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Production, Characterization, and Functional Properties of Protein Concentrate from Momordica cochinchinensis Seeds

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Abstract:

Momordica cochinchinensis protein concentrate (MCPC) was obtained from partially defatted *M. cochinchinensis* seeds through isoelectric precipitation and subsequent physical separation. The effects of various parameters in ultrasound-assisted extraction were investigated to optimize protein yield. The optimal extraction conditions were identified as pH 11, ultrasound amplitude of 25%, sonication time of 20 minutes, and a material-to-solvent ratio of 1:25. MCPC and partially defatted Gac flour (PDS) were analyzed for proximate composition, amino acid profile, antioxidant capacity, and functional properties, including protein solubility, water- and oil-binding capacities, emulsifying ability, foaming capacity and stability, and gelation behavior. The results showed that MCPC contained more than 81% protein, comprising both essential and non-essential amino acids, with glutamic acid (4.47 g/100 g) and aspartic acid (2.28 g/100 g) as the predominant residues. MCPC also exhibited moderate yet significant antioxidant activity compared with commonly studied plant proteins. Comprehensive characterization of the defatted seed and its protein concentrate revealed distinct modifications in physicochemical and functional attributes following protein isolation, underscoring the potential of MCPC as a high-quality plant-based protein source for food applications.

Keywords: Momordica Cochinchinesis seed, protein concentrate, Ultrasound-assisted extraction, Functional Properties.

1. Introduction

The *Momordica Cochinchinensis Spreng,* as known as Gac, belongs to the Curcurbitaceae family, and is a tropical climbing plant commonly grown in Southeast Asia and Northeastern Australia [1]. The outer skin of the Gac fruit is covered with small spines while the flesh (dermis) is dense and light orange in color. The seeds are usually brown or black, surrounded by aril. In the Gac fruit, the pulp accounts for the highest proportion (49–75%), the red skin surrounding the seeds accounts for about 6–31%, and the skin accounts for about 17% [2]. Gac fruit has many different uses from seeds to fruit, the aril is cooked with rice to get its color and oil to make gac sticky rice, used in festivals in Vietnam. In Thailand, immature green Gac fruit is often used as a vegetable [3]. Gac seed is the mature dry seed, which is inedible and mostly considered as waste in the food industry. However, in traditional Asian medicine, gac seed is used to treat various conditions such as inflammation, liver and spleen and has been used in traditional Asian medicine to treat ulcers and inflammation [4]. According to [5], the gac seed kernel has a very high protein content, showing great potential for application in the food and value-added products sectors. The outstanding composition of the gac seed kernel is the high oil and protein content, showing great potential in focusing on exploiting these two groups of substances with biological and economic value. In addition to its high fat content, gac seeds also contain biological proteins and peptides that have been isolated and studied for application in the medical field, including MCCTI-1 [6], MCoCC-1, MCoCC-2 [7], MCoTI-I and MCoTI-II [8], MCo-3 to MCo-6 [9]. Of these, MCCTI-1 protein is a trypsin inhibitor isolated from gac seeds by extraction using 5% acetic acid solvent, ammonium sulfate precipitation, ion exchange chromatography and high performance liquid chromatography (HPLC) [6]. Small molecular weight proteins (17 kD) in the seeds have neuroactive activities that can be applied to treat

neurodegenerative diseases such as dementia, Alzheimer's and Parkinson's [10]. In addition, Cochinin B, a ribosome-inactivating protein with a molecular weight of 28 kDa exhibited potent antitumor activity on human cervical, embryonic kidney, and small cell lung carcinoma cell lines [11].

Plant-based protein powders have attracted significant consumer attention in recent years, driven by their growing demand for sustainable and health-conscious dietary options. This interest has led to the development of a diverse range of plant-based protein sources with superior nutritional and functional properties. Recent reports have highlighted the protein content and amino acid composition of many commercially available plant-based proteins [12] . With the best our knowledge, for Gac seed protein extraction studies mainly focus on the isolation of short protein chains and biological peptides with applications mainly in the fields of medicine and biology. Although gac seeds have a very high protein content and are currently considered a waste material, there are no published reports on the specific content of essential amino acids in gac seed protein, protein compositions, as well as their potential applications in food. The aim of this research is to product the protein concentrate from gac seeds and evaluated for proximate composition and functional properties

2. Materials and methods

2.1. Materials

Gac fruits were purchased from local market in Ho Chi Minh City. Partially defatted Gac seed powder was prepared as follows: Firstly, the remained pulp on the seeds was manually removed. The seeds were then washed with potable water and the kernels were manually separated from the seeds. The kernels were ground and subsequently dried at 40°C to a moisture content of 10%. Chemical compositions of the Gac seed powder were as follows (% on dried basis): 2.31% ash, 54.19% lipid, 27.11% protein, 16.39% total carbohydrate. The partially defatted Gac seed powder was prepared in our lab using a Heat press at 120°C. After pression, the solid phase was separated, dried at 40°C to a moisture content of 5% and then ground to particles with a size less than 0.5 mm. The resulting powder was stored at 4 °C until further use for protein extraction.

All chemicals and reagents were analytical grade compounds purchased from Fisher Scientific.

2.2. Production of Gac protein concentrate

Partially defatted Gac seed flour (PDS) was used as the starting material for the preparation of protein concentrate through isoelectric precipitation followed by centrifugation. A 10 g portion of PDS (on a dry matter basis) was subjected to ultrasound-assisted extraction (UAE) to enhance the release of proteins from the plant matrix. The primary objective of this process was to maximize protein recovery efficiency.

Ultrasound-assisted extraction (UAE) was performed under varying water-to-flour ratios, pH levels, ultrasonic durations, and ultrasonic amplitus to identify the optimal conditions for maximum protein recovery. Partially defatted seed flour was mixed with water at flour to water ratios of 1/10, 1/15, 1/20, 1/25, 1/30 and 1/35. The pH of each suspension was adjusted to pH 7, 8, 9, 11, 11, 12 using 1.0 N NaOH and 1.0 N HCl with ultrasonic time of 5-30 min and ultrasonic amplitut of 20-45%. Suspensions were centrifuged and protein concentration in each supernatant was determined by Kjehdal method using 6.25 as conversion factor. The obtained flour suspension was stired at room temperature for 1 h, then centrifuged at 5000 rpm for 20 min. The supernatant was collected and adjusted to pH 4.5 (the isoelectric pH) with 1.0 N hydrochloric acid (HCl). The suspension was centrifuged at 5000 rpm for 20 min. The supernatant was discarded and the precipitate was collected and wash 3 times with distill water and dried at 40°C.

2.3. Determination of functional properties of MCPC and PDS

2.3.1. Water holding capacities

Water holding capacity of MCPC and PDS was determined according to the modified method described by Y. Zhang et al. (2021) [13]. Approximately 0.1 g of the powder was weighed into centrifuge tubes, and 5 mL of distilled water adjusted to pH 2–12 using HCl or NaOH (0.1 N) was added. The mixture was vortexed for 3 min, allowed to stand for 30 min, and then centrifuged at 5000 rpm for 20 min. The supernatant was decanted, and the residue was weighed. Water holding capacity was calculated according to the following equation:

$$WHC\ (\%) = \frac{Weight\ of\ water\ retained\ in\ the\ sample}{Initial\ sample} \times 100$$

2.3.2. Oil binding capacities

Oil absorption capacity of MCPC and PDS was determined according to the modified method described by Y. Zhang et al. (2021) with slight modifications [13]. Approximately 0.1 g of the sample powder was placed in a centrifuge tube and mixed with 5 mL of vegetable oil. The mixture was vortexed for 3 minutes, allowed to stand for 30 minutes, and subsequently centrifuged at 5000 rpm for 20 minutes. After centrifugation, the supernatant was carefully removed, and the remaining residue was weighed. The oil absorption capacity was then determined using the following equation:

$$OAC\ (\%) = \frac{Weight\ of\ water\ retained\ in\ the\ sample}{Initial\ sample} \times 100$$

2.3.3. Emulsifying capacity

Emulsifying capacity of MCPC and PDS was determined according to the modified method described by Yasumatsu et al. (1972) [14]. Approximately 0.7 g of the material was weighed into centrifuge tubes, and 10 mL of distilled water adjusted to pH 2–12 using 1 N NaOH or 1 N HCl was addd. Subsequently, 10 mL of refined soybean oil was added, and the mixture was homogenized at 5,000 rpm for 20 min to form an emulsion. Emulsifying capacity (EC) was calculated using the following equation:

$$EC\ (\%) = \frac{Ht}{He} \times 100$$

Where, EC = emulsifying capacity (%), Ht = height of the emulsified layer after centrifugation (mm), and He = total height of the mixture in the tube (mm).

2.3.4. Foaming capacity and stability

Foaming capacity and foam stability of MCPC and PDS were determined according to the method described by Deng et al. (2011) with slight modifications [15]. Approximately 1 g of the powder was dispersed in 50 mL of distilled water, and the pH was adjusted from 2 to 12. The suspension was homogenized for 3 min using a high-speed homogenizer and then quickly transferred into a 100 mL graduated cylinder to record the volume before and after homogenization. Foaming capacity (FC) was calculated immediately at 0 min, while foam stability (FS) was determined after 120 min at 25 °C. FC and FS were calculated according to the following equations:

$$FC\ (\%) = \frac{V - V_0}{V_0} \times 100$$

$$FS\ (\%) = \frac{V_2}{V_0} \times 100$$

Where, FC = foaming capacity (%), FS = foam stability (%), V_0 = initial liquid volume (mL), V = volume immediately after homogenization (mL), and V_2= volume after 120 min (mL).

2.3.5. Protein solubility (PS)

Protein solubility of MCPC and PDS was determined according to the method described by Lixin Huang et al. (2019) with slight modifications [16]. Approximately 0.125 g of sample was dispersed in 50 mL of distilled water, and the pH was adjusted in the range of 2–12 using 0.1 N HCl or 0.1 N NaOH. The suspension was stirred for 30 min at room temperature and centrifuged at 4000 rpm for 10 min. The protein content in the supernatant and total protein were measured by the Kjeldahl method. Protein solubility was calculated using the following equation:

$$Solubility\ (\%) = \frac{Protein\ content\ in\ supernatant}{Total\ protein\ content\ in\ sapmle} \times 100$$

2.3.6. Gelation ability

The minimum gelation concentration was determined following the method described by Sathe et al. (1981) [17], with slight modifications. Sample solutions were prepared at protein concentrations of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, and 20% (w/v) in 5 mL of distilled water. The test tubes containing the protein solutions were heated in a boiling water bath at 95–100 °C for 30–60 minutes, then immediately cooled under running tap water, followed by further cooling at 4 °C for 2 hours. The minimum gelation concentration was defined as the lowest protein concentration at which the sample did not flow or slide when the test tube was inverted, indicating the formation of a self-supporting gel. This value was determined experimentally by observing the gel strength and flow behavior of each sample upon inversion.

2.4. Physicochemical properties of MCPC

2.4.1. Chemical composition

The moisture, ash, lipid, and protein contents of MCPC and PDS were analyzed following the standard procedures of the Association of Official Analytical Chemists (AOAC) [18]. The protein content was quantified using the Kjeldahl method, applying a nitrogen-to-protein conversion factor of 6.25. The total sugar content was determined according to the AOAC method 974.06.

2.4.2. Protein fraction

The extraction procedure was adapted from Deng et al. (2019) with minor modifications [19]. In brief, PDS was first mixed with distilled water at a 1:10 (w/v) ratio and stirred for 2 h at room temperature. The resulting suspension was centrifuged at 5000 × g for 10 min, and the supernatant was collected as the crude albumin fraction. The remaining residue was re-suspended in 0.5 M NaCl, followed by centrifugation, and the obtained supernatant was designated as the globulin fraction. Subsequently, the residue was extracted with 70% ethanol under continuous stirring, centrifuged, and the supernatant was identified as the prolamin fraction. Finally, the glutelin fraction was isolated from the remaining residue using 0.1 M NaOH. The protein content of each fraction was determined using the Kjeldahl method.

2.4.3. Amino acid compositions

The amino acid contents of MCPC were determined using HPLC and expressed as g amino acid/100 g protein according to AOAC (994.12). The obtained chromatogram was represented in Supplementary information (Fig 1S).

2.4.4. SDS-PAGE separation of protein compositions

SDS-PAGE experiments were performed using a Mini-PROTEAN 3 Cell (Bio-Rad, USA). The separation was carried out by running on polyacrylamide gels (7.3 cm in height) consisting of 4% stacking gel (4% acrylamide/Bis, 0.125 M Tris, and 0.1% SDS) and 12% separating gel (12% acrylamide/Bis, 0.375 M Tris, and 0.1% SDS). Electrophoresis was performed for 1.5 hours at 90V for the first 10 minutes, increasing to 110V until the separation was finished using a running buffer containing 15.1 g/L Tris, 72 g/L glycine, and 5 g/L SDS. After finishing running, gels were stained overnight in stain solution containing 0.3% (w/v) Coomassie Brilliant Blue, 0.25% (v/v) EtOH, and 7% (v/v) acetic acid for at least 1.5 hours. Then gels were washed with a washing solution composed of 40% (v/v) MeOH and 7% (v/v) acetic acid.

For SDS-PAGE analysis, the protein powder was dissolved up to the concentration of 10 mg/mL in buffer solution containing 0.032 M Tris, 0.017 M SDS, 4% (v/v) 2-mercaptoethanol, 0.1% (v/v) bromophenol blue, 51% (v/v) glycerol, and H_2O. The protein sample was ultrasonicated in an ultrasonic bath for 30 minutes, then denatured at 95°C in a water bath for 10 minutes before centrifuging at 13000 rpm for 10 minutes at room temperature. The sample was run at 10-20-25-30-35 μg on a 12% polyacrylamide gel. GangNam-STAIN™ Prestained Protein Ladder (iNtRON Biotechnology DR, South Korea) was carried out at the same time as the samples

2.4.5. DPPH Radical Scavenging Activity

The antioxidant activity was evaluated using the DPPH radical scavenging assay. In brief, samples at various concentrations were extracted with distilled water and treated ultrasonically for 15 minutes. Subsequently, 1 mL of the extract was mixed with 5 mL of 0.08 mM DPPH solution prepared in water and incubated in the dark at room temperature for 30 minutes. The absorbance of the reaction mixture was recorded at 517 nm. A standard calibration curve was generated using Trolox (0–200 μM), and the antioxidant activity was expressed as micromoles of Trolox equivalents (μmol TE) per gram of protein.

3. Results and Discussion

3.1. Protein fractions

The protein components of Gac seeds were isolated and quantified based on their solubility in various solvent systems. The analysis showed that glutelin (NaOH-soluble) and globulin (salt-soluble) were the major fractions, accounting for 48.08% and 27.68% of the total protein content, respectively. Conversely, albumin (water-soluble) and prolamin (ethanol-soluble) represented only 12.41% and 9.66% and 2.17% of the protein was nonextractable. These results suggest that glutelin and globulin serve as the main storage proteins in Gac seeds. The present findings contrast with those reported for another Momordica species, Momordica charantia, in which albumin was identified as the dominant protein fraction (49.3%), followed by globulin (29.3%) and glutelin (3.1%), while prolamin was absent [19].

Therefore, this research emphasizes optimizing the alkaline extraction process and evaluating the functional characteristics of the obtained protein concentrate to explore its potential utility in food applications.

3.2. Ultrasound-assisted Extraction of protein from Defatted Gac Seed

3.2.1 Effect of solid-to-solvent ratio on protein extraction yield

The effect of solid-to-solvent ratio on extraction yeild was evaluated at five levels under fixed conditions (pH=9; ultrasound amplitude =30 % and ultrasound time: 15 min) and presented in Fig 1.

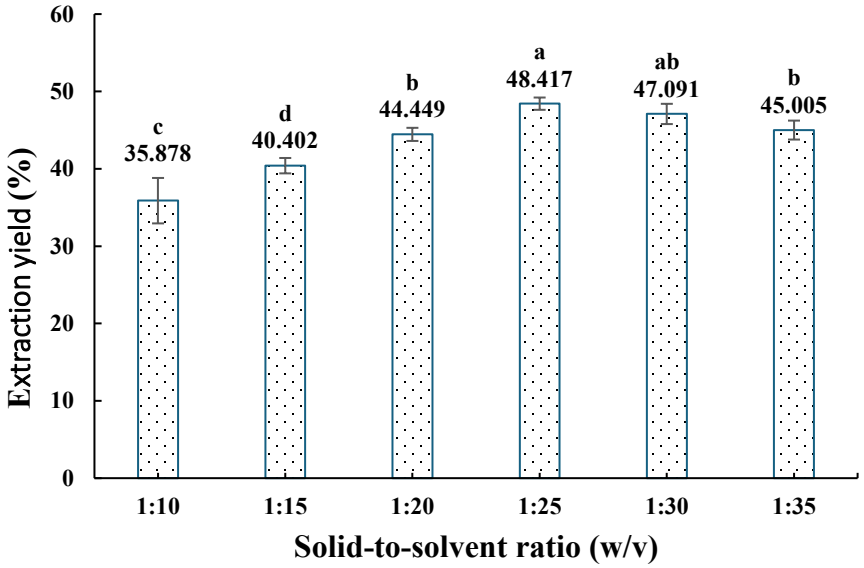


Figure 1: Effect of solid-to-solvent ratio on protein extraction yield

As illustrated in Figure 1, the extraction yield increased progressively with higher solvent-to-material ratios, rising from 1:10 to 1:25 and reaching a maximum of 48.42%, which was significantly higher ($p < 0.05$) than those obtained at lower ratios. This enhancement can be attributed to improved diffusion of proteins into the solvent, reduced protein re-precipitation in a more diluted medium, and more efficient propagation of ultrasonic waves [20] [21]. However, a further increase in the solvent ratio to 1:30 and 1:35 resulted in a slight decline in yield, likely due to excessive dilution, which decreased ultrasonic energy density and cavitation efficiency [21]. Similar observations have been reported in studies on protein extraction from other sources. Nguyen et al. (2019) found that protein yield from defatted peanut (Arachis hypogaea L.) increased up to a 1:25 ratio before stabilizing [22], while Dong et al. (2011) reported notable improvements in protein extraction from rapeseed (Brassica napus L.) as the solvent ratio increased from 1:10 to 1:25 [23]. Based on these findings, a material-to-solvent ratio of 1:25 was identified as the optimal condition for protein extraction in this study.

3.2.2. Effect of pH on protein extraction yield

The variation in protein extraction yield as a function of solution pH under fixed conditions (ultrasound amplitude = 30%, solid-to-solvent ratio = 1:25, and ultrasound time = 15 min) is shown in Figure 2.

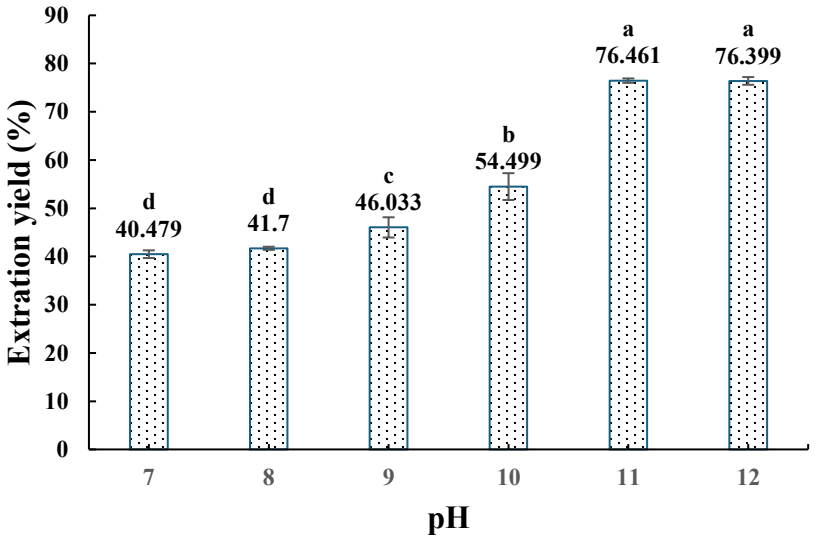


Figure 2: Effect of pH on protein extraction yield

The extraction yield increased steadily as the pH rose from 7 to 11, reaching a maximum of 76.46% at pH 11, with significant differences observed among treatments ($\alpha = 0.05$). This enhancement is likely due to improved protein solubility under alkaline conditions, where a greater deviation from the isoelectric point (pI) enhances electrostatic repulsion, promotes protein unfolding, and disrupts associations with polysaccharides and lipids, thereby facilitating protein release [24]. Beyond this point, at pH 12, no significant improvement was observed compared to pH 11, which may be attributed to excessive denaturation or partial hydrolysis leading to reduced solubility and protein aggregation [24]. Similar pH-dependent patterns have been reported for other oilseeds. Celik et al. (2019) observed that protein extraction from defatted sour cherry seeds increased with pH, reaching a maximum yield of 76.9% at pH 12, although pH 10 was identified as optimal to balance extraction efficiency and structural preservation [25]. Therefore, pH 11 was determined to be the optimal condition for protein extraction from Gac seeds, providing both high yield and structural stability.

3.2.3 Effect of ultrasound conditions on protein extraction yield

The effect of ultrasound amplitude and ultrasound time on extraction yeild was evaluated and presented in Fig 3.

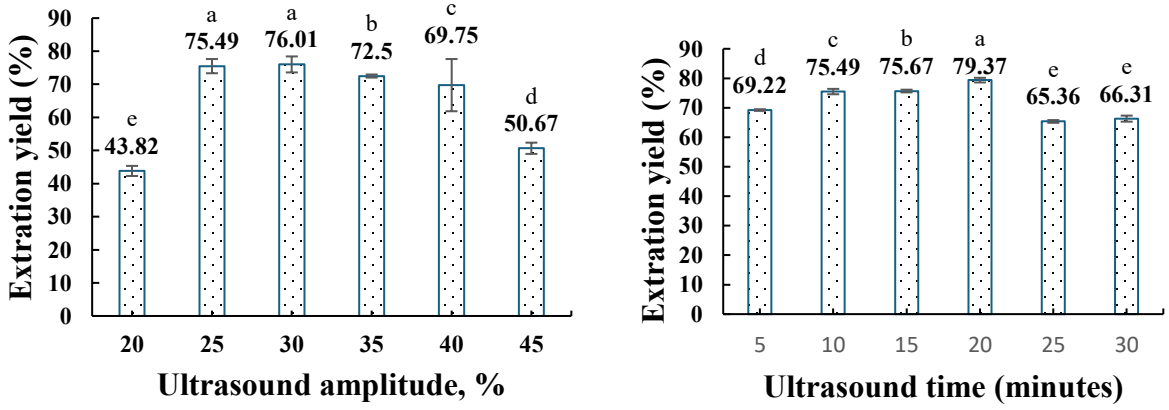


Figure 3: Effect of ultrasound amplitude (A) and ultrasound time (B) on protein extraction yield

Figure 3A illustrates the effect of ultrasound amplitude on protein extraction yield. Increasing the amplitude from 20% to 25% significantly enhanced the yield from 43.82% to 75.49% ($\alpha = 0.05$), attributed to intensified cavitation and cell wall disruption that facilitated protein release. At 30% amplitude, the yield reached a plateau, indicating saturation of the extraction process. This trend aligns with the observations of Villalón-López et al. (2025), who reported a similar threshold effect when ultrasound energy density increased from 132 to 150 kJ/L [26]. Beyond 30%, further increases in amplitude (30–45%) led to a pronounced decrease in yield, likely resulting from excessive cavitation that generated intense shock waves, shear forces, and localized heating, promoting protein denaturation and fragmentation [27]. Moreover, the formation of excessive cavitation bubbles may have hindered efficient energy transfer and elevated the bulk temperature, thereby diminishing extraction efficiency [28]. Comparable findings were reported by Sert et al. (2022), who observed that moderate ultrasound amplitude (50%) enhanced protein recovery from pumpkin seed press cake, while excessive amplitude levels induced denaturation and yield reduction [29]. Since no significant difference was detected between 25% and 30% amplitudes, 25% amplitudes was selected as the optimal condition for subsequent experiments, ensuring high extraction efficiency while minimizing energy consumption and the potential for protein degradation.

As shown in Figure 3B, increasing the sonication time from 5 to 20 minutes significantly enhanced the protein extraction yield, reaching a maximum of 79.37% at 20 minutes ($\alpha = 0.05$). This improvement can be attributed to intensified cavitation effects, where repeated bubble formation and collapse generate strong shear forces that disrupt Gac seed cell walls, thereby facilitating protein release and improving solubilization in the alkaline medium [26]. However, extending the sonication time beyond 20 minutes (20–30 min) resulted in a notable decline in yield. Prolonged sonication may lead to the formation of free radicals, structural degradation, or protein aggregation due to the exposure of hydrophobic regions following protein unfolding, resulting in insoluble complexes [30]. Similar behavior has been reported in other plant sources. Fatima et al. (2023) observed that ultrasound-assisted protein extraction from Moringa leaves increased up to 20 minutes before declining with longer treatment times [30]. Likewise, Zhang et al. (2023) reported that protein yield from Dictyophora rubrovolvata mushroom caps rose between 10 and 25 minutes, then decreased beyond 25 minutes due to protein aggregation [31]. Therefore, a sonication time of 20 minutes was identified as the optimal condition for protein extraction from Gac seeds, providing high recovery efficiency while minimizing the risk of protein denaturation and aggregation.

3.3. Determination of functional properties of MCPC

3.3.1 Protein solubility

Solubility is a crucial functional characteristic of proteins, as it strongly influences their water-holding capacity, emulsifying properties, and gelling behavior, thereby determining their suitability for various applications. The protein solubility of defatted Gac seed flour (PDS) and MCPC as a function of pH was investigated, and the results are presented in Figure 4.

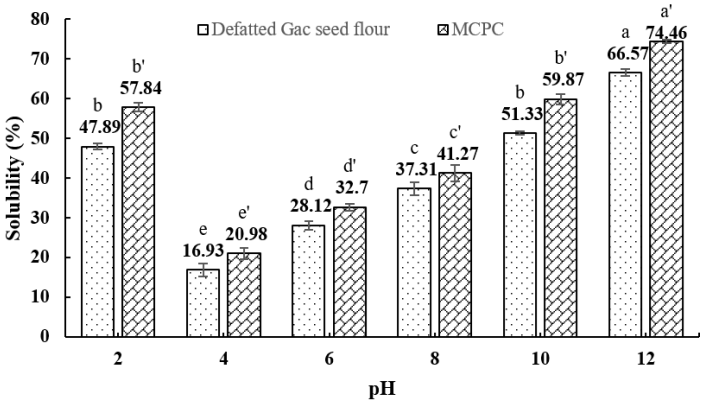


Figure 4. Effect of pH on protein solubility of PDS and MCPC

As shown in Figure 4, the protein solubility of PDS and MCPC varied significantly with pH ($\alpha = 0.05$). The lowest solubility was recorded at pH 4 (5.16%), corresponding to the isoelectric region, whereas solubility increased under both acidic (10.01% at pH 2) and alkaline conditions, reaching a maximum of 18.51% at pH 12. Across all pH levels, MCPC consistently exhibited higher solubility than PDS.

The minimum solubility at pH 4 reflects protein aggregation at the isoelectric point [30]. As the pH deviates from this point, increased ionization of charged groups enhances electrostatic repulsion and exposes hydrophilic residues, thereby improving solubility [32]. The rise in solubility at pH 2 is consistent with observations in soybean protein, where strong positive surface charges promote protein–water interactions. Similar pH-dependent trends have been reported for walnut [31], almond, and rambutan seed proteins [33], indicating that Gac seed protein follows a common plant protein behavior—exhibiting minimal solubility near the isoelectric point and enhanced solubility under both acidic and alkaline conditions.

3.3.2 Water holding capacity and oil adsorption capacity

Water-holding capacity (WHC) is a critical functional property of proteins, as it strongly affects the texture, viscosity, and mouthfeel of various food products such as soups. Figure 5 illustrates the influence of pH on the WHC of Gac MCPC and PDS.

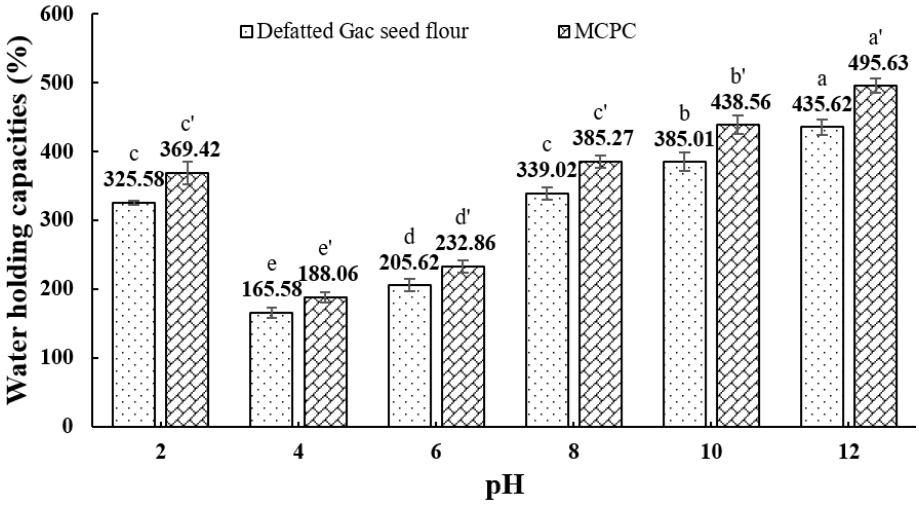


Figure 5. Water holding capacities of MCPC

The WHC decreased from 369.42% at pH 2 to 188.06% at pH 4, then increased progressively, reaching 459.63% at pH 12 ($\alpha = 0.05$). The lowest WHC observed at pH 4 corresponds to the protein's isoelectric point, where diminished electrostatic repulsion favors protein aggregation and reduces the availability of water-binding sites. Under alkaline conditions, protein unfolding and the ionization of carboxyl groups expose additional hydrophilic sites, thereby enhancing water retention [34]. Similar pH-dependent behavior has been observed for mung bean protein isolate [35]. These findings highlight the potential of Gac seed protein for use in bakery, processed meat, and plant-based meat products that require high water-holding capacity [36].

Oil-binding capacity (OBC) is a key functional property that reflects the interaction between proteins and lipids, primarily influenced by the presence of hydrophobic amino acid residues and the protein's ability to form a structural matrix that entraps oil. The results revealed that MCPC exhibited a significantly higher OBC (166.23%) compared to PDGS (155.01%). This improvement can be attributed to the purification process, which eliminated non-protein impurities and increased the exposure of hydrophobic groups on the protein surface, thereby enhancing the affinity and interaction with oil molecules [34]. The higher OBC observed in MCPC suggests its strong potential for

use in lipid-rich food systems such as sausages, baked goods, processed meats, and meat analogs, where proteins function as flavor carriers and contribute to texture enhancement. Therefore, MCPC can serve not only as a valuable nutritional protein source but also as a functional ingredient, providing an effective means of valorizing Gac seed by-products that are typically underutilized.

3.3.3 Emulsifying capacity

The emulsifying capacity of MCPC was evaluated under varying pH conditions and compared with that of PDGS, as presented in Figure 5.

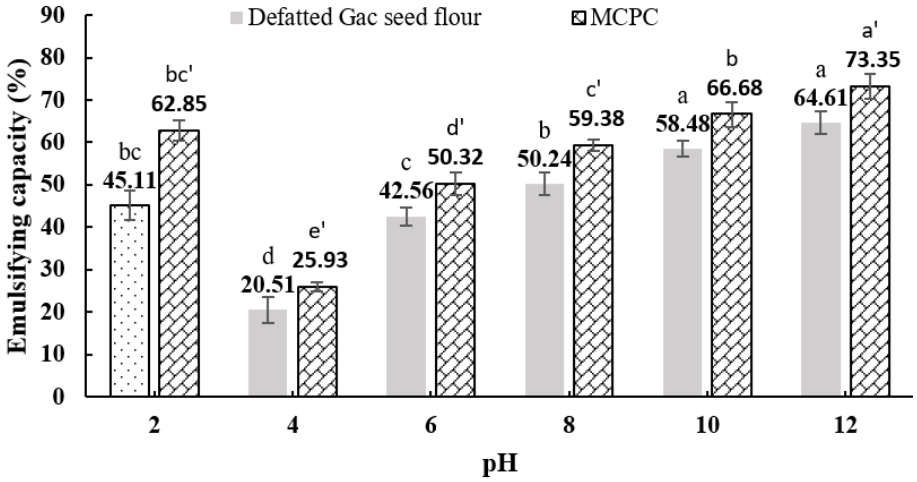


Figure 6: Emulsifying capacity of MCPC

As shown in Figure 6, the emulsifying capacity reached its minimum at pH 4 (25.93%) and increased significantly under both acidic (pH 2) and alkaline conditions, attaining a maximum of 73.35% at pH 12 ($\alpha = 0.05$). The reduced emulsifying activity near pH 4 corresponds to the isoelectric point, where diminished surface charge promotes protein aggregation and restricts adsorption at the oil–water interface [37]. In contrast, at alkaline pH, increased negative surface charges enhance electrostatic repulsion and induce protein unfolding, thereby improving interfacial coverage and stabilizing emulsion formation [38]. These results demonstrate the potential of Gac seed protein as a natural emulsifying agent for use in acidic or alkaline food systems, such as beverages and sauces.

3.3.4 Foaming capacity and stability

The foaming capacity and stability of MCPC and PDGS across different pH levels are shown in Figure 8.

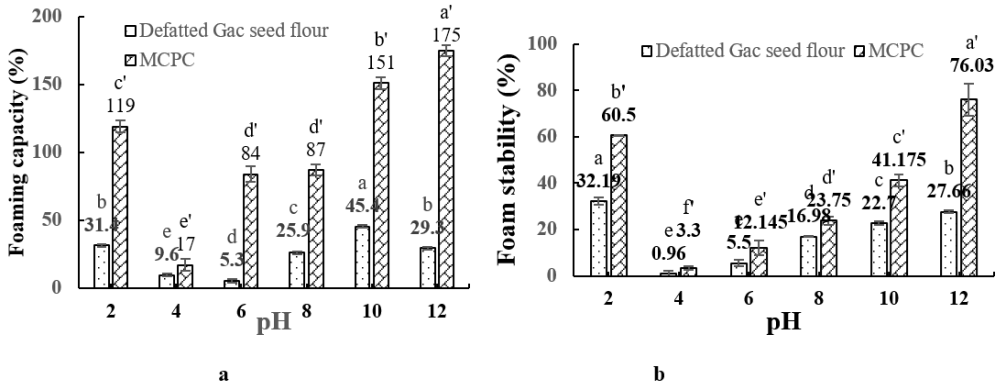


Figure 7. Foaming capacity (a) and foaming stability (b) of MCPC and PDGS

The lowest foaming capacity (17%) was recorded at pH 4, corresponding to the isoelectric point (pI), where diminished electrostatic repulsion promotes protein aggregation and restricts their migration to the air–water interface. In contrast, foaming capacity increased markedly under both acidic (pH 2) and alkaline (pH 8–12) conditions, likely due to enhanced net surface charges, improved solubility, and partial protein unfolding that exposes hydrophobic regions, facilitating adsorption and film formation at the interface [39]. The highest foaming capacity (175%) was observed at pH 10, suggesting that strong alkaline conditions promote extensive protein denaturation and the exposure of hydrophobic and sulfhydryl groups, leading to the formation of flexible, cohesive protein films that stabilize the foam structure [40].

Foam stability is an essential functional attribute that influences both the sensory characteristics and technological performance of foamed food products. As shown in Figure 9, the foam stability of Gac seed protein varied significantly with pH ($\alpha = 0.05$). A pronounced decline was observed from pH 2 (60.5%) to pH 4 (3.3%), corresponding to the isoelectric point, where reduced electrostatic repulsion facilitates protein aggregation and results in weak interfacial films. Beyond this point, foam stability increased markedly with rising pH, reaching a maximum of 76.03% at pH 12. This improvement can be attributed to enhanced protein charge, better dispersion, and the formation of more cohesive interfacial films stabilized by hydrophobic interactions and hydrogen bonding [41]. These observations are consistent with the findings of Khalid et al. (2015), who reported similar pH-dependent behavior in cowpea protein isolates [42]

3.3.5 Gelation ability

Gelation ability is a critical functional property of proteins, representing their capacity to associate and form a three-dimensional network structure upon exposure to heat or environmental stimuli. This property not only determines the suitability of proteins for various food applications but also directly influences their sensory characteristics and functional value.

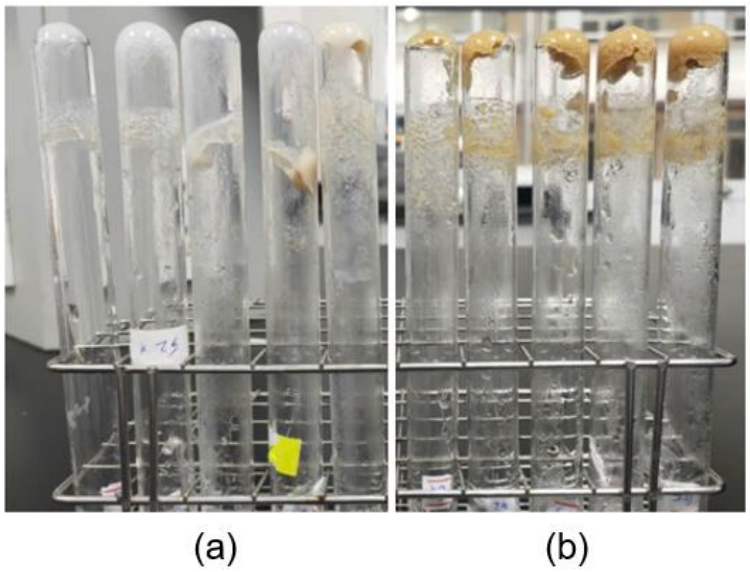


Figure 8. Gelation forming ability evaluation results of commercial soy protein powder (a) and protein from gac seeds (b)

In this study, the gelation ability of protein extracted from Gac seeds was evaluated and compared with that of commercial soy protein powder to assess its potential as a novel functional ingredient. The results revealed a clear

distinction between the two samples. Gac seed protein exhibited gel formation at a minimum concentration of 2%, whereas the commercial soy protein showed no distinct gelation at any tested concentration. This indicates that Gac seed protein possesses a strong capacity to form intermolecular networks, suggesting robust interactions between protein molecules under thermal treatment. The superior gelation performance of Gac seed protein at low concentrations highlights its potential applicability in the food industry, particularly in products that require a stable gel matrix, such as sausages, surimi, and plant-based meat analogs.

3.4. Physicochemical properties

3.4.1. Chemical composition of MCPC

The proximate compositions of PDGS and MCPC were analyzed and are presented in Table 1.

Table 1. Chemical composition of defatted Gac seed flour and MCPC

Components (%)	Defatted Gac Seed Flour	MCPC
Moisture	6.25±0.06[a]	2.75±0.24[b]
Protein	13.5±1.06[b]	81.77±0.71[a]
Lipid	44.09±0.24[a]	2.60±0.56[b]
Starch	24.60±1.41[a]	1.75±0.00[b]
Total sugars	5.32±0.10[a]	0.46±0.00[b]
Ash (%)	2.01±0.04[b]	9.46±0.12[a]

The analytical results revealed that the defatted Gac seed contained up to 44.1 g of protein per 100 g, comparable to other oilseeds known for their high protein content following oil removal. When compared with common oilseed residues, Gac seed residue exhibited a protein content equivalent to or exceeding that of soybean meal (25.7–52.4%), peanut meal (40–50%), and sunflower meal (29–43.4%) [43] [44]. These findings suggest that Gac seed residue is not merely a by-product but a valuable protein-rich resource. The high protein content of both defatted Gac seed flour and MCPC demonstrates the feasibility of extracting protein for industrial applications. Thus, Gac seed can be considered a promising novel source of plant-based protein.

Comprehensive characterization of the defatted seed and its protein concentrate further revealed notable alterations in physicochemical and functional properties following protein isolation.

3.4.2. Amino acid compositions

The amino acid composition plays a crucial role in determining the biological and nutritional properties of proteins. The amino acid profiles of albumin and glutelin are presented in Table 2.

Table 2. Amino acid compositions of MCPC

Amino acid	Content (g/100g)	FAO/WHO recommendation (mg/g)
Axit aspartic	2.28	
Threonine	0.72	28
Serine	1.21	
Axit glutamic	4.47	
Proline	1.13	
Glycine	1.38	
Alanine	1.24	
Cystine	0.5	
Valine	1.23	42
Methionine	0.36	
Isoleucine	1.19	42
Leucine	2.14	48
Tyrosine	1.08	
Phenylalanine	1.26	

Lysine	0.86	42
Histidine	0.84	
Arginine	3.25	

Analysis revealed that Gac seed protein contains both essential (EAAs) and non-essential amino acids (NEAAs), with glutamic acid (4.47 g/100 g) and aspartic acid (2.28 g/100 g) being the most abundant [38]. Arginine was also present in high concentration (3.25 g/100 g), suggesting potential health-promoting benefits [45]. Among the essential amino acids, leucine (2.14 g/100 g) and valine (1.23 g/100 g) were relatively abundant, while methionine (0.36 g/100 g) and lysine (0.86 g/100 g) were identified as limiting amino acids. This composition aligns with previous reports indicating that lysine, methionine, and cysteine are typically the most restricted amino acids in plant-derived proteins [46]. Comparable amino acid distribution patterns have been observed in various plant protein sources, where methionine and lysine commonly serve as the first limiting amino acids.

3.4.3. Protein compositions

The protein compositions of MCPC was characrestic using SDS-PAGE analysis. The obtained results were presented in Figure 9.

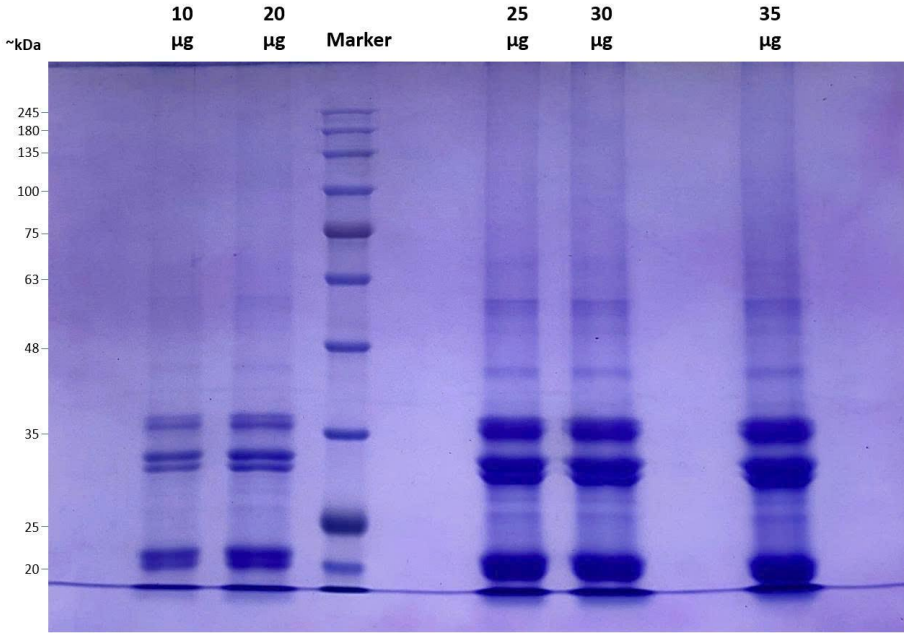


Figure 9. SDS-PAGE analysis of MCPC

SDS-PAGE analysis showed that the gac seed protein bands were mainly distributed in the range of 20 to 45 kDa, with the most abundant fraction below 25 kDa. This range corresponds to previously reported trypsin inhibitors such as MCCTI-1 [6], MCoCC-1, MCoCC-2 [7], MCoTI-I and MCoTI-II [8], MCo-3 to MCo-6 [9]. Additional bands at 25–30 kDa were consistent with the ribosome-inactivating protein Cochinin B (28 kDa) described by Chuethong et al. (2007) [9]. Furthermore, protein fractions in the range of 30–40 kDa were also detected. According to the study of Balachandran et al. (1997), it was mentioned that most of the wheat sieve fluid protein has a weight in the range of 10–40 kDa, which is also consistent with the study of the protein mass of gac seeds [11].

3.4.4 DPPH Radical Scavenging Activity

The antioxidant activity of Gac seed protein was evaluated using the DPPH radical scavenging assay and expressed as Trolox Equivalent Antioxidant Capacity (TEAC) (Table S1). Quantification was performed using a Trolox calibration curve (Fig. S2). As presented in Table S1, TEAC values increased with protein concentration, ranging from 9.05 ± 0.20 µg Trolox/mL at 50 µg/mL to 23.63 ± 0.19 µg Trolox/mL at 200 µg/mL, demonstrating a clear dose-dependent relationship. The progressive increase in TEAC values indicates that MCPC exhibits measurable

antioxidant potential, likely attributable to its amino acid composition—particularly the presence of tyrosine, tryptophan, methionine, and cysteine residues capable of donating electrons and scavenging free radicals [47]. Although the TEAC values obtained were lower than those reported for hydrolyzed soybean protein (~30–40 μmol Trolox/g protein) [48] and sunflower seed protein (~28 μmol Trolox/g) [49], the observed concentration-dependent trend aligns with findings from other plant-derived antioxidant peptides, such as those from mung bean protein hydrolysates [50].

Overall, these results suggest that MCPC possesses moderate but notable antioxidant activity relative to commonly studied plant proteins. Given that Gac seeds are typically underutilized byproducts, their exploitation as a protein source with antioxidant potential presents a valuable opportunity for incorporation into functional foods and nutraceutical formulations.

4. Conclusion

This study successfully produced a protein concentrate from defatted Momordica cochinchinensis (Gac) seeds using ultrasound-assisted extraction followed by isoelectric precipitation. The compositional analysis revealed that glutelin was the dominant protein fraction, followed by globulin, albumin, and prolamin. Optimization of extraction parameters identified the most effective conditions for maximizing protein yield while minimizing denaturation as a solid-to-solvent ratio of 1:25, pH 11, ultrasound amplitude of 25%, and sonication time of 20 minutes. Evaluation of functional properties showed that the Gac seed protein concentrate (MCPC) possessed excellent oil-binding capacity and emulsifying activity, suggesting its suitability for use in lipid-rich and emulsion-based food systems. However, its relatively low foaming capacity indicates limited applicability in products that require high aeration. The solubility of MCPC was strongly influenced by pH, with markedly enhanced solubility under alkaline conditions, which may broaden its potential applications in various processed food formulations. Overall, MCPC represents a promising plant-based protein ingredient that not only valorizes Gac seed by-products but also offers valuable nutritional and functional properties for the food industry. Future research should focus on detailed amino acid profiling, exploration of bioactive functionalities, and assessment of its performance within specific food matrices to establish its commercial viability.

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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