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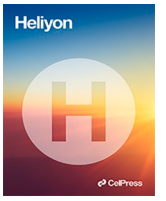

Research article

Physicochemical, structural, and functional characterization of guar meal protein isolate (*Cyamopsis tetragonoloba*)

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ABSTRACT

Guar korma and churi protein isolates were assessed for their physicochemical, nutritional, functional, structural, and digestibility properties for their application in the food industry. The water extracted protein isolate of guar korma showed a protein content of 89.7 % and a yield of 48.7 %. Water extracted protein isolate of guar korma showed an excellent protein efficiency ratio, essential amino acid/total amino acids (34.35 %), amino acid score, and protein digestibility corrected amino acid score values, suggesting the existence of high-quality proteins. Water extracted protein isolate of guar korma contains all the essential amino acids except Methionine and Cysteine, according to World Health Organization recommendations for children and adults. The protein profiling of water extracted protein isolate of guar korma was analyzed using 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis and indicated the presence of eight major protein bands in the range of 17–100 kDa. *In vitro* digestibility of water extracted protein isolate of guar korma showed the complete digestion of the abundant protein bands within 15 min. Further, the foaming capacity, water/oil holding capacity, and emulsifying stability of water extracted protein isolate of guar korma were comparable with soy protein isolate. Fourier Transform Infrared and Circular Dichroism spectral analysis revealed the presence of several aromatic groups and β-sheets, random coils respectively in water extracted protein isolate of guar korma. The morphological nature of the guar protein isolate was characterized by Scanning Electron Microscopy. Overall, these findings support that water extracted protein isolate of guar korma has excellent functional and nutritional properties and could be a potential alternative plant protein in food industries.

1. Introduction

The guar or cluster bean is a drought-resistant and native African plant belonging to the Fabaceae family, mainly cultivated in northwest India [1,2]. The guar seed is composed of 43–47 % germ, 30–33 % hull, and 27–30 % (w/w) endosperm [3]. Globally guar seed production is 1.0–1.6 million tons per annum, and India is the world's biggest cultivator contributing 80 % to the world market,

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followed by Pakistan (15 %). In India, Rajasthan is the major guar producer (70 %), followed by Haryana (12 %), Gujarat (11 %), and Punjab (3 %).

Guar meal (GM) is an industrial spent in the guar gum process; it contains the germ and husk portion of the guar seeds and includes 30–60 % crude protein. It is produced after the separation of endosperm from the germ and hull of the guar seeds *Cyamopsis tetragonolobus* [4]. GM is available in two forms: Guar meal korma and churi. The study by Nidhina and Muthukumar [5] revealed that heat treatment of guar meal would significantly reduce anti-nutritional factors. For the functional applications of plant-based proteins in food industries, in addition to providing all the essential amino acids, they should have good functional properties like solubility, emulsifying capacity, water, and oil-holding capacities, gel foaming capacity, foaming ability, rheological property, etc., [6].

Globally the demand for plant-based protein products has increased due to their nutritional value and adoption of vegan culture. Plant-based proteins have become the best replacement for animal-based proteins like egg and whey proteins. Plant-based protein supplements and nutritional powders are mainly soy, pea, and wheat proteins. However, soy and wheat-based products market growth has been restrained due to the existence of anti-nutritional factors and allergens. Hence, the demand for novel plant-based products has increased in the food and beverage industries. It emphasizes to explore substituting traditional concentrates with nutrient-rich, low-priced agro-industrial by-products for innovative usage of proteins in nutritious and healthy food formulations.

Limited research has been conducted to enhance the applicability and utilization of GM or seed protein in human foods. A few studies have reported on guar meal's extraction, characterization, functional properties and protein isolate [5,7]. Currently, GM has negligible application in the food industry for human consumption due to the presence of anti-nutritional factors such as trypsin inhibitors, saponins, residual gum, lignin, polyphenols, few organic acids, and aldehydes with foul smell and bitter taste [8]. Collectively, to the best of our knowledge, there is no comprehensive data on guar meal (GM), especially protein isolate, to support its potential optimal utilization in the food industry. Hence, the present study aims to investigate the physicochemical, nutritional, functional, digestibility, and structural properties of guar korma and churi protein isolate ensuring they are free of anti-nutritional factors. This study could contribute to incorporating guar korma protein isolate as a plant-based protein substitute, thus enhancing the nutritional value and promoting the value-added utilization of guar meal.

2. Materials and methods

2.1. Materials

Guar korma and churi were obtained from Durga Enterprises, Rajasthan, India. The analytical grade chemicals used in the present study were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck Specialities Private Ltd. (Mumbai, India), and Himedia Laboratories Private Ltd. (Mumbai, India). Other chemicals and solvents used in this study were analytical grades. All the chemicals were stored as per supplier instructions.

2.2. Proximate composition analysis

Moisture, ash, fat, crude fiber, and carbohydrate contents of guar korma and churi were calculated by the standard methods of the Association of Official Analytic Chemists [9]. The protein contents of guar korma and churi were estimated using the automatic Kjeldahl apparatus by the AOAC method (N × 6.25). All experiments were carried out in triplicates, and the results were expressed as mean ± standard deviation (SD).

2.3. Preparation of protein isolates

Protein isolates of guar korma and churi were prepared according to the method reported by Khalil [7] with minor modifications. In order to remove the anti-nutritional factors, defatted samples were autoclaved for 20 min, dried at room temperature, and stored at −20 °C until analysis. 100 g of defatted sample was suspended in 1 L of distilled water or salt-alkali mixture containing 5 % NaCl and 0.1 N NaOH separately. The suspension pH was adjusted to 9.0 with 1 M Na_2CO_3, stirred for 2 h, then centrifuged at 8000 rpm for 15 min, 4 °C. The extraction step was repeated with the residue to obtain higher yields. The supernatants were pooled and adjusted to pH 4.5 with 4 N HCl for both guar korma and churi and incubated at 4 °C for 1 h. The formed precipitate was collected by centrifugation at 8000 rpm for 15 min, then washed twice with distilled water and solubilized by adjusting pH to 7.0 with 1 N Na_2CO_3. The protein isolate was dialyzed against distilled water for 12 h at 4 °C using a 12 K molecular weight cut-off dialysis membrane (Sigma-Aldrich, USA) before freeze drying. The freeze-dried protein isolates were stored at −20 °C for further analysis. The automatic Kjeldahl method determined the protein content in guar korma and churi protein isolates [9]. The protein yield was estimated using the following formula.

$$Yield\ (\%) = \frac{Wt.\ of\ protein\ extract \times percent\ protein\ in\ the\ protein\ extract}{Wt.\ of\ guar\ meal\ seed\ flour \times percent\ protein\ in\ the\ guar\ meal\ seed\ flour} \times 100$$

2.4. Nutritional properties

2.4.1. Amino acid analysis

The amino acid composition of defatted guar korma and churi, water extracted protein isolate from korma (WPIK) and water

extracted protein isolate from churi (WPIC) was determined using an automated amino acid analyzer (Sykam S-433, Germany) with a cationic separation column (LCA K-06; 150 × 4.6 mm). Samples were hydrolyzed using 6 N HCl containing 0.1 % phenol at 110 °C for 24 h in the presence of nitrogen gas. The hydrolyzed samples were dried under a vacuum and suspended in a sample diluting buffer (Sykam, Germany). The suspensions were filtered (0.2 μm), and filtrates were used for amino acid analysis. The instrument was calibrated with a standard mixture of amino acids before analysis. Tryptophan concentration was estimated according to the method described by Pinter and Molnar [10].

The amino acid composition of defatted guar korma, churi, WPIK, and WPIC was used to predict the nutritional quality of the protein samples, as given below.

a) $\textit{Amino acid score}\ (\%) = \frac{mg\ of\ amino\ acid\ per\ gram\ test\ protein}{mg\ of\ amino\ acid\ per\ g.\ \ of FAO/WHO\ standard\ pattern} \times 100$:

b) The ratio of essential amino acids (E) to the total amino acids (T) of the test protein:

$$E \Big/ T\ (\%) = \frac{Ile + Leu + Lys + Met + Cys + Phe + Tyr + Thr + Trp + Val + His}{Ala + Asx + Arg + Gly + Glx + His + Ile + Leu + Lys + Met + Cys + Phe + Tyr + Pro + Ser + Thr + Trp + Val} \times 100$$

c) The protein efficiency ratio (PER) was calculated using the following equations formulated by Alsmeyer et al. [11]:

$$\text{PER} = -0.684 + 0.456\ (\text{LEU}) - 0.047\ (\text{PRO}) \text{ - - - - - - - - -} \quad 1$$

$$\text{PER} = -0.468 + 0.454\ (\text{LEU}) - 0.105\ (\text{TYR}) \text{ - - - - - - - - -} \quad 2$$

$$\text{PER} = -1.816 + 0.435\ (\text{MET}) + 0.780\ (\text{LEU}) + 0.211\ (\text{HIS}) - 0.944\ (\text{TYR}) \text{ - - - -} \quad 3$$

2.4.2. Protein digestibility corrected amino acid score (PDCAAS)

The amino acid composition of protein isolates was used to calculate PDCAAS. The PDCAAS was calculated using the below formula.

$$PDCAAS = \frac{mg\ of\ limiting\ amino\ acid\ in\ 1\ gram\ of\ test\ protein}{mg\ of\ same\ amino\ acid\ in\ 1\ gram\ of\ FAO/WHO\ standard\ pattern} \times invitro\ protein\ digestibility\ (\%) \times 100$$

2.5. Analysis of functional properties

2.5.1. Protein solubility

The solubility of freeze-dried water and salt alkali-extracted protein isolates from guar korma (SAPIK) and churi (SAPIC) were assessed at pH 2–12. The protein isolates were dissolved in different buffers (100 mM), HCl–KCl buffer for pH 2.0; citrate phosphate buffer for pH 3.0, 4.0, 5.0, 6.0; phosphate buffer for pH 7.0 and 8.0; Glycine-NaOH buffer for pH 9.0, 10.0; borate buffer for pH 11.0 and 12.0 at a final concentration of 1 mg/mL. The dispersion was incubated at 4 °C for 1 h with occasional stirring. After incubation, the dispersion was centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was collected, and the protein content was assayed by the Bradford Coomassie Brilliant Blue method. The protein solubility percentage was determined by dividing the protein content in the supernatant by the total protein in the dispersion and multiplying by 100 [12].

2.5.2. Emulsifying property

Emulsion activity (EA) and emulsion stability (ES) of freeze-dried protein isolates (WPIK, WPIC, SAPIK, and SAPIC) and soy protein isolate (SPI) were determined by a turbidimetric method described by Mu et al. [13]. Sunflower oil and 0.1 % protein solution, pH 8.0 (1:3), were mixed in a centrifuge tube. The mixture was homogenized using a mechanical homogenizer at maximum settings for 1 min to form an emulsion. Fifty microliters of the emulsion was collected from the bottom of the tube and added to a glass tube containing 0.1 % SDS (5 mL) at 0 and 10 min after homogenization. The optical density (OD_{500}) of these diluted solutions was determined using a spectrophotometer against the blank. The following equation calculated EA and ES:

$$EA\ \left(m^2 \Big/ g\right) = \frac{2 \times T \times A_0\ \times dilution\ factor}{C \times \Phi \times L \times 10000}$$

$$ES\ (min) = \frac{A_0}{(A_0 - A_{10})} \times 10$$

Where:

T = 2.303; dilution factor = 100

C – Weight of protein per unit volume (g/ml).

L – Width of the optical path (0.01 m).

Φ – Oil volumetric fraction (0.25).

A_0- Absorbance at 0 min.
A_{10}-Absorbance at 10 min.

2.5.3. Foaming property

Foaming properties such as foam stability (FS) and foam capacity (FC) of freeze-dried protein isolates (WPIK, WPIC, SAPIK, and SAPIC) and SPI were estimated using the method by Deng et al. 1 % (w/v) of protein solution (5 mL) was whipped thoroughly using a homogenizer for 5 min, and the volume of solution was measured immediately using a measuring cylinder [14]. The sample volume was noted before and after whipping, and the solution was incubated for 120 min at room temperature. FC and FS were expressed as percentage volume increase and calculated per the formula below.

$$Foam\ capacity\ (\%) = \frac{[Volume\ after\ stirring\ -\ Volume\ before\ stirring]\ in\ ml}{[Volume\ before\ stirring]\ in\ ml} \times 100$$

$$Foam\ stability\ (\%) = \frac{[Volume\ after\ 120\ min.\ standing\ -\ Volume\ before\ stirring]\ in\ ml}{[Volume\ after\ stirring\ -\ Volume\ before\ stirring]\ in\ ml} \times 100$$

2.5.4. Bulk density

The bulk density of freeze-dried protein isolates (WPIK, WPIC, SAPIK, and SAPIC) and SPI were measured by the method described by Khalid et al. [15]. The freeze-dried protein isolate sample was taken in a measuring cylinder and gently tapped the measuring cylinder for tight packing of the sample. The volume occupied by the sample was measured, and the bulk density was expressed as g/mL.

2.5.5. Water and oil holding capacity

Water and oil holding capacities of freeze-dried protein isolates (WPIK, WPIC, SAPIK, and SAPIC) and SPI were analyzed as reported by Beuchat [16]. 10 mL of distilled water or refined sunflower oil was added to the 1 g sample, mixed for 30 s, and incubated at room temperature for 30 min. The suspension was centrifuged at 8000 rpm for 20 min. The supernatant was measured, and water and oil holding capacities (WHC and OHC) were calculated as water or oil (mL) retained per gram of sample.

WHC = volume of water added to sample – volume of supernatant

OHC = volume of oil added to sample – volume of supernatant

2.6. SDS-PAGE analysis

Denaturing SDS-PAGE analysis was performed for crude, defatted, autoclaved, water-extracted, and salt-alkali extracted guar korma. It was carried out to determine the molecular weights of the proteins and peptides. Protein samples were dissolved in phosphate buffer (0.1 M, pH-8) and loaded in 12 % SDS-PAGE gel for peptide analysis. Electrophoresis was performed at constant voltage (80 V) for 120 min at room temperature using a SDS Tris-glycine buffer system. The protein bands were visualized after staining the gel with Coomassie brilliant blue (CBB) R-250 (0.1 %). The apparent molecular weights of the proteins were determined using a pre-stained protein ladder (Hi-media, India).

2.7. In vitro digestion

2.7.1. In vitro protein digestibility (IVPD) of guar protein isolates

In vitro protein digestibility (IVPD) of water-extracted protein isolate of guar korma was assessed using the pepsin-pancreatic enzyme method [17] with minor modifications. 100 mg of protein sample was dissolved in 15 mL of 0.1 N HCl having 1.5 mg/mL of pepsin (Sigma, USA) and incubated for 3 h at 37 °C in a water bath. 0.1 N HCl without pepsin was used as blank. After incubation, pepsin hydrolysis was terminated by neutralization upon adding 0.5 M sodium hydroxide. Then, pancreatic digestion was started by adding pancreatin (0.53 mg/mL) in 0.2 M phosphate buffer (pH 8.0). The sample was incubated at 37 °C for 24 h in a water bath, followed by enzyme inactivation at 100 °C for 5–10 min. The supernatant was collected (8000 rpm for 20 min) and the total protein content in the supernatant was estimated using the Kjeldahl method. Casein was used as the standard, and finally, IVPD was determined by the following equation:

$$Digestibility\ (\%) = \frac{N_s - N_b}{N_s} \times 100$$

Where N_s = nitrogen content in the sample, N_b = nitrogen content in the blank.

2.7.2. Gastric digestion assay of WPIK

Simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) digestion stability assays were performed for WPIK according to the method of Fu et al. [18]. SGF was prepared by mixing 3.2 mg/mL pepsin dissolved in 30 mM sodium chloride at pH 1.2 and was

prepared as described in the United States Pharmacopoeia [19]. 0.2 mL aliquots of SGF were dispensed in 1.5 mL microcentrifuge tubes and incubated for 10 min at 37 °C in a water bath. After incubation, 10 μl of the test protein sample at 5 mg/mL concentration in 0.03 M NaCl was added to each SGF solution and incubated at 37 °C. Samples were taken at 0, 15, 30, 45, and 60 min intervals, and 75 μl of 1 N NaOH was dispensed to each sample to terminate the reaction. Then 20 μl of each sample was mixed with 4 × laemmli buffer, boiled for 10 min in a water bath, and electrophoresed on 12 % tris-glycine SDS-PAGE. The gel was stained with CBB R-250, and protein bands were visualized after de-staining. Similarly, SIF was prepared by mixing pancreatin (10 mg/mL) in 0.05 M KH_2PO_4, pH 7.5.64 μl aliquots of SIF were dispensed in 1.5 mL microcentrifuge tubes and incubated at 37 °C for 10 min in a water bath. Then 10 μl of a test protein sample at a concentration of 5 mg/mL in 0.05 M KH_2PO_4, pH 7.5, was added to each SIF solution and incubated at 37 °C. Samples were taken at 0, 15, 30, 45, and 60 min intervals and analyzed by tris-glycine SDS-PAGE as mentioned above.

2.8. Structural and morphological characteristics of protein isolates

2.8.1. Circular Dichroism (CD)

Circular dichroism spectra were scanned at the far UV region 190–260 nm (bandwidth was 1.0 nm, path length was 100 mm) using an automatic spectropolarimeter (Jasco 715, Jasco Corp). Freeze-dried WPIK and WPIC protein samples were dissolved in 10 mM phosphate buffer (pH 8.0) to a final concentration of 0.3 mg/mL. Three scans were recorded and averaged to obtain the spectrum. The spectra were corrected by subtracting the spectrum of the buffer. The results were expressed as mean residue ellipticity (θ) in deg. $cm^2/dmol^{-1}$. The concentration of protein samples was determined by the Kjeldhal method [9]. The secondary structures of protein samples were estimated using SELCON3.

2.8.2. Scanning electron microscopy (SEM)

Microscopic structural morphologies of crude, defatted, autoclaved korma, and WPIK were examined under SEM. Samples were mounted on round aluminum stubs with double sticky tape and then sputter-coated with 10 nm of gold. Then the samples were analyzed in SEM (LEO, Cambridge, UK/435VP) at an accelerating potential of 20 kV. Typical SEM images (5000x and 1000x) were obtained for further analysis.

2.8.3. Fourier transform infrared spectra (FTIR)

The chemical composition of crude, defatted, autoclaved korma, and WPIK was analyzed using FTIR spectra. The spectral analysis was performed using FTIR (IFS 25, Bruker, Germany). One microgram of each sample was placed in contact with Platinum ATR at 25 °C, and the FTIR spectrum of each sample was recorded in wavelengths ranging from 3500 to 500 cm^{-1} at room temperature.

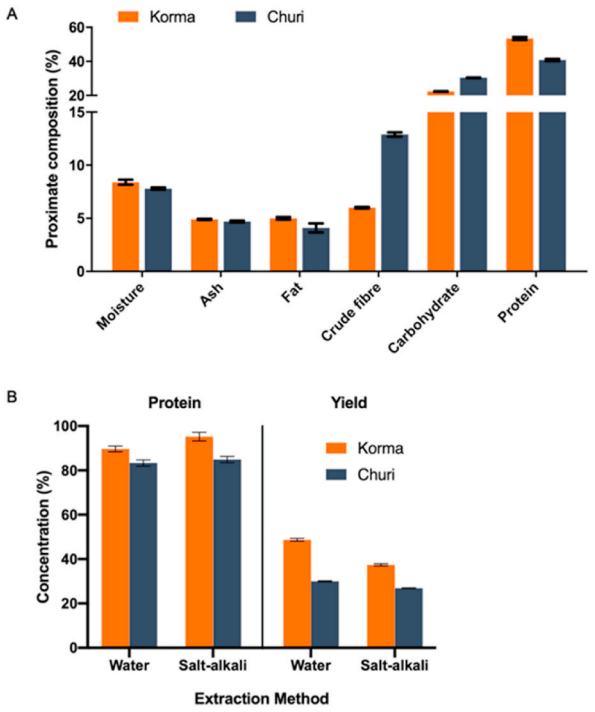

Fig. 1. Proximate composition, protein content, and yield of guar korma and churi **(A)** Proximate composition of crude guar korma and churi **(B)** Protein content and yield of water and salt-alkali extracted protein isolates from guar korma and churi.

2.9. Statistical analysis

All tests were conducted in triplicate, and the data were reported using three values. One-way ANOVA was carried out using SPSS 22.0 software (SPSS Inc., Chicago, USA). Significant differences were defined at the $P \leq 0.05$ level between the samples.

3. Results and discussion

3.1. Proximate composition analysis

The proximate composition of guar korma and churi were shown in Fig. 1A. Guar korma had higher protein content (53.4 %) than guar churi (40.8 %). Gaur korma and churi showed a higher protein content when compared to flax seed meal (39.0 %), Canola meal (36.1 %), and Kenaf meal (26.2 %). But guar korma had superior protein content than soybean meal (48.3 %) and defatted Simarouba meal (47.7 %) [20–22]. Moisture and ash content were higher in korma than in churi. Moisture content in guar korma was comparable to industrial guar korma (8.2 %) but lower than kenaf meal (9.34 %), soybean meal (11.56 %), common vetch seeds (11.92 %), and canola meal (11.35 %) [5,21,22]. The moisture content in korma and churi was higher than in wheat (5.89 %), rice (3.96 %), and flax seed meal (6.65 %) [21,23,24]. Ash content in guar korma and churi was comparable to industrial guar meal but lower than flax seed

Table 1
Nutritional properties of guar korma, churi, and their water-extracted protein isolates.

Amino acid	Amino acid composition (g/100 g)					
	Guar Meal		Protein Isolate		FAO/WHO	
	Korma	Churi	WPIK[b]	WPIC**	Child	Adult
Ile	2.40	2.27	3.10	3.13	2.80	1.30
Leu	4.82	5.04	6.10	6.22	6.60	1.90
Lys	3.66	4.91	4.90	4.70	5.80	1.60
His	3.34	2.23	2.54	2.44	1.90	1.60
Met	1.13	0.81	1.20	1.01		
Cys	0.59	1.13	0.62	0.51		
TSAA	1.72	1.94	1.82	1.52	2.50	1.70
Phe	2.96	2.50	4.10	4.01		
Tyr	3.02	2.39	3.84	4.51		
TAAA	5.98	4.89	7.94	8.52	6.30	1.90
Thr	2.15	1.40	3.10	3.45	3.40	0.90
Trp	2.61	3.33	1.93	2.45	1.10	0.50
Val	2.18	1.15	3.63	3.90	3.50	1.30
TEAA	28.86	27.16	35.06	36.33		
Arg	18.07	18.96	14.57	13.50		
Asp & Asn	10.89	11.36	11.20	11.28		
Glu & Gln	28.53	31.28	22.47	21.70		
Ser	4.80	4.59	5.02	5.07		
Pro	3.21	3.14	3.92	4.03		
Gly	5.71	5.28	5.82	6.10		
Ala	2.45	1.48	4.00	4.51		
TNEAA	73.66	76.09	67.00	66.19		
Amino acid score (%)						
His	171.56	113.90	131.16	125.39		
Ile	83.55	78.68	108.62	109.17		
Leu	71.30	73.92***	90.68	91.94***		
Lys	61.64**	82.10	82.88**	79.05**		
SAA[a]	67.30	75.53	71.42[b]	59.33[b]		
AAA ##	92.63	75.25	123.65	132.00		
Trp	237.20	302.72	175.46	222.73		
Val	60.83[b]	32.03[b]	101.75	107.88		
Thr	61.85***	39.92**	89.46***	99.16		
Predicted protein efficiency ratio (PER)						
PER-1	1.36	1.46	1.92	1.97		
PER-2	1.40	1.57	1.90	1.88		
PER-3	0.30	0.69	0.38	0.0		
E/T (%)	28.15	26.31	34.35	35.44		
PDCAAS (%)						
2–5 years age	–	–	59	49		
10–12 years age	–	–	67	56		
Adults	–	–	86	72		

WPIK[b] – Water extracted protein isolate of korma; WPIC**– Water extracted protein isolate of churi; TSAA – Total sulfur-containing amino acids (Met + cys); TAAA – Total aromatic amino acids (Phe + Tyr) TEAA – Total essential amino acids; TNEAA – Total non-essential amino acids.

[a] Sulfur containing amino acid: Met + Cys; ## Aromatic amino acid: Phe + Tyr.

[b] First limiting amino acid; **Second limiting amino acid; ***Third limiting amino acid.

meal and canola meal [5,21]. Guar korma showed higher fat content (5.0 %) than churi (4.1 %). These results were similar to Nidhina and Muthukumar findings [5], where they reported industrial guar korma and raw churi had 5.4 and 4.1 % fat content, respectively. Crude fibre content was higher in guar churi (12.9 %) than in korma (6.0 %). The fibre content of guar churi was similar to that of the Canola meal (11.54 %), and in guar korma, it was identical to that of flax seed meal (5.27 %) [21]. Guar churi had higher total carbohydrate content (30.4 %) than korma (22.36 %), and these results were comparable to earlier study reported by Nidhina and Muthukumar [5]. The carbohydrate content in korma and churi was lower than that of kenaf seed meal (57 %) [25] and flax seed meal (34–38 %) [26]. Based on these findings, guar korma was selected over guar churi for further biochemical and functional characterization studies as it contains significantly higher protein content.

3.2. Selection of the guar protein isolates extraction method

Protein isolates were prepared separately from guar korma and churi by water and salt-alkali extractions using the isoelectric pH precipitation method. The salt-alkali extracted protein concentrates exhibited significantly higher protein content. The salt-alkali extracted guar korma protein isolate had 95.2 % ± 1.96 protein, while its water extracted counterpart contained 89.3 % ± 1.3 protein. These findings were comparable to results obtained for guar seed protein isolate, with a protein content of 88.2 %, and hemp protein isolate, with 85.9 % protein [7,27]. Whereas salt-alkali and water-extracted protein isolate of guar churi showed 84.9 % ± 1.42 and 83.3 % ± 1.41 protein content, respectively (Fig. 1B). The yield of total protein in WPIK (48.7 %) was higher than SAPIK (37.4 %), WPIC (30 %) and SAPIC (26.9 %). The water extraction process yielded significantly higher protein recovery than salt-alkali extraction. The water extraction is an economically feasible method for large-scale extraction, whereas salt-alkali is challenging due to salt recycling at larger scale. Hence, water extraction in alkali conditions (pH 9.0) at room temperature could be a viable method for the large-scale production of guar protein isolate.

3.3. Nutritional properties

3.3.1. Amino acid analysis

The quality of protein isolate was determined using the amino acid composition of guar korma, churi, and their water extracted protein isolates (WPIK and WPIC) shown in Table 1 and compared with recommendations for adults and children [28]. Guar korma, churi, WPIK, and WPIC are rich in Glx, Arg (Glu and Gln), and Asx (Asp and Asn). These results were similar to the earlier studies on guar meal protein and lupin protein isolates [3,7,29]. WPIK and WPIC exhibited relatively higher amounts of essential amino acids than defatted guar korma and churi (Table 1). The increased content of some amino acids in WPIK and WPIC may be attributed to the water-alkali extraction process, which facilitates higher protein solubility and stability along with the isoelectric precipitation method. Similar results were also observed by El-Adawy et al. [30]. Millan et al. showed higher content of total amino acids of lupin protein isolate prepared by water-alkali extraction followed by isoelectric precipitation [31]. Among all the essential amino acids, Leu was abundant amino acid, followed by Lys and Phe. Among all fractions, Met and Cys were rich in guar churi, followed by WPIK, guar korma, and WPIC. The higher content of aromatic amino acids was present in WPIC, followed by WPIK, guar korma, and churi. The contents of Ile, His, aromatic amino acids, Trp, and Val in WPIK and WPIC were higher than FAO/WHO [28] recommendations for children and adults. WPIK contains all the essential amino acids in adequate amounts for adults and thus could be used as a nutritional supplement [28]. WPIK and WPIC showed higher amounts of Lys than rice (3.7 g/100 g) protein and lower than lupin protein isolate (5.9 g/100 g) [32]. Amino acid analysis revealed that sulfur-containing amino acids were limiting for WPIK and WPIC, whereas Val was the limiting amino acid for guar korma and churi. Hence, fortifying limiting amino acids in small quantities will improve the biological value of WPIK and WPIC as desirable nutritional proteins for food formulations.

3.3.2. Nutritional properties of guar protein isolates

The nutritional property of a protein can be assessed by its amino acid composition against the standard pattern of amino acids. The amino acid scores (%), predicted protein efficiency ratio (PER), and the ratio of essential amino acids to the total amino acids (E/T%) are used to quantify the nutritional quality of guar korma, churi, WPIK, and WPIC. The amino acid score denotes limiting amino acids in the test sample. Amino acid score analysis revealed that Val was the first limiting amino acid in guar korma and churi, whereas, for WPIK and WPIC, it was the sulfur-containing amino acids (Table 1). It was well reported that legume proteins show deficiency of sulfur containing amino acids, namely cys and Met. Lys was the second limiting amino acid in guar korma, WPIK, and WPIC, whereas it was Thr for churi. The third limiting amino acid was Thr in guar korma and WPIK, whereas it was Leu in churi and WPIC. Similar results were observed in Simarouba meal and beach pea legume protein [20,33]. Low levels of Lys were also reported in lotus seed protein and watermelon seeds [34,35].

There was a significant difference in the (E/T%) among churi, korma, WPIK, and WPIC. Proteins with E/T values greater than 36 % are considered nutritionally beneficial. The E/T (%) for churi, korma, WPIK, and WPIC was 26.31, 28.15, 34.35 and 35.44 %, respectively (Table 1). The E/T (%) of WPIC and WPIK were similar to the watermelon seed proteins and the albumin fraction of lotus seed protein [34,35]. WPIC showed a better E/T (%) and can be used as an excellent source of protein for feeding children and infants.

PER is considered the best indicator for evaluating protein quality. The PER values for WPIK and WPIC were predicted using the equation [1–3]. The PER values of WPIK and WPIC were 1.92 and 1.97, respectively (Table 1), and these are higher than PER values of cowpea protein (1.21) and pigeon pea protein (1.82) [36]. According to Friedman, a protein with a PER 1.5–2.0 was considered a medium-quality protein [37]; a PER value of 2.0 and above is considered nutritionally high-quality. Since WPIK and WPIC PER values are close to 2.0, they may be regarded as nutritionally high protein quality.

3.3.3. Protein digestibility corrected amino acid score (PDCAAS)

PDCAAS was calculated to determine the protein quality based on the amino acid requirements and its ability to digest it. PDCAAS (%) value of WPIK was 59 % for the 2–5 age group, 67 % for the 10–12 age group, and 86 % for adults. WPIC showed PDCAAS values of 49, 56, and 72 % for the 2–5 age group, 10–12 age group, and adults, respectively (Table 1) [28]. WPIK and WPIC showed higher PDCAAS values than legumes such as peanut (70 %), wheat (42 %), maize (46 %), and cowpea flour (46.74 %). It indicates that WPIK

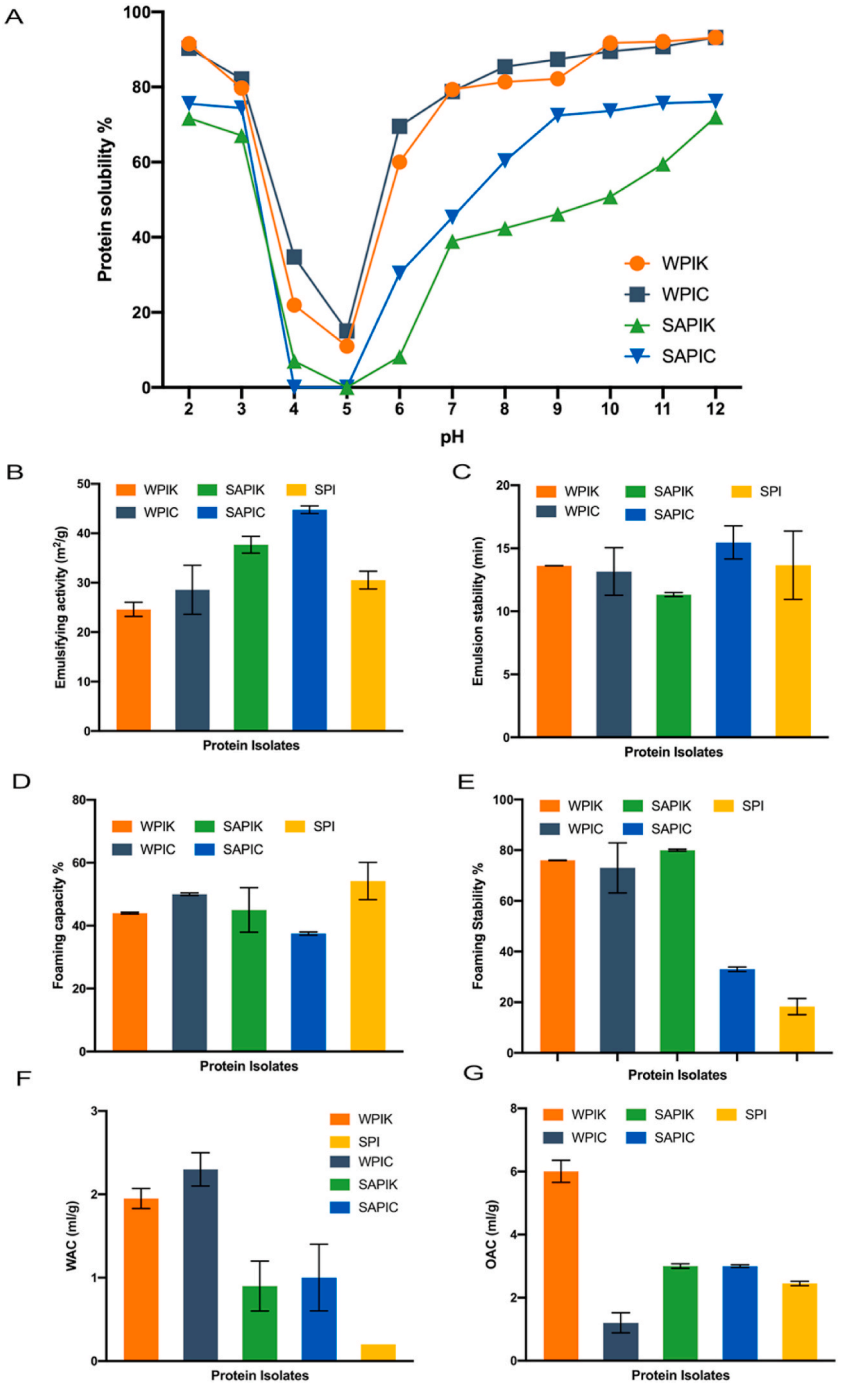

Fig. 2. Functional properties of water and salt-alkali extracted protein isolates from guar korma and churi. (A) Solubility profile of different fractions of guar korma and churi protein isolates at different pH. **(B)** Emulsifying activity and **(C)** Emulsion stability of guar korma and churi protein isolates. **(D)** Foaming capacity and **(E)** Foaming stability of protein isolates of guar korma and churi **(F)** Water absorption capacity and **(G)** Oil absorption capacity of protein isolates of guar korma and churi.

and WPIC contained higher amounts of digestible proteins rich in essential amino acids. However, PDCAAS values of WPIK and WPIC were lower than soy (91 %) [38–40].

3.4. Analysis of functional properties

3.4.1. Protein solubility

Protein solubility is a vital functional property, as it affects the color, sensory, and texture properties of food products and suggests the types of foods or beverages in which the protein is supplemented. The pH-dependent solubility profiles of guar korma and churi protein isolates were shown in Fig. 2A. Water and salt alkali-extracted protein isolates from guar korma and churi showed the least solubility at pH 4–5, due to its isoelectric pH. Similar results were also reported in other legumes such as soybean (pH 4–5), pea (pH 4–6), and common vetch (pH 4–5) [23,24]. The solubility of water and salt-alkali extracted protein isolates was increased at pH below 4 and above 6. The maximum solubility was observed at pH 12 for the WPI of korma (93 %) and churi (93 %), SAPI of korma (72 %), and churi (76 %). Water and salt-alkali extracted protein isolates of korma and churi showed increased solubility between pH 2.0 and 3.0, which suggests that these protein isolates may be recommended as supplements in acidic beverages. WPI and SAPI of korma and churi showed a 'U' shaped solubility pH graph curve similar to pea, soybean protein, bitter melon seed protein, and sunflower seed protein [23,41]. Higher solubility of a protein is correlated with a lower number of hydrophobic amino acids, electrostatic repulsion and raised charge, and ionic hydration at a pH lesser or greater than the isoelectric pH. The extraction, denaturation, and salting methods greatly influence the protein solubility by altering the hydrophobicity/hydrophilicity ratio of the amino acids on the protein surface and influencing its surface characteristics, respectively [42].

3.4.2. Emulsifying property

Emulsifying activity (EA) of a substance is described as the volume of oil that can be emulsified by protein before phase inversion or collapse of emulsion occurs, where emulsion stability (ES) is the capability of substance droplets to remain dispersed without coalescence over a defined period. Proteins are selected as emulsifiers over low molecular weight substances in food applications, especially in manufacturing formulated food products. The emulsifying activity of a protein is influenced by many factors, including protein hydrophobicity, solubility, molar mass, the presence of carbohydrates, pH, temperature, charge, conformational stability, and ionic strength [42,43]. The presence of salts, pH, and several physical processes like cream formation, coalescence, and flocculation influences the emulsion stability of a protein.

Emulsifying activity and emulsion stability of WPIK, WPIC, SAPIK, SAPIC, and SPI were represented in Fig. 2B & C. The emulsion properties of protein isolates (WPIK, WPIC, SAPIK, and SAPIC) were compared with SPI. SAPIC showed the highest emulsifying activity (44.77 $m^2/g \pm 0.78$), followed by SAPIK (37.68 $m^2/g \pm 1.69$), SPI (30.52 $m^2/g \pm 1.79$), WPIC (28.56 $m^2/g \pm 4.95$), and WPIK (24.60 $m^2/g \pm 1.43$). The increased emulsification capacity of SAPIC and SAPIK can be attributed to their high surface charge and solubility at higher pH. SAPIC exhibited the highest emulsion stability (15.47 min ±1.3), while SPI, WPIK, WPIC, and SAPIK showed 13.66, 13.62, 13.16, and 11.33 min emulsion stabilities, respectively. The emulsifying activity of SAPIC was similar to soybean meal (44.10 m^2/g) and higher than SPI and SPI-Acacia gum conjugate [13,21]. Khattab and Arntfield reported emulsifying activity of raw (33.60 m^2/g), roasted (25.95 m^2/g), and boiled (20.50 m^2/g) flax seed meal which are lower than SAPIC and SAPIK [21]. Emulsifying stabilities of WPIC and WPIK were similar to pumpkin seed protein (alkali fraction) and SPI (14.95 min) [12,13].

3.4.3. Foaming properties

The foaming properties of proteins are generally evaluated by two indices, foaming capacity (FC) and foaming stability (FS). The primary role of proteins in foams is to lower the interfacial tension and form continuous and cohesive films. An ideal protein foaming agent should stabilize foams rapidly and effectively at lower concentrations, act as an operative foaming agent at various pH ranges, and resist foaming inhibitors such as fat, alcohol, and flavorings. Several factors, such as the source of the protein, solubility, pH, processing methods, concentration, temperature, and foaming methods, determine the foaming properties of proteins (Kinsella, 1984).

The foaming properties of the WPI and SAPI of guar korma and churi are presented in Fig. 2D & E. The foaming capacity of WPIK (44 %) and WPIC (50 %) were more significant than the SAPI fractions but were lower when compared to SPI (54.2 %) (Fig. 2D & E), this could be due to lesser surface tension at the air-liquid interface [15]. SAPIK (80 %) showed the highest foaming stability, followed by WPIK (76 %), WPIC (73 %), SAPIC (33 %), and SPI (18.3 %). The foaming capacity of WPIK and SAPIK were higher than the raw (25 %), autoclaved (10 %), boiled (5 %), and roasted industrial guar korma (5 %) [5]. The foaming stability of proteins depends on the properties of interfacial membranes formed by protein-protein interactions. In contrast, foaming capacity relies on the ability of proteins to absorb at an air-water interface. Hence, the foaming capacity of protein molecules may not necessarily correlate to foaming stability [44]. The foaming stability of guar korma and churi proteins are higher than the pumpkin seed proteins reported by Vinayshree and Vasu, indicating their suitability for incorporation in soft drinks and beverages [12].

3.4.4. Bulk density

Bulk density is the weight of the powder present in a defined volume. It is expressed in g/cm^3, kg/m^3, or g/100 mL. Bulk density is an essential factor that controls the product's packaging requirements. High bulk density powders occupy less space and reduce packaging, storage, and transport cost per kg of the material. The bulk density of protein powder is influenced by several factors, including the density of solids, the amount of occluded air, and the amount of air between the particles [45]. WPIK, WPIC, SAPIK, SAPIC, and SPI showed a bulk density of 0.215, 0.4, 0.212, 0.340, and 0.45 g/mL, respectively. The bulk density divergence among protein isolates may be due to the structural changes caused by different extraction methods. The bulk densities of churi isolates were

higher than korma isolates. They were similar to albumin (0.330 and 0.360 g/mL) and prolamine (0.310 g/mL) fractions of Mateera and watermelon seed protein [34]. SPI showed a bulk density of 0.45 g/mL, which is comparable with WPIC.

3.4.5. *Water holding capacity (WHC) and oil holding capacity (OHC)*

WHC is the measure of the total amount of water molecules that can bind to the protein isolates against gravity. WHC of proteins is influenced by several factors, including the composition of amino acid, protein conformation, ionic strength, solubility, temperature, pH, and the concentration of surfactants, tannins, lipids, and carbohydrates associated with proteins [46].

WHC of WPIK and WPIC were 2–1.9 and 2.3–2.0 mL/g (Fig. 2F), respectively, which was higher than the SAPIK (0.9–0.8 mL/g) and SAPIC (1 mL/g). The higher WHC in WPIK and WPIC could be due to the presence of its high phosphate groups and other polar groups, which could interact with water molecules to enhance the hydration of proteins [47]. WHC of WPIK and WPIC were comparable to guar protein isolate, bitter and sweet lupin seed protein isolates, sesame seed protein isolate, Ginkgo seed protein isolate, and cowpea protein isolate [14,15,17,30,48]. Protein isolates with WHC from 1.49 to 4.72 mL/g were recommended for their application in viscous foods. Hence, the WPI of guar korma and churi could be essential ingredients in foods such as bakery products, baked doughs, gels, and sausages where a high water-holding capacity is desired.

The OHC value was significant as a measure of the protein absorption capacity of oil, which can reflect the hydrophobic capacity of protein. OHC of WPIK was 5–6 mL/g which is higher than the WPIC (1–1.2 mL/g), SAPIK (3 mL/g), SAPIC (2.8–3 mL/g), and SPI (2.45 mL/g) (Fig. 2G). Similarly, Khalil showed an OHC of 3.57 mL/g for guar protein isolate [7]. OHC of WPIK, WPIC, SAPIK, and SAPIC were higher than the bitter lupin protein isolate (2.81 mL/g), sesame seed protein isolate (1.5 mL/g), cowpea protein isolate (1.10 mL/g), and ginkgo protein isolate (2.95 mL/g) [14,15,30,48]. These results indicate that WPIK and WPIC had good water and oil

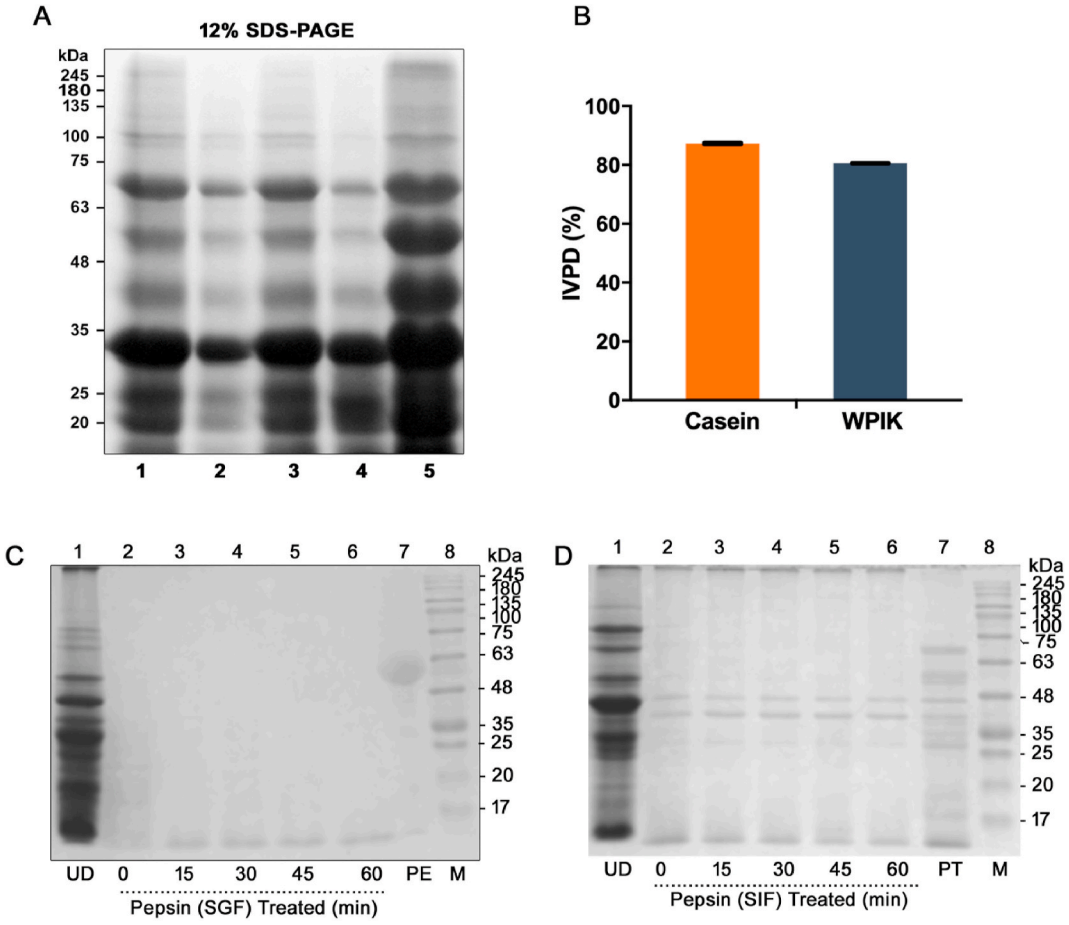

Fig. 3. SDS-PAGE analysis and *invitro* digestion of guar korma (A) (Lane 1) SDS-PAGE band pattern of crude guar korma, (Lane 2) defatted guar korma, (Lane 3) autoclaved defatted guar korma, (Lane 4) water extracted protein isolate from guar korma (WPIK), (Lane 5) salt-alkali extracted protein isolate from guar korma (SAPIK). **(B)** *In vitro* protein digestibility of WPIK and casein. **(C).** *In vitro* digestion of WPIK in SGF. (Lane 1) Undigested WPIK (UD), (Lane 2–6) samples treated with SGF at 0, 15, 30, 45, and 60 min, (Lane 7) (PE) pepsin enzyme, and (Lane 8) pre-stained protein ladder. **(D)** *In vitro* digestion of WPIK in SIF. (Lane 1) is Undigested WPIK (UD), (Lane 2–6) samples treated with SIF at 0, 15, 30, 45, and 60 min, (Lane 7) PT (mixture of five pancreatin enzymes), (Lane 8) pre-stained protein ladder. (Original images of Fig. 3A, C and D are submitted as supplementary data S3-A, B, C respectively).

holding capacities. Therefore WPIK with higher OHC might be used in food industries for mayonnaise, cake batters, bakery products, doughnuts, sausages, soups, and salad dressings.

3.5. Protein profiling by SDS-PAGE analysis

SDS-PAGE of crude, defatted, autoclaved, water, and salt-alkali extracted protein isolates of guar korma was performed to examine the effect of extraction methods on the molecular weight distribution of protein and shown in Fig. 3A. All fractions exhibited a similar protein profile, indicating that extraction processes such as defatting, autoclaving, and isoelectric precipitation did not cause protein denaturation or proteolysis. The protein profile of all fractions resolved into eight protein bands in the range of 100–17 kDa (Fig. 3A).

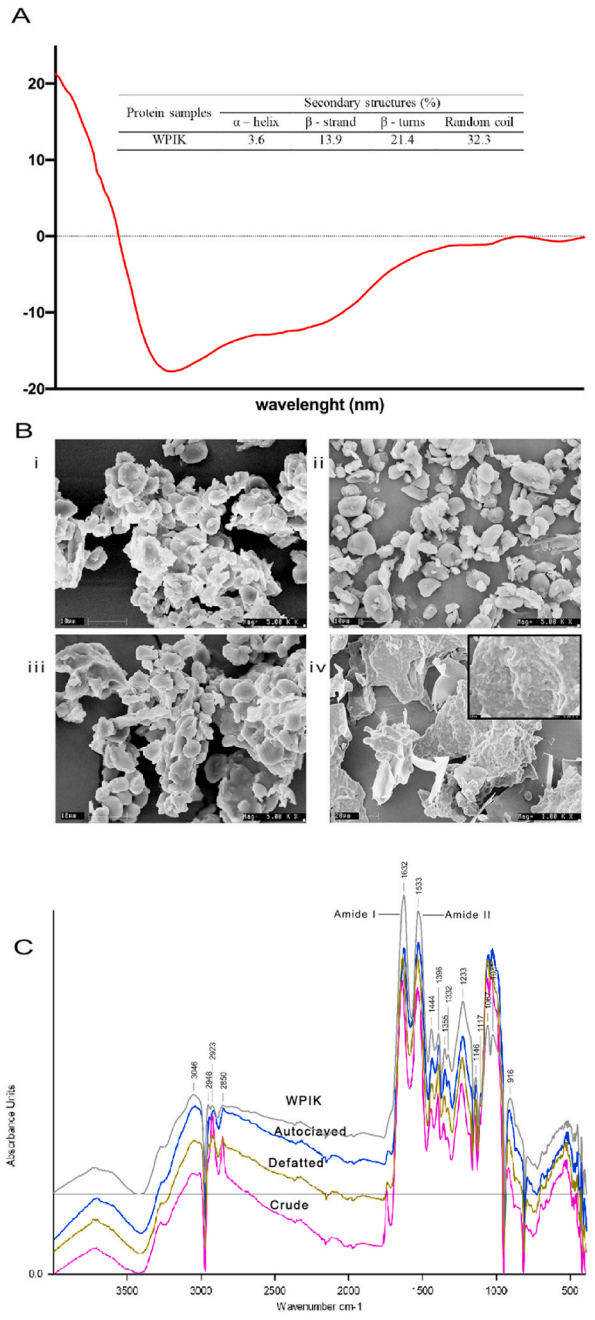

Fig. 4. Structural and morphological characteristics of guar protein isolate (A) Circular dichroism (CD) spectral analysis of WPIK **(B)** Scanning electron microscopic images of differentially treated guar korma. (i) Crude korma, (ii) Defatted korma, (iii) Autoclaved korma, and (iv) WPIK. (C) Fourier transform infrared spectra (FTIR) of crude korma, defatted korma, autoclaved korma, and WPIK.

Similarly, protein isolates from field peas and kidney beans also showed protein bands in the range of 95–14 kDa and 93-17 kDa, respectively [6]. Guar korma-derived samples exhibited darker bands in the 70-17 kDa range. The differential intensity of protein bands between guar korma may be attributed to the effect of the extraction methods on protein. Salt-alkali extracted protein isolates from guar korma showed darker bands in the range of 100–17 kDa than water extracted protein isolates. A similar observation was also reported by Vinayashree and Vasu [12], where they showed alkali fraction of pumpkin seed protein exhibited darker bands than the water fraction. Water extracted protein isolates from guar korma showed darker bands in the 70-17 kDa range.

3.6. In vitro digestion

3.6.1. In vitro protein digestibility

The *in vitro* protein digestion assay condition resembles the natural digestion process, which uses physiological and biochemical properties of gastric and intestinal fluids. It is an efficient and reliable method to quantify the bioavailability of protein isolate when consumed. *In vitro* digestion of WPIK was performed using a pepsin-pancreatic enzyme system, simulated intestinal fluid, and simulated gastric fluid at 37 °C. Fig. 3B shows *in vitro* protein digestibility of WPIK and casein. WPIK exhibited 80.6 % *in vitro* digestibility, whereas the standard of casein showed 87.3 % (Fig. 3B). *In vitro* digestion of WPIK was higher than other legumes such as soy protein isolate (52 %), cowpea (73 %) and pigeon peas (59 %) [33,49]. The higher *in vitro* digestibility of WPIK may be due to rich amounts of digestible proteins and lower or negligible amounts of anti-nutritional factors such as phytates, tannins and protease inhibitors which contribute to decreased digestibility.

3.6.2. Gastric digestion assay of WPIK

SGF and SIF digestion stability assay was performed to check the degree of digestibility of WPIK. SGF contains pepsin, an acidic proteolytic enzyme (pH 1.2), whereas SIF as pancreatin (a combination of amylase, lipase, trypsin, ribonuclease, and protease). SDS-PAGE analysis showed that WPIK was completely digested within 15 min in both SGF (Fig. 3C) and SIF (Fig. 3D) treatments. The complete digestion of dense protein bands in WPIK into smaller fragments within 15 min indicates that WPIK may not exhibit any allergenic activity [18]. Similarly, Sinagawa-Garcia et al. and Vinayashree and Vasu showed complete digestion of transgenic amarantin maize and pumpkin seed protein within 15 min when treated with SGF and SIF [12,50]. These results showed complete digestion of WPIK in the human gastrointestinal tract.

3.7. Structural and morphological characteristics of guar protein isolates

3.7.1. Circular Dichroism (CD)

The CD is an ideal process for evaluating the secondary structure of proteins, and it is defined as the differential absorption of left-handed and right-handed circularly polarized light [51]. A far-UV CD spectrum was commonly used to analyze the secondary structure arrangement of WPIK and WPIC. The CD spectral profiles and the secondary structure composition of WPIK and WPIC were presented in Fig. 4A. The spectra of WPIK and WPIC showed a positive band near 190 nm with a zero crossing around 198 nm and a negative band near 200 nm. β-turns (around 21 %) and random coil (about 32 %) were the predominant structures in WPIK and WPIC. No significant difference in the secondary structure composition of WPIK and WPIC was observed. A similar trend of a higher amount of random coil than β-turn and β-sheet were observed in Akebia trifoliata var. *australis* seed protein [43], and he attributed this trend to the alkali extraction of protein isolate (Du et al., 2012). Similar results were also observed in freeze-dried quinoa protein [52], salt and alkali fractions of pumpkin seeds [12], buckwheat seed protein, and common vetch glutelin protein (CVGTP) [24,53].

3.7.2. Scanning electron microscopy (SEM)

The microstructures of crude, defatted, autoclaved korma and WPIK were studied by SEM, and images were depicted in Fig. 4B. The SEM results indicate that crude, defatted, and autoclaved korma exhibited heterogeneous particles with smooth and globular surface topography. SEM analysis revealed that no significant changes happened during different treatments of guar korma, such as defatting and autoclaving. WPIK showed flatter and tighter microstructures with strong angles varied from crude, defatted, and autoclaved korma. A similar microstructure was also reported in wheat protein [54], cottonseed meal [55], protein isolate (PI) of Purslane seeds and ultra sound pretreated quinoa protein [56,57]. The smooth surface of WPIK may be due to the higher content of protein [58] and the alkali extraction process [59].

3.7.3. Fourier transform infrared spectra (FTIR) analysis

FTIR analysis indicates the molecular structural changes of the molecules using the vibration of functional groups. FTIR spectrum (3500–500 cm^{-1}) of crude, defatted, autoclaved korma, and WPIK reveals absorption bands of major functional groups (Fig. 4C). The spectra bands 2948, 2947, 2923, and 2922 cm^{-1} correspond to the alkane compound due to asymmetric CH_2 stretching. The 2947 and 2948 cm^{-1} were present in defatted, autoclaved korma, and WPIK but absent in crude korma. The spectra band 1744 cm^{-1} corresponds to triglycerides due to C–O stretching of esters observed in a crude korma. This band was shifted to 1742 cm^{-1} with reduced intensity, indicating the reduction of triglycerides due to the defatting process. This triglyceride spectra band was absent in autoclaved korma and WPIK, suggesting low or no triglycerides in the sample. Similar results were also observed in the FTIR analysis of crude okra seed flours [60]. The spectra band 1635 cm^{-1} was due to N–H bending/C–N stretching corresponding to the amide-1 band of the protein. The C–O and C–N stretches were observed at 1537, 1536, and 1533 cm^{-1}, corresponding to the amide-2 band of the protein. However, the bending and stretching vibrational bands of amide-1 and amide-2 are important for the determination of secondary structure of

protein [61]. The spectra band 1061 and 1036 cm^{-1} present in crude korma were due to C=N stretch, which suggests the presence of primary amine groups. The intensities of these bands were greater in crude, defatted, and autoclaved korma than WPIK.

4. Conclusion

The present comprehensively analyzed the biochemical, functional, and nutritional properties of the water extracted protein isolate of guar korma. WPIK and WPIC contained higher amounts of Arg, Asx, and Glx. The essential amino acid composition of protein isolates were as per FAO/WHO reference protein limits, indicating its potential suitability as valuable nutritional and functional components in various food applications. The contents of Ile, His, aromatic amino acids, Trp, and Val in WPIK and WPIC were higher than FAO/WHO (2007) recommendations for children and adults. WPIK contains all the essential amino acids in adequate amounts for adults. WPIK and WPIC revealed satisfactory PER, E/T, amino acid score, and PDCAAS values. *In vitro* digestion of WPIK showed the complete digestion of dense protein bands into smaller fragments. WPIK exhibited favorable functional properties required for using it as a functional ingredient in various food applications. The results of microstructures, flatter and tighter with strong angles like structure in WPIK might be improve protein solubility and digestibility. Collectively, our results demonstrated that WPIK could be an excellent source of plant protein for a rich source of amino acids and essential amino acids with excellent functional properties. Consequently, WPIK holds the potential to serve as a viable plant-based protein alternative in health-oriented food formulations for the creation cost effective innovative food products.

Data availability

The authors declare that the data supporting the findings of this study are available within the article. The raw/derived data supporting the findings of this study are available from the corresponding author at request.

Conflict of interest

The authors declare no competing interests.

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Ethics declarations

Not applicable.

CRediT authorship contribution statement

Bhavya Kotnala: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Vijayaraj Panneerselvam:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Data curation. **Arun Kumar Vijayakumar:** Writing – review & editing, Validation, Resources, Funding acquisition, Data curation, Conceptualization.

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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Appendix A. Supplementary data

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