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Exploring Functional Properties of Mantis Shrimp (*Miyakella nepa*) Protein Powder for Sustainable Food Applications

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ABSTRACT

The experiment was conducted from January to August, 2023 at the Central Institute of Fisheries Education, Mumbai, India, to investigate the potential of mantis shrimp (*Miyakella nepa*) as a resource for developing value-added food products. The protein powder from alkaline solubilization showed a higher water-holding capacity (2.39 ml g^{-1}) than phosphate buffer extraction (0.79 ml g^{-1}), while phosphate buffer extraction exhibited greater oil-holding capacity (2.04 g g^{-1}) compared to alkaline solubilization (1.81 g g^{-1}). Emulsion capacity was 47.02% for alkaline solubilization and 52.38% for phosphate buffer extraction, with phosphate buffer extraction demonstrating better emulsion stability (38.98%) after heating at 80°C for 30 minutes. For foaming properties, alkaline solubilization had a higher foaming capacity (201.66%), whereas phosphate buffer extraction showed superior foaming stability (32.87%). Both powders achieved maximum solubility at pH 12, with solubility at pH 7 being 22.44% for alkaline solubilization and 9.25% for phosphate buffer extraction. Bulk density was 0.30 g ml^{-1} for alkaline solubilization and 0.34 g ml^{-1} for phosphate buffer extraction, indicating slight variations in physical characteristics between the two protein powders. The differences observed in water and oil-holding capacities indicated variations in hydrophilic and hydrophobic interactions within the extracted proteins. Emulsion and foaming characteristics reflected their potential use in formulated food products requiring stable emulsions or aerated textures. Differences in bulk density could influence powder handling, packaging, and application in various product formulations, highlighting functional versatility between extraction methods.

KEYWORDS: Bulk density, functional properties, mantis shrimp, protein powder

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1. INTRODUCTION

The fish industry is expected to produce 160.8 mt of fish by-and co-products annually by 2030. This projected growth highlights the urgent need for sustainable management of fish by-and co-products, presenting an opportunity to enhance the sector's environmental sustainability (Ribeiro et al., 2024). Fish proteins derived from dried fish are increasingly recognized for their exceptional nutritional value and functional properties, making them valuable ingredients in functional and ready-to-eat food products. Although fish is a widely available and nutritious protein source, its strong odor often causes rejection by some people (Kumoro et al., 2022). Dried fish, however, is a concentrated source of high-quality protein, essential amino acids, and vital micronutrients such as iron, zinc, calcium, and omega-3 fatty acids, all crucial for human health (Byrd et al., 2021). These proteins provide highly digestible amino acids, but their practical application is often limited by high production costs (Sahana et al., 2024). Incorporating fish protein powders into ready-to-eat products can significantly enhance their protein content and nutritional quality (Shaviklo, 2015).

Firstly, incorporating fish proteins into food products improves their nutritional value. Secondly, it promotes efficient utilization of fishery resources and increases per capita fish protein consumption (Vakily et al., 2009; Shavikalo, 2012). Fish proteins can also serve as valuable ingredients in formulations for protein enrichment by the food industry (Zatta Cassol et al., 2024). Additionally, edible crustaceans hold significant nutraceutical potential and applications in the pharmaceutical industry. Their exoskeletons contain chitin, a natural biopolymer of considerable value (Samia and Hoda, 2019).

Among low-value by-catches, mantis shrimp make a notable contribution, particularly in trawling operations (Barragan-Mendez et al., 2020). Due to limited commercial importance, mantis shrimp are often discarded, thrown overboard, or used for fish meal, poultry feed, or manure (Govindan, 1984). However, some studies have explored value-added products from mantis shrimp, such as protein (Mathew et al., 1982), chitosan (Madhavan and Ramachandran, 1975), and mince-based products like cutlets and wafers (Zynudheen et al., 2009; Gedam et al., 2024). Other products include fish fingers, crackers, pickles, silage (Bijoy et al., 2016), protein powder-fortified noodles (Viji et al., 2022), wheat sticks, and potato croquettes (Samia and Hoda, 2019).

Consuming bioactive components, such as protein isolates, is an effective way to harness their benefits. Functional attributes of these ingredients, including high solubility, emulsifying properties, and gel formation, are critical for successful application (Hall et al., 2017). Shrimp-derived proteins have shown desirable functional properties in various studies. For instance, protein hydrolysates from non-penaeid shrimp (*Acetus indicus*) exhibit excellent solubility, foaming, emulsification, water retention, and oil binding, showcasing their potential in functional foods and nutraceuticals (Dhanabalan et al., 2020).

Proteins containing carotenoids have also been extracted from shrimp processing waste, demonstrating properties suitable for human food and animal feed (Dayakar et al., 2022; Dayakar et al., 2023). Shrimp protein hydrolysates from *Litopenaeus vannamei* have further exhibited functional and antioxidant properties (Latorres et al., 2018), and can be incorporated into food products to enhance functionality and prolong shelf life (Adeyeye et al., 2008).

Moreover, proteins from shrimp species such as Penaeus notabilis have shown high solubility, making them advantageous in beverages (Kinsella, 1976) and low-acid foods such as meat products (Olaofe et al., 1993). Their water absorption and oil emulsification capacities make them suitable for viscous food formulations, such as soups, sausages, and cakes.

Despite these advances, a comprehensive understanding of specific protein types and functional properties derived from mantis shrimp remains lacking. Present study was aimed to investigate the potential of mantis shrimp (*Miyakella nepa*) as a resource for developing value-added food products

2. MATERIALS AND METHODS

The experiment was conducted during January to August 2023 at Central Institute of Fisheries Education, Mumbai, Maharashtra, India.

2.1. Sample collection and preparation

Mantis shrimps (*Miyakella nepa*) were procured from Versova landing center, Mumbai, from March to May 2023. These were caught by single-day trawlers and dol nets. Shrimps were packed in iceboxes (shrimp:ice=1:1) and transported to the lab within one hour. Shrimps were washed with ice-cold potable water, and total length (cm) and weight (g) were measured. Meat was extracted using scissors for meat yield calculation.

2.2. Proximate composition

Moisture, crude protein, fat, and ash of fresh shrimp muscle were analysed using standard methods (Anonymous, 2023).

2.3. Muscle protein fraction of mantis shrimp

Shrimp muscle (20 g) was homogenized in 200 milliliter 0.05 M phosphate buffer (pH 7.5; 15.6 millimolar sodium hydrogen phosphate and 3.5 millimolar potassium dihydrogen phosphate) using Polytron PT2100

homogenizer. Homogenate was centrifuged at 5000×g for 15 minutes (R24 Remi Instruments). Residue was re-homogenized in buffer and centrifuged again. Combined supernatant was treated with trichloroacetic acid (5 percent) to precipitate proteins, which were collected by filtration as sarcoplasmic protein fraction (Hashimoto et al., 1979).

Residue from sarcoplasmic extraction was homogenized with 0.5 M potassium chloride phosphate buffer (pH 7.5; 0.45 M potassium chloride, 15.6 millimolar sodium hydrogen phosphate, and 35 millimolar potassium dihydrogen phosphate) and centrifuged. Process was repeated, and the combined supernatant was the myofibrillar protein fraction. Final residue contained alkali-soluble and stroma proteins. Nitrogen content in all fractions was determined using micro-Kjeldahl method, and protein composition was calculated.

2.4. Preparation of muscle protein powder

2.4.1. Alkaline solubilisation process

Shrimp meat was homogenized with ice-cold deionized water (1:9) using Polytron PT MR 2100 homogenizer for 3 minutes. One molar sodium hydroxide raised pH to 11.5. Homogenate was centrifuged at 8000 rpm for 20 minutes at 4 degree Celsius, forming three layers: top fat layer, middle soluble protein supernatant, and bottom insoluble protein. Supernatant was filtered through cheesecloth and Whatman number 1 paper to remove neutral lipids, skin, bone, and connective tissue. pH was adjusted to 5.5 (isoelectric point) using one normal hydrochloric acid. Centrifugation at 8000 rpm for 15 minutes at 4 degree Celsius separated the precipitate (Hultin et al., 2020). Spray drying (SM Scitech, Calcutta, India) was used for protein powder preparation (inlet 160 degree Celsius; outlet 80 degree Celsius; feed rate 5 milliliter per minute). This powder was designated as P_1 (Gong et al., 2016).

2.4.2. Phosphate buffer method

Shrimp meat was homogenized with 0.5 M potassium chloride phosphate buffer (pH 7.5) and centrifuged at 5000×g for 15 minutes. Process was repeated. Supernatant was spray-dried as per alkaline solubilisation method. This powder was designated as P_2 (Hashimoto et al., 1979).

2.5. Physico-functional characteristics

2.5.1. Bulk density

Bulk density was measured by filling protein powder into a pre-weighed 10 milliliter graduated cylinder up to 10 ml mark, tapping 20 times, and weighing the filled cylinder (Joshi et al., 2011). Results were reported in g ml^{-1}.

2.5.2. Water holding capacity (WHC)

Water holding capacity was determined by dispersing 10 mg ml^{-1} protein powder in 20 ml water, vortexing for 30 seconds, standing for 6 hours at room temperature, and centrifuging at 2800×g for 30 minutes. Supernatant was filtered (Whatman number 1) and volume measured. Water holding capacity was expressed as milliliter of water absorbed per gram of protein sample (Diniz and Martin, 1997).

2.5.3. Oil holding capacity (OHC)

Oil holding capacity was determined by mixing 0.5 g protein powder with 10 ml mustard oil, vortexing for 30 sec., standing for 30 min at 25°C, and centrifuging at 2800×g for 25 min. Supernatant was decanted, and weight difference before and after decanting was recorded. Oil holding capacity was expressed as grams of oil absorbed per gram of protein sample (Shahidi et al., 1995).

2.5.4. Emulsifying properties

Emulsifying capacity and stability were measured using method of Yasumatsu et al. (1972). Emulsion was prepared with 10 mg ml^{-1} sample, 50 ml cold water (4°C), and 50 ml sunflower oil. Emulsion was homogenized using Polytron PT MR 2100 and divided into two equal portions. One tube was centrifuged at 4000×g for 10 min. Second tube was heated at 80°C for 30 min, cooled, and centrifuged similarly. The height of the emulsified layer, as a percentage of the total height of material in the unheated tubes, was used to calculate the emulsifying activity and stability using:

Emulsifying capacity (%)=(Height of emulsion layer/ Height of whole layer)×100

Emulsifying stability (%)=(Height of emulsion layer after heating/ Height of whole layer)×100

2.5.5. Foaming properties

Foaming capacity and stability were measured as per Yasumatsu et al. (1972) and Thiansilakul et al. (2007). Sample solution (10 mg ml^{-1}) was homogenized at 10000 rpm for 3 min, transferred to a 50 ml graduated cylinder, and volume recorded at 0 and 60 minutes. The FC (%) and FS (%) of each sample were calculated using the equations, respectively:

$FC\ (\%)=(V_T/V_0)\times100$

$FS\ (\%)=F_t/V_t)/(F_T/V_T)\times100$

Where V_0=Initial volume, V_T=Volume at 0 min., V_t=Volume at 60 min., F_t=Foam volume at 0 min., F_t=Foam volume at 60 min.

2.6. Protein solubility

Protein solubility was measured following Diniz and Martin (1997). Sample was homogenized in 5 5% SDS (1:9), heated at 85°C for 1 hour, and centrifuged at 3500 rpm for 10 min at room temperature. Supernatant was collected, and protein content was determined by Biuret method using bovine

serum albumin as standard. Protein solubility (percent) was calculated as:

Protein Solubility (%)=(Protein content in the supernatant/ Total protein in the sample)×100

3. RESULTS AND DISCUSSION

3.1. Length and weight of mantis shrimp

The length and weight and yield (%) of meat of mantis shrimp are detailed in Table 1. From the results it was observed that, the length of mantis shrimp varied between 10.4–14.8 cm and a weight ranged from 11.22–34.59 g. The length and weight characteristics of mantis shrimp found in the present study are in consistent with the findings of (Ramezani et al., 2016). The yield of meat of mantis shrimp were found to be in the range of 30.59–56.59% and and the results are in line with the observations made by (Wardianto et al., 2012) for *Harposquilla raphidea* (39.91% to 40.28%). Since the mantis shrimps were caught from the open sea, it was difficult to get uniform size catch and the differences in length and weight of mantis shrimp might also be attributed to season, growth stage, reproduction, feeding ground, etc.

Table 1: Length and weight of mantis shrimp

Parameters	Mantis shrimp	Range
Total length (cm)	11.77±1.41	10.4–14.8
Total weight (g)	18.19±7.28	11.22–34.59
Meat weight (g)	7.07±2.78	4.01–13.22
Meat yield (%)	39.09±5.97	30.59–56.59

Data are expressed as mean±S.D. (n=20)

phosphate buffer-extracted protein was slightly higher than that of whey protein (0.5 ml g^{-1}) (Kaushik et al., 2016). The OHC values for alkaline-solubilized protein were akin to the OHC of quinoa protein (1.88 ml g^{-1}) (Elsohaimy et al., 2015). On the other hand, the OHC value for the phosphate buffer-extracted protein was slightly lower than that of soya protein (2.81 ml g^{-1}) (Foh et al., 2012). It's worth noting that the OHC values for both methods were lower than those reported by Kumarakuru et al., 2018. Furthermore, the improved capacity of protein isolates to retain and absorb

Table 2: Proximate composition (%) of mantis shrimp (wet weight basis)

Species	Moisture	Protein	Fat	Ash	Carbohydrates*
Miyakella nepa	81.47± 0.29	15.06± 0.03	0.66± 0.01	2.26± 0.21	0.55± 0.13

Data are expressed as mean ±S.D. (n=3). Carbohydrate was calculated by difference

3.2. Proximate composition of mantis shrimp

The findings of proximate composition of mantis shrimp are given in Table 2. From the results, it can be revealed that mantis shrimp meat exhibits a moisture content of 81.47%, which are in consistent with the reports of Ramezani et al., 2016 (81%). Moreover, mantis shrimp was shown to possess a protein content of 15.06%, surpassing the previously reported figures of Ramezani et al., 2016 (12.82%). Similarly, the fat content (0.66%) and ash content (2.26%) values exceeded the earlier reports by Ramezani et al., 2016. Variations in the body's chemical composition, particularly concerning protein, fat, carbohydrate, moisture, and ash content, can be attributed to factors like differences in food type and composition, fluctuations in environmental conditions, including temperature and salinity, changes in fish density, and physiological processes (Jassim et al., 2014).

3.3. Muscle protein fractions of mantis shrimp

In the present study, various protein fractions in mantis shrimp muscle, viz. sarcoplasmic, myofibrillar, and stroma proteins were analysed and shown in Figure 1. The myofibrillar content (55.74%) constituted the major fraction, followed by sarcoplasmic protein (38.14%). Alkali-soluble protein and stroma protein together accounted for 6.10%. These findings closely resembled the protein fractions observed in white muscle of sardine, where sarcoplasmic proteins constituted 33–39%, myofibrillar proteins 58–61%, alkali-soluble proteins 1–5%, and stroma proteins 1–2% (Hashimoto et al., 1979). In contrast, the protein composition of flower tail shrimp (*Metapenaeus dobsonian*) displayed higher myofibrillar protein (76.67%) and lower sarcoplasmic protein (16.82%) compared to mantis shrimp (Laly et al., 2019).

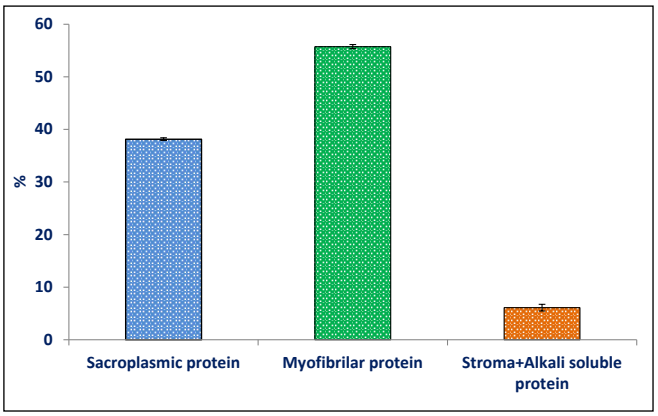

Figure 1: Muscle protein fractions of mantis shrimp, *Miyakella nepa*

3.4. Proximate composition of protein powder

The proximate composition of protein powder isolated from mantis shrimp by two methods is depicted in Table 3. The protein content of the alkaline solubilization method and

Table 3: Proximate composition of protein powder from mantis shrimp.

Protein powder	Moisture	Protein	Fat	Ash
P_1	2.38±0.15	78.00±0.62	1.03±0.10	15.89±0.40
P_2	2.25±0.13	71.97±0.33	2.62±0.11	11.85±0.63

Data are expressed as mean±S.D. (n=3)

phosphate buffer method was determined to be 78% and 71.97%, respectively. The higher protein content in the alkaline solubilization method could be attributed to the effective removal of lipids and other insoluble components during the production of fish protein powder. This mantis shrimp protein powder displayed higher protein content to *Brycinus nurse* protein isolates (50.40%) (Kasozi et al., 2018). However, the values of protein observed in the present study were lower than those reported for catfish protein isolate (86.74%) (Haryati et al., 2020) and for pony (89.70%) and mackerel (87.27%) fish protein isolate (Kumarakuru et al., 2018). The fat content of the protein extracted through the alkaline solubilization method exhibited a similar value to the fat content of protein isolates from mackerel and pony fish, while the fat content of protein isolate obtained via the phosphate buffer method was comparable to sardine protein isolate (Kumarakuru et al., 2018). In contrast, the ash content in the present study was higher than that reported by (Haryati et al., 2020) and (Kumarakuru et al., 2018). The elevated ash content in the alkaline solubilization method was attributed to the addition of acid and base during the pH adjustment of the protein slurry (Chalamaiah et al., 2010).

3.5. Physico-functional characteristics of mantis shrimp protein powder

3.5.1. Bulk density

The physico-functional characteristics of protein powder are presented in Table 4. Bulk density was a concept that related to the ratio of weight to volume, often expressed as g ml^{-1}. It provides insights into the material's density, with lower values indicating bulkier characteristics. Bulk density was influenced by various factors, including the method of preparation, drying process, particle fineness, and moisture content of the product. As shown in Table 4, it was clear that P_1 and P_2 exhibit significant differences in their bulk densities, measuring 0.34 g ml^{-1} and 0.30 g ml^{-1}, respectively. The protein obtained through the phosphate buffer method displays a lower bulk density compared to that from the alkaline solubilization method. This difference in bulk density could be attributed to the relatively lower protein content in the phosphate buffer approach. The findings of this study aligned with the bulk density values of tilapia protein isolate at 0.34 g ml^{-1} (Foh et al., 2012) and cape hake fish isolate at 0.34 g ml^{-1} (Pires et al., 2012). These

Table 4: Physico-functional properties of mantis shrimp protein powder

Functional properties	P_1	P_2
Bulk density (g ml^{-1})	0.34±0.00	0.30±0.00
Water holding capacity (ml g^{-1})	2.39±0.00	0.79±0.00
Oil holding capacity (g oil g^{-1})	1.81±0.10	2.04±0.07
Emulsion capacity (%)	47.02±1.03	52.38±1.03
Emulsion stability (%)	27.67±1.03	38.98±0.51
Foaming capacity (%)	201.66±2.88	170±0.00
Foaming stability (%)		
15 mins	$117.47±0.25^d$	$69.35±1.40^d$
30 mins	$80.41±0.54^c$	$43.91±0.91^c$
45 mins	$62.43±1.15^b$	$35.54±0.79^b$
60 mins	$24.17±0.00^a$	$32.87±2.74^a$

Data with different superscripts (a) is significantly different between the treatments (p<0.05) are expressed as mean±S.D. (n=3). P_1: Alkaline olubilisation method; P_2: Phosphate buffer method, the mean value in the same column

values showed a narrower range when compared to the bulk density of salmon protein powder (0.49 g ml^{-1}), herring protein powder (0.59 g ml^{-1}), and cod protein powder (0.60 g ml^{-1}). The reduced bulk density in salmon protein powder could be attributed to its relatively lower protein content compared to the other two protein powders (Abdollahi and Undeland, 2019).

3.5.2. Water and oil holding capacity

WHC was a physical property that characterizes a food's ability to retain water within its three-dimensional protein structure (Hermanson and Akesson, 1975). Proteins had both hydrophilic and hydrophobic properties, allowing them to interact with water and oil in food products (Butt and Rizwana, 2010). The WHC and OHC of the protein powders are presented in Table 4. The alkaline solubilization method exhibited a higher water-holding capacity (2.39 ml g^{-1}) compared to the phosphate buffer extraction method (0.79 ml g^{-1}). Variations in the WHC of protein powder could be influenced by factors such as solution pH, ionic strength, and the use of acids and bases. The inclusion of acids and bases in the alkaline solubilization method could reduce the protein content of the sarcoplasm and promote the extraction of myofibrillar protein, which was instrumental in retaining water during gel formation (Foh et al., 2012; Lone et al., 2015). In contrast, the higher (2.04 g g^{-1}) OHC was demonstrated in phosphate buffer extraction method than the alkaline solubilization method (1.81 g g^{-1}).

The WHC of the alkaline solubilization method in this study was similar to the findings of (Foh et al., 2012) for tilapia protein isolate (2.51 ml g^{-1}), while the WHC of

water and oil enhanced its applicability in the development of various food products, including cakes, sausages, and mayonnaise, thereby enhancing their functional attributes (Kumarakuru et al., 2018).

3.5.3. Emulsifying properties

Proteins, being surface-active substances, facilitate the formation of oil-in-water emulsions due to their water solubility and the presence of hydrophilic and hydrophobic functional groups (Gbogouri et al., 2004). According to the present results, it was observed that the emulsion capacity and stability of mantis shrimp proteins varied based on the extraction method employed. The phosphate buffer extraction method exhibited a higher emulsion capacity of 52.38%, surpassing the alkaline solubilization method (47.02%). Similarly, concerning emulsion stability, the phosphate buffer extraction method demonstrated a superior value of 38.98% compared to the alkaline solubilization method (27.67%). The enhanced emulsion capacity and stability of P_2 can be attributed to its lower solubility, potentially resulting in increased exposure of hydrophobic groups on the protein surface. This exposure enhances the emulsifying properties of P_2. The emulsifying characteristics of proteins can be elucidated based on their surface properties and how efficiently the isolate reduced the interfacial tension between the hydrophobic and hydrophilic components in food. The emulsification process involved the absorption of proteins onto the surface of oil droplets during homogenization, thereby preventing phase separation (Moschakis et al., 2010).

3.5.4. Foaming properties

The foaming capacity and stability of the protein obtained through the alkaline solubilization method measured at 201.66% and 24.17%, respectively. These values surpass the foaming capacity and stability of the protein derived from the phosphate buffer extraction method (Table 4). The observed foaming capacity value was notably higher than the values reported by for tilapia protein isolate (102.31%) (Foh et al., 2012; Kumarakuru et al., 2018). This enhanced foaming property can be attributed to the effects of the acid/alkaline dissolution processes, which influenced protein unfolding, consequently contributing positively to the protein's foaming ability (Galvez-Rongel et al., 2014).

3.5.5. Solubility

The results presented in Figure 2 were in line with the findings of solubility in a vacuum oven dried rainbow trout protein isolate (Lone et al., 2015). At a pH of 7, it was observed that P_1 exhibited better solubility compared to P_2. This difference in solubility may be attributed to the increased exposure of hydrophobic groups on the protein surface of P_2. This surface property was also connected to the improved emulsifying properties of P_2. Both protein powders displayed a solubility curve characterized by a typical U-shaped pattern. The solubility of protein powder at a pH of 7 was determined to be 22.44% for P_1 and 9.25% for P_2, surpassing the reported solubility of hake protein powder (4%) (Pires et al., 2012) and fish protein powder (9–11%) (Abdollahi and Undeland, 2019). It's important to note that both protein powders exhibited their maximum solubility at pH 12. These results suggested that extreme pH levels, such as 12, had an impact on protein solubility by exposing more charged and polar groups to the surrounding water (Kristinsson and Rasco, 2000).

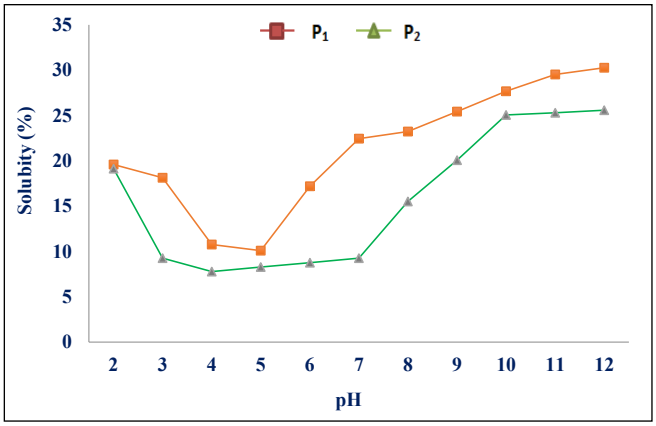

Figure 2: Solubility of mantis shrimp protein powder obtained by pH shift method and phosphate buffer method. P_1: pH shift method, P_2: Phosphate buffer method. Values are expressed as the mean value of three independent replications (n=3)

4. CONCLUSION

The study successfully demonstrated the potential of mantis shrimp (*Miyakella nepa*) as a sustainable source for developing protein powder through two extraction methods- alkaline solubilization and phosphate buffer extraction. Alkaline solubilization produced protein powder with superior water-holding and foaming capacities, while phosphate buffer extraction resulted in better oil-holding capacity, emulsion properties, and foaming stability. Both powders exhibited maximum solubility at pH 12, with alkaline solubilization showing better solubility at neutral pH.

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