.1. Water holding capacity (WHC) and oil holding capacity (OHC)
WHC and OHC of all the protein fractions derived from banana peel powder are presented in Table 3. WHC of albumin showed the highest value than globulin, prolamin, and glutelin. However, WHC of prolamin was found negligible. These results validated the higher protein content in the contemplated proteins, which had more significant WHC than
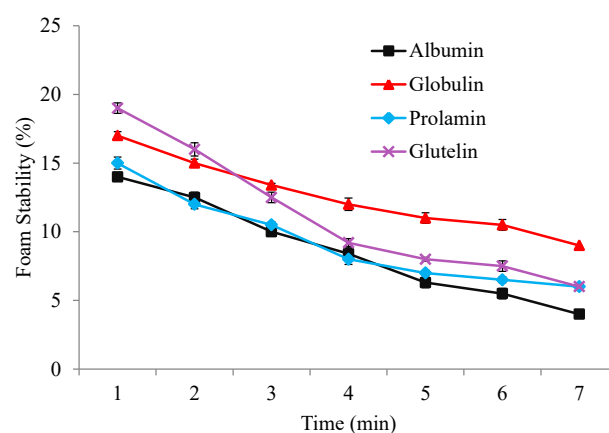

Fig. 1. Foaming stability of banana peel protein fractions.

non-protein fractions or fractions with low protein availability (Borchani et al., 2021). Significant differences were found between the values of water holding capacity for albumin, globulin, prolamin, and glutelin (p < 0.05). These variances were observed might be due to the differences in solubility, molecular weight, and intermolecular interactions of protein fractions (Lawal, Adebowale, Ogunsanwo, Sosanwo, & Bankole, 2005). WHC alludes to the capacity of the protein to guzzle water and hold against gravitational force. Water and protein interactions are significant due to the consequences for the flavor and texture of food. These interactions influence the basic property of viscous foods like soup and custards. Hence, these food products need to absorb water without protein disintegration to provide body, thickness and viscosity (Tounkara et al., 2013).

Regarding oil holding capacity (OHC), water-soluble albumin and NaOH soluble glutelin were the predominant protein fractions in banana peel powder (1.97 ± 0.12 g/g and 1.73 ± 0.11 g/g, respectively) without any significant difference between them. Further, there was no significant difference between the OHC of albumin and globulin. It has been reported that protein powders with small particle sizes hold more oil than protein powders with larger particle sizes (Kinsella & Melachouris, 1976). This might be the reason behind the predominant OHC of albumin, glutelin, and globulin. OHC of albumin showed the similarity with the result reported by Sathe, Deshpande, & Salunkhe (1982); they also found OHC of plant-based proteins mainly depends on protein concentration and protein-lipid-carbohydrate interactions. OHC is an essential functional property associated with a physical entrapment that acts as a flavor retainer and increases the palatability of foods (Akpossan et al., 2015; Ulloa et al., 2017).

3.5.2. Emulsifying capacity
The emulsion capacity of banana peel protein fractions is shown in Table 3. The highest emulsion capacity was observed in the albumin fraction, followed by glutelin, globulin, and prolamin fractions, with no significant difference (p < 0.05) between albumin and glutelin fractions. The emulsion capacity of albumin and glutelin was found higher without any significant difference, which might be due to the availability of a higher quantity of small fraction of soluble proteins as identified by the particle size distribution of albumin and glutelin. Zhu et al. (2018) reported the higher emulsion activity by the proteins with the availability of a greater quantity of small protein particles, which were reported to be capable of adsorbing on the oil–water interface.

3.5.3. Foaming capacity and foaming stability
Proteins used to be treated as a good foaming agent because protein can easily diffuse in the air–water interface and form a cohesive and elastic film by partial unfolding (Tounkara et al., 2013). Foams are often used to improve the texture, consistency, and appearance of any food
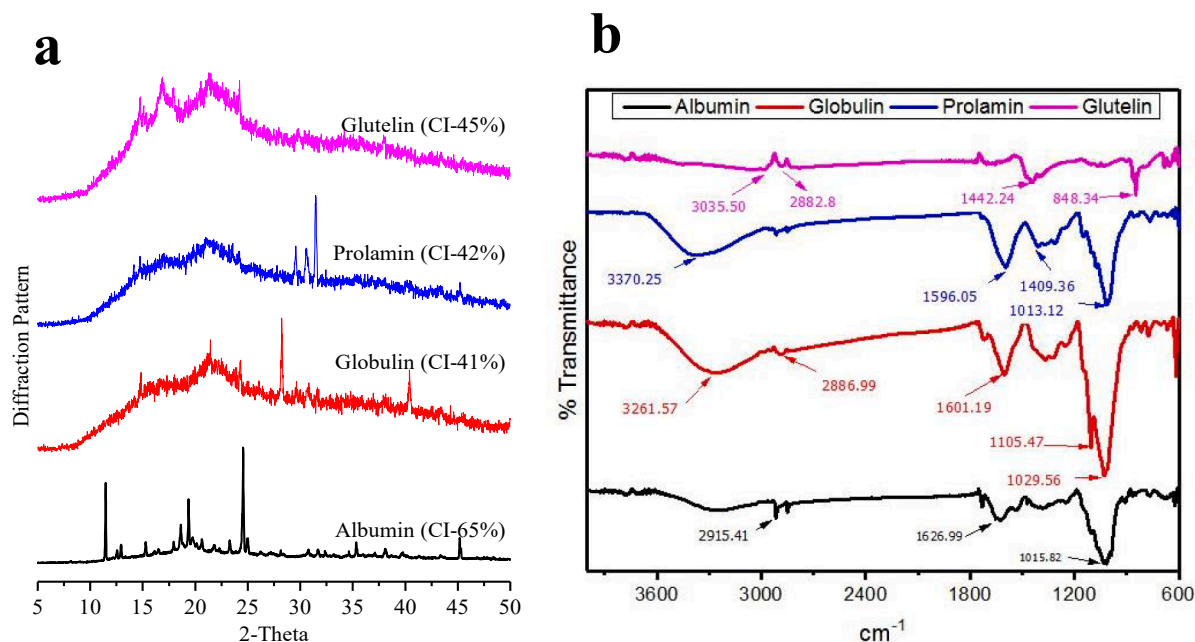
Fig. 2. X-ray diffraction pattern and FTIR spectroscopy of banana peel derived albumin, globulin, prolamin, and glutelin protein fractions.

item. On the other hand, to have good foam stability, protein molecules must form persistent intermolecular polymers wrapping the air bubbles, because intermolecular cohesiveness and elasticity are the two major factors needed for proper foam stability (Mohamed, Zhu, Issoufou, Fatmata, & Zhou, 2009). The foaming capacity of banana peel protein fractions is shown in Table 3. The foaming capacity of glutelin showed the highest value, followed by globulin, prolamin, and albumin. The higher values seem to be due to increased solubility and net charges of protein fractions, which weakened the hydrophobic interactions and increased protein flexibility. The foam stability of globulin was the highest among all four fractions. The foam stability decreases from 17 to 9, 19 to 6, 15 to 6, and 14 to 4% for globulin, glutelin, prolamin, and albumin, respectively (Fig. 1).

3.5.4. Dispersibility index

Results in the present study showed the highest dispersibility (951.55 ± 3.83 g/kg) for glutelin protein fraction and lowest dispersibility (701.38 ± 3.07 g/kg) for albumin protein fraction (Table 3). Though glutelin showed the highest dispersibility, prolamin also showed dispersibility (924.49 ± 3.82 g/kg) comparable to glutelin fraction, whereas dispersibility of albumin was found much lower compared to the other three fractions. Dispersibility of proteins is affected by temperature, pH, and degree of agitation of solvent (Kinsella & Melachouris, 1976). The dispersibility decreases with increasing the concentration of protein (Akpossan et al., 2015); therefore, protein content increment is not favorable for better dispersibility. Dispersibility of all the protein fractions was found significantly different (p < 0.05) from each other. However, each fraction showed a good dispersibility value might be due to the complete defatting of banana peel powder before protein extraction (Akpossan et al., 2015).

3.5.5. Wettability

Wettability mainly depends on particle size, surface polarity, topography, the texture of particles, surface area, density, porosity, and microstructure of protein particles (Hägerdal & Löfqvist, 1978). The wettability of albumin, globulin, prolamin, and glutelin are presented in Table 3. Albumin, globulin, and glutelin protein fractions showed minimum wettability values (<10 min) without any significant difference

(p < 0.05). Contrarily, the wettability of the prolamin fraction (14.32 ± 0.25 min) was significantly (p < 0.05) higher than the other three fractions due to the use of 70% aqueous ethanol for prolamin extraction as ethanol is immiscible with water. Wettability acts as a significant property when protein powders are scattered to prepare beverages and batters. The minimum wettability of albumin, globulin, and glutelin was due to defatted banana peel powder, which reduced the wettability time (Akpossan et al., 2015).

3.6. Thermal properties

Thermal properties (onset temperature, denaturation temperature. end temperature, and denaturation enthalpy) of all the protein fractions are shown in Table 3. The denaturation temperature of all the protein fractions was found within 100 °C, comparable with several plant proteins (Scilingo & Añón, 1996). Denaturation temperature below 100 °C is strong evidence behind using a moisture-free sample as Kitabatake, Indo, and Doi (1989) reported that denaturation temperature sometimes crossed 100 °C due to the higher moisture content in the sample. Results showed that the denaturation temperature and enthalpy of albumin was the highest (82.08 °C and 78.41 J/g), followed by globulin (74.12 °C and 47.03 J/g), glutelin (48.7 °C and 13.17 J/g), and prolamin (30.31 °C and 6.45 J/g). This might be due to the protein concentration difference among the fractions (Guimarães et al., 2012). In addition, the crystalline structure of albumin (confirmed by X-ray analysis) also restricted the thermal destruction, which appeared as increased denaturation temperature. The direct relationship between the enthalpy of denaturation and protein concentration showed that extraction techniques and further processing steps did not instigate protein denaturation. In contrast, an inverse relationship between enthalpy and protein concentration (which is not acceptable) has been reported due to protein denaturation during extraction and processing (Guimarães et al., 2012).

3.7. Structural properties

3.7.1. X-ray diffraction

X-ray diffraction is presently used as the main method to analyze the crystal structure and size and variations of crystal size among various
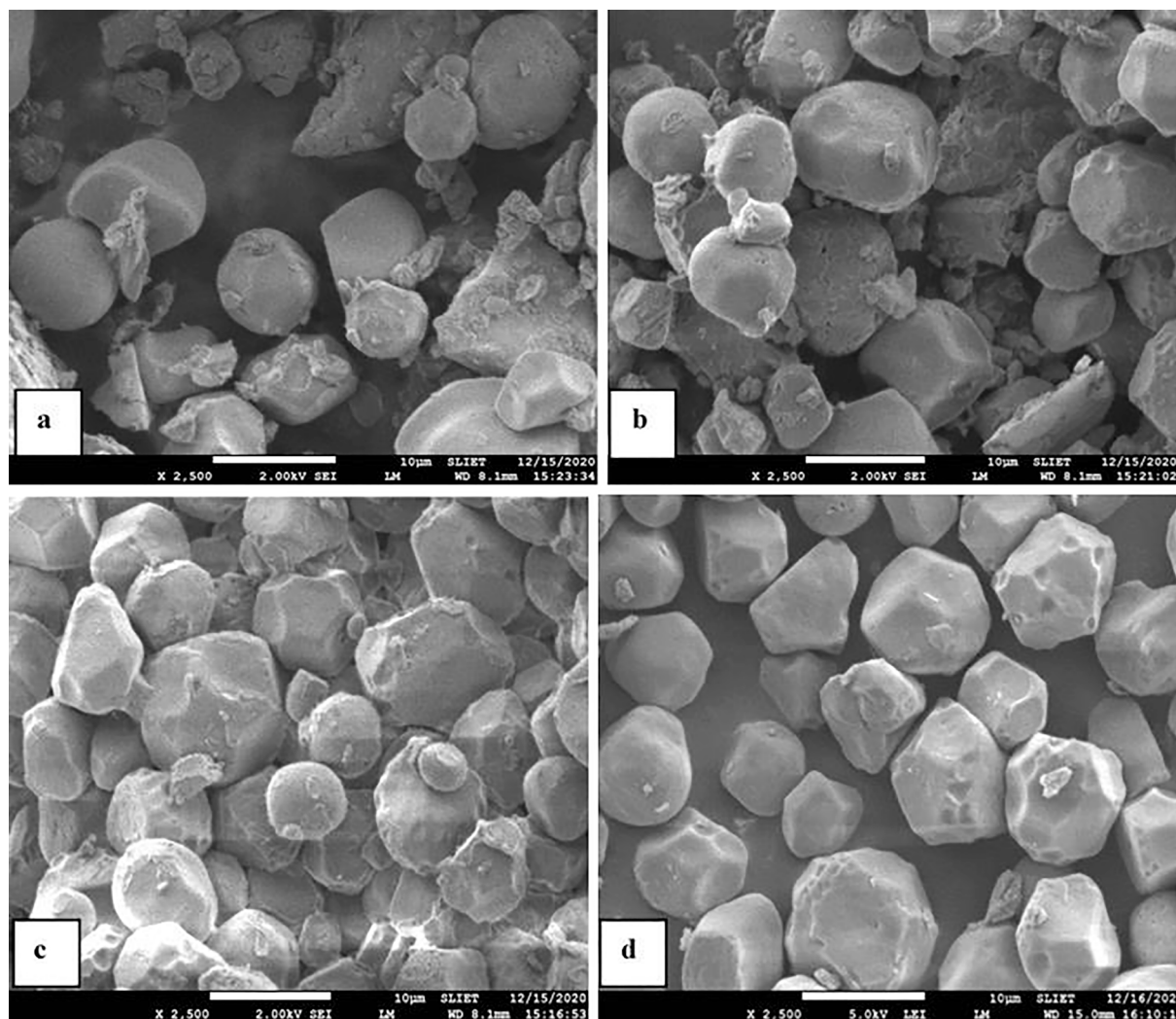
Fig. 3. SEM image of banana peel derived (a) Albumin, (b) Globulin, (c) Prolamin, and (d) Glutelin protein fractions.

protein isolates and fractions. The intensity of diffraction and angle of diffraction (2θ) are the two main factors responsible for crystal size. Higher diffraction angle and lower diffraction intensity represent the smaller crystal size (Yu, Yang, Sun, Zhang, Bi, & Yang, 2015). Diffractograms of albumin, globulin, prolamin, and glutelin are shown in Fig. 2a. Albumin fractions showed several sharp peaks with varying intensity, indicating crystalline behavior with a crystallinity index of 65%. However, major peaks were found at 2θ of 18°, 19°, 45° (low intensity), and 12°, 20° (high intensity). However, the other three fractions showed amorphous nature (41–45%) with broad peaks between 15 and 20°. A similar peak at a lower diffraction angle was reported for phosphate-modified peanut protein (Yu et al., 2015). The results of XRD for albumin are to some extent matching with soy protein crystalline nature with three peaks at 8.5°, 19.5°, and 24.4° (Wang, Tang, Li, Yang, Li, & Ma, 2008). In terms of the size of the crystal, the albumin protein fraction was large, followed by glutelin, globulin, and prolamin.

3.7.2. FTIR spectroscopy

FTIR is a broadly used method for determining the secondary structure of the protein as well as information regarding the protein's structural composition (Kong & Yu, 2007). Spectrums of FTIR (600–4000 cm^{-1} region) for albumin, globulin, prolamin and glutelin protein fractions derived from banana peel powder are shown in Fig. 2b and major peaks are presented in Table 3. The difference in transmittance pattern of all protein fractions confirmed the structural

difference due to the presence of different functional groups. However, all four fractions showed one or two clear peaks between 1670 and 1242 cm^{-1}, which is an indication of presence of Amide I, Amide II or Amide III (Chávez-Murillo, Veyna-Torres, Cavazos-Tamez, de la Rosa-Millán, & Serna-Saldívar, 2018; Kong & Yu, 2007). From these typical bands, absorbance bands at 1626.99, 1601.19, and 1596 cm^{-1} in albumin, goblin, and prolamin, respectively, resembled stretching vibration of C=O of the amide-I (Borchani et al., 2021). However, a typical band at 1442 cm^{-1} by glutelin confirmed the Amide III, which did not appear for other fractions (Borchani et al., 2021). A weak band at 2882.80 and 2915 cm^{-1} can be attributed to the stretching vibration of C—H of amide B or CH$_3$ group, was appeared in all protein fractions (Jin et al., 2014).

From other bands, the broad bands at 3370.25 cm^{-1} and 1409.36 cm^{-1} for prolamin protein fraction clearly resembled stretching vibration of –OH group. The strong band at 3261.57 cm^{-1} indicates the contribution of the amino group and also indicates that banana peel derived globulin has less interaction with starch than globulin from other sources (Guo et al., 2013). Bands at 3035.50 cm^{-1} and 848.34 cm^{-1} might be occurred due to the presence of inorganic ions, as NaOH solution was used for glutelin extraction. A strong signal at near 1015 cm^{-1} represents the stretching vibration of C—O in saccharide, which comes under the fingerprint region (1000–1400 cm^{-1}), which was evident in all protein fractions except glutelin. However, there was no band (not even small bands) found in the region of 800–1200 cm^{-1}, which indicates the absence of interactions of protein fractions with

starch. These patterns indicate the absence of polysaccharides and confirm the low carbohydrates content and richness in protein content in albumin and other protein fraction. The low carbohydrate content in protein is an indication of high purity and is possibly responsible for better functional properties of albumin. Additionally, in the case of globulin, the intensity of absorbance was found to increase at the bands near 1105.27, 1029.56, 816.92, 776.66, 665.92, and 616.05 cm^{-1}. Guo et al. (2013) also reported a similar kind of increased intensity pattern at the same region for globulin fraction derived from aged rice compared to globulin derived from fresh rice.

3.7.3. Surface morphology

The SEM image of albumin showed spherical structures and flattened surfaces as shown in Fig. 3a with smooth morphology. Chavan, McKenzie, and Shahidi (2001) and Zhu, Yi, and Li (2011) also reported smooth and homogeneous morphology for albumin extracted from beach pea and peach kernel. Morphology of globulin was also found similar with albumin except for some globular granules (Fig. 3b) were found in globulin fraction. Sun, Wu, Ma, Min, Lai, and Wu (2017) also reported similar morphology for globulin extracted from the mulberry leaf. Morphology of prolamin was found cloudy in shape (Fig. 3c). Sun et al. (2017) also reported similar cloudy-shaped prolamin with holes on their surfaces extracted from the mulberry leaf. Glutelin protein fraction showed flattened surfaces (Fig. 3d) with irregular structures. Glutelin extracted from mulberry leaf was also found with a rough surface and irregularly formed networks (Sun et al., 2017). The granular size of protein from micrograms was found in the range of 4.18 to 18.45 (mean: 9.07 μm) for albumin, 8.47 to 15.59 (mean: 9.07 μm) for globulin, 4.17 to 12.37 (mean: 7.28 μm) for glutelin, and 4.13 to 13.05 (mean: 6.87) for prolamin. However, these findings are not in line with the size determined by light scattering, which might be due to the formation of soluble protein aggregate in the aqueous solution, which measured the average hydrodynamic size of the aggregate (Xiong et al., 2018). Nevertheless, the smaller particle size of glutelin with rough and flattened microstructure promoted the dissolution rate in the aqueous phase and resulted in higher foaming capacity and dispersibility value Kumarakuru et al. (2018). In contrast, the smooth and homogeneous morphology of albumin was responsible for increased WHC, OHC, and EC (Mao & Hua, 2012).

4. Conclusion

In the present study, four different protein fractions from banana peel were characterized based on physicochemical, functional, and structural properties. The total protein content after adding all the four fractions was found 92.6 ± 2.05% and total recovery of protein was 9.12 ± 0.24 g/100 g, which was approximately 89.76% to the crude protein of banana peel powder. Albumin protein fraction showed the highest yield and solubility among all the fractions. The particle size and color difference of all the protein fractions were found significantly different with each other except between albumin and glutelin. Albumin protein fraction was found predominant in terms of water holding capacity, oil holding capacity, and emulsifying capacity. In contrast, foaming capacity and dispersibility value of glutelin were the highest among all the protein fractions. The wettability of albumin, globulin and glutelin fractions were comparable; however, prolamin showed maximum wettability. Thermal characteristics of protein fractions showed a comparable relation with several plant proteins. However, due to the crystalline structure, albumin showed the highest thermal resistance with a denaturation temperature of 82.08 °C. X-ray diffraction analysis confirmed the amorphous nature of all proteins fractions except albumin. Apart from a single band centered at 1015.82 cm^{-1}, FTIR spectra verified the richness of protein in the albumin fraction by showing no peaks in the region of 800–1200 cm^{-1}. However, all four fractions showed one or two clear peaks between 1670 and 1242 cm^{-1}, which is an indication of presence of Amide I, Amide II or Amide III. The surface

morphology of banana peel protein fractions demonstrated different microstructures for different protein fractions, which might be the reason behind the different functional properties of all the protein fractions. However, the size analyzed from micrograms were very small as compared to size determined by laser scattering due to formation of soluble protein aggregate in the aqueous solution. Conclusively, the higher albumin content in waste banana peel showed the path to reduce the dependency on animal sources for albumin.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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