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Biochemical, antioxidant and functional characterisation of edible jellyfish, *Rhopilema hispidum* from Indian coast

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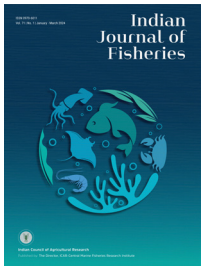

Abstract

Rhopilema hispidum specimens collected from the south-west coast of India were evaluated fo their nutritional composition, bioactivity, functional properties, and storage stability. The exuded body fluid was pooled, filtered, and centrifuged prior to characterisation. Proximate analysis revealed a whole body moisture, protein, ash and fat content of 96.3±0.95, 2.03±0.08, 1.15±0.32, and fat content of 0.023±0.01%, respectively. The gross energy content estimated was 15.71 kJ g_{DM}^{-1} for whole jellyfish and 13.68 kJ g_{DM}^{-1} for the body fluid. Fatty acid profiling identified palmitic acid (1.553±0.21 mg per 100 g) as the primary saturated fatty acid, followed by eicosapentaenoic acid (EPA; 1.181±0.23 mg per 100 g). The exuded body fluid demonstrated strong antioxidant potential in ABTS, DPPH, ferric reducing power, and metal chelating assays. Functional properties of the body fluid exhibited significant concentration dependent enhancement with foaming capacity increasing from 23.26±0.19 to 33.16±0.24%, foaming stability from 19.06±0.15 to 31.24±0.57%, emulsifying activity index (EAI) from 7.07±0.25 to 15.38±0.43 m^2 g^{-1}, and emulsion stability index (ESI) from 10.11±0.11 to 19.82±1.01 min. During refrigerated storage (2±1°C), TVB-N and TMA-N levels were tracked up to day 5, and lipid oxidation (PV) was monitored through day 9. Based on volatile base nitrogen limits, the acceptable shelf life of chilled *R. hispidum* was established as 5 days.

Introduction

Jellyfish, classified under the phylum Cnidaria, are gelatinous zooplankton widely distributed throughout the marine water column. Coastal regions around the world frequently experience large scale aggregations or swarms of jellyfish (Baliarsingh *et al*., 2020). In recent years, such blooms have become increasingly common in estuarine and backwater ecosystems, often resulting in substantial ecological and economic concerns. The dense biomass associated with these blooms interferes with fishing operations, causing gear damage and reducing catch efficiency. Certain jellyfish species further intensify these impacts, leading to disruptions in routine fishing activities and posing serious challenges to the livelihoods of local fishers (Bosch-Belmar *et al*., 2020). In various European countries, jellyfish are primarily viewed as a problem species due to their negative impact on marine-related industries (Edelist *et al*., 2021). In contrast, many Asian nations have long acknowledged the bioactive potential of jellyfish, incorporating them into traditional medicine and culinary practices (Summat *et al*., 2023). With jellyfish populations on the rise, there is growing interest in harnessing this underutilised marine resource for beneficial applications. Anthropogenic activities such as overfishing and coastal development, along with environmental shifts including changes in sea temperature, salinity, and oceanic currents influenced by global climate patterns, have led to a noticeable increase in jellyfish populations and the recurrence of blooms in marine ecosystems (Pitt *et al*., 2018). These blooms carry substantial ecological and economic impacts, particularly through their disruption of coastal fisheries and the resulting challenges faced by fishing communities (Lucas *et al*., 2014).

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Rather than viewing jellyfish solely as a marine nuisance, there is a growing perspective that recognises them as an underexplored biological resource with considerable potential. The Chinese pharmacopeia documents the nutritional and therapeutic properties of jellyfish, underscoring their historical use in traditional medicine (Domenico *et al*., 2016). Emerging research has identified medusozoan jellyfish as a valuable source of essential nutrients (Leone *et al*., 2015), along with unique bioactive compounds and potential lead molecules for pharmaceutical development. Interest is also expanding in their application as a novel food ingredient, particularly in Western markets (Leone *et al*., 2019). Global food habits are evolving, with rising interest in local terrestrial and aquatic foods. Seafood consumption is increasing, and jellyfish are gaining acceptance as a novel marine delicacy (Bonaccorsi,*et al*., 2020).

Jellyfish consumption is well established in several Asian and South-east Asian countries, where it is valued as a culinary delicacy. Among the ten recognised edible species, *Rhopilema hispidum* has been frequently cited for its prominence in regional diets (Chinnadurai *et al*., 2019). This species also holds importance in international trade, contributing to foreign exchange earnings through its export (Kumawat *et al*., 2023). While China pioneered the large scale processing of edible jellyfish (Duarte *et al*., 2022), broader commercial processing efforts have only recently begun to expand globally. Jellyfish are recognised for their favourable nutritional attributes, offering low calorie and fat content along with a valuable source of protein and collagen. Several species are considered safe for human consumption and are widely incorporated into traditional culinary preparations. Moreover, collagen extracted from jellyfish has been shown to possess bioactive properties, such as promoting wound healing and exhibiting antioxidant activity (Felician *et al*., 2019).

This study focuses on assessing the nutritional composition, antioxidant activity and functional properties of the edible jellyfish *R. hispidum*

Materials and methods

Sample collection and body fluid extraction

Fresh edible jellyfish (*R. hispidum*) specimens (Total wet weight = 12.5kg; Individual bell diameter = 18–24 cm; Individual wet weight = 750–900 g) collected off the south-west coast of India were transported to the laboratory in an insulated ice box utilising a two layer icing system. Upon arrival, the specimens were removed from the ice, de-iced, and thoroughly rinsed with chilled distilled water to eliminate sand, mucus and surface contaminants. To extract the naturally exuded body fluid, whole intact specimens were placed on clean stainless steel mesh screens over ice jacketed collection trays held under controlled refrigeration at 4±1°C for exactly one hour. The exuded body fluid was collected continuously, yielding an average specimen-to-fluid volume ratio of approximately10:1 (w/v). To ensure uniform sample composition for subsequent analytical assays, the exuded fluid collected from all 15 specimens was pooled. The pooled body fluid was immediately filtered through a double layer muslin cloth to remove cellular debris, followed by centrifugation at 4,000 g for 15 min at 4°C to precipitate remaining suspended solids. The clear supernatant was stored at -18℃ in airtight containers until further biochemical and functional characterisation.

Nutritional profiling

Proximate composition and energy factor

Proximate composition was analysed according to standard procedures established by AOAC (2019). Moisture content was determined using the oven drying method. Crude protein was quantified through the Kjeldahl method by estimating total nitrogen, while lipid content was measured using petroleum ether extraction. Ash content was determined by incinerating the sample at 550 ± 2°C for 5 h. Carbohydrate content was calculated using the following formula:

Carbohydrate (%) = 100 − (Moisture + Protein + Ash + Fat)

Spoilage associated nitrogenous compounds, including trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N), were measured using Conway's microdiffusion method (Conway, 1950). Lipid oxidation was assessed by determining the peroxide value (PV) following the method described by Yildiz *et al*. (2003).

Sampling was conducted in triplicate (n=3, whole jellyfish specimens per sampling interval) on designated storage days. For spoilage associated nitrogenous markers, total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) analyses were performed on days 1, 3, and 5. For lipid oxidation tracking, peroxide value (PV) was evaluated at days 1, 3, 5, 7, and 9. At each sampling point, tissue samples were collected to assess biochemical deterioration until the established quality threshold limits were reached.

In jellyfish, conventional methods for estimating energy content may yield inaccurate results due to the retention of 'water of hydration' after drying and the naturally low energy density of these organisms. Given the water of hydration content of 11.7%, a modified approach for calculating gross energy density, as suggested by Doyle *et al*. (2007) was adopted:

$$\text{Revised gross energy in kJ g}^{-1} = [(\text{Protein} \times a) + (\text{Lipid} \times b) + (\text{Carbohydrate} \times c)] \times d$$

where: a = 23.9 b = 39.5; c = 17.5; d = Correction factor of water of hydration, 1.13

Fatty acid profiling

The fatty acid profiling was carried out and the trans esterification was done according to Sreelakshmi *et al*. (2019) and the study was executed using a gas-liquid chromatograph (Perkin Elmer Clarus 580) equipped with a flame ionization detector with column Elite 225 in the presence of hydrogen (40 ml min^{-1}) and air (450 ml min^{-1}). Nitrogen was admitted and the flow rate was adjusted to 0.5 ml−1min^{-1}. The separated fatty acid was then compared with the standard fatty acid methyl esters to identify the compound (Supleco FAME Mix, Sigma-Aldrich Co., St. Louis, USA).

DPPH activity

The DPPH free radical scavenging activity of the body fluid was evaluated following the method of Yen and Wu (1999). Body fluid samples were prepared in double-distilled water at a concentration of 2 mg ml^{-1}. A 1.5 ml aliquot of each sample was mixed with 1.5 ml of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution prepared in 99.5% ethanol, and the mixture was vortexed thoroughly using a cyclomixture at high speed. The reaction mixtures were then incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 517 nm using a double beam spectrophotometer. Trolox (10–100 µg ml^{-1}) was used as a standard to construct the calibration curve (R^2>0.996), and IC_{50} values were calculated from the dose–response plots. Repeatability of the assay was confirmed with %RSD values below 4%. DPPH radical scavenging activity was calculated as:

$$\text{DPPH free radical scavenging activity} = 1 - \frac{Abs_{sample}}{Abs_{control}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) of the jellyfish body fluid was evaluated based on the method described by Oyaiza (1986). Briefly, 1ml of sample was mixed with 2.5 ml of 0.2M phosphate buffer and 1% of 2.5 ml of potassium ferricyanide. This mixture was then incubated for 30 min, after which 2.5 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction. An aliquot (2.5 ml) of the resulting mixture was then mixed thoroughly with 2.5 ml of distilled water and 0.5 ml of ferric chloride solution, followed by incubation for 10 min. Absorbance was measured at 700 nm using double beam spectrophotometer. Butylated hydroxyanisole (BHA) was used as the reference antioxidant. Higher absorbance values indicated greater ferric reducing activity. The assay was conducted in triplicate (R^2=0.992).

ABTS radical scavenging activity

The ABTS radical scavenging property of jellyfish body fluid was determined according to the method of Re *et al*. (1999) with slight modifications. Initially a stock solution of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was prepared by adding 5 ml of 7 mM ABTS solution with 88 µl of 140 mM potassium persulfate, and introduced into dark at room temperature for 12-16 h. A volume of stock solution was diluted with phosphate buffer (PB 5 mM, pH 7.4) containing 0.15M NaCl for preparing working solution of ABTS radicals in order to obtain an absorbance of 0.70±0.02 at 734 nm (R^2=0.993). The ionic reduction was calculated using the following equation:

$$\text{ABTS (\%)} = 1 - \frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Fatty acid profiling

The metal chelating activity of the fluid was determined as described by Decker and Welch (1990). Initially, 1 ml of the sample (5 mg ml^{-1}) was mixed with 3.7 ml of distilled water, which was then allowed to react with a mixture containing 0.1 ml 2Mm $FeCl_2$ and 0.2 ml of 5 Mm ferrozine. The solution was kept undisturbed for 10 min followed by measuring the absorbance at 562 nm (R^2>0.990). The metal chelating ability was calculated using the equation:

$$\text{Metal chelating activity} = 1 - \frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Functional properties of jellyfish fluid

Foaming properties

Functional properties of the jellyfish body fluid were analysed in terms of foaming and emulsion properties in accordance with Sathe and Salunkhe (1981). Body fluid concentrations 0.1, 0.5, 1% were prepared. For the forming capacity assay, 20 ml of each sample was whipped using a homogeniser at 13500 rpm for 2 min, to incorporate air. The whipped sample was immediately transferred to 100 ml measuring cylinder and the foam volume was noted (RSD<4%). The foaming capacity was calculated using the following formula:

Foaming capacity = ((Volume after whipping) - Volume before whipping))/ ((Volume before whipping)) × 100

Following whipping, the sample allowed to stand undisturbed at 20℃ for 30 min, after which the foam volume was recorded. Foam stability was determined using the formula:

Foaming stability = ((Volume after standing) - (Volume before whipping))/ (Volume before whipping) × 100

Emulsion properties

The emulsifying properties such as emulsion activity index (EAI) and emulsion stability index (ESI) were calculated as per Pearce and Kinsella (1978). Body fluid concentrations at 0.1, 0.5 and 1% were prepared and 30 ml of each sample was mixed with10 ml of refined sunflower oil and homogenised at 20500 rpm for 1min. Immediately after homogenisation, 50 µl of the emulsion was withdrawn from the bottom of the container at 0 and 10 min and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the mixture was analysed at 500 nm using a double beam spectrophotometer, immediately after emulsion formation (A_0) and after 10 min (A_{10}). The EAI and ESI were calculated as using the following equations:

$$EAI = 2T\Phi C$$

where T is turbidity ($T = 2.303A_{500}/l$; A_{500} = Absorbance at 500 nm; l = Path length); Φ = Oil volume fraction (0.25); and C = Protein concentration.

$$EAI\ (m^2 g^{-1}) = \frac{2 \times 2.3.3 \times Abs_{500nm}}{0.25 \times \text{Fluid weight (g)}}$$

$$ESI\ (min) = \frac{A_0 \times \Delta t}{\Delta A}$$

where, ΔA = Absorbance at 0min (A_0) - Absorbance after 10 min (A_{10}) and Δt = 10 min

Shelf life study

To evaluate the biochemical stability of *R. hispidum* during chilled storage, whole cleaned jellyfish specimens (n=15) were individually packaged in clean, food grade polyethylene bags and stored under controlled refrigerated conditions at 2±1°C. The storage temperature was continuously monitored using a calibrated digital temperature probe. The product was periodically withdrawn to study the nutritional and biochemical changes during storage.

Statistical analyses

All data were derived from triplicate analysis with relative standard deviation (RSD) values maintained below 5%, ensuring analytical consistency. Fatty acid quantification *via* GC-FID was validated using known standards and linear calibration curves (R^2>0.995), enhancing the reliability of the reported values. The data obtained were analysed using One way ANOVA. Each experiment was carried out in triplicate, and data were first tested for normality before proceeding with statistical analysis. Data given are mean±standard deviation. Duncan multiple comparison tests was used to determine the significant difference between the means of the triplicate values using IBM SPSS v 20 (SPSS Inc., Chicago, IL, USA).

Results and discussion

While prior research has extensively characterised the edible jellyfish meat post-desalting or drying, no significant efforts have focused on analysing the naturally exuded body fluid as a potential nutrient source. This study addresses this gap, contributing valuable data to ongoing global efforts toward circular marine bioproduct utilisation and functional food diversification under the EU Blue Growth and UN SDG 14 frameworks. By examining both the whole body tissue and the exuded fluid, the study offers new insights into the broader utility of jellyfish, not only as gelatinous marine organisms but also as valuable biological resources that can support sustainable utilisation. The proximate composition of the body tissue and exuded fluid was analysed independently, and the results are summarised in Table 1.

The proximate composition of the whole body showed a high moisture content of 96.3±0.95%, followed by 2.03±0.08% protein, 1.15±0.32% ash, 0.023±0.01% fat. A comparable pattern was observed in the body fluid, which contained 98.04±0.61% moisture, 1.10±0.19% protein, 1.02±0.45% ash, 0.013±0.01 % fat (Table 1). These values align with previous reports in jellyfish species such as *Aurelia aurita* (Abdulla *et al*., 2015) and *Cotylorhiza tuberculata* (Leone *et al*., 2015). The high water content in jellyfish tissues contributes to their buoyancy and inherently low energy density, as described by Khong *et al*. (2016). Similar compositional profiles have also been reported in earlier studies (Raj *et al*., 2019). Once dried, the samples exhibited a marked increase in the concentration of protein and minerals, highlighting their nutritional potential despite their highly diluted composition in the fresh state.

An important observation was the inverse relationship between ash and protein content in both the body tissue and the exuded fluid. This pattern may reflect the tissue specific distribution of inorganic ions, which can influence protein solubility and structural stability. The elevated ash content indicates significant presence of divalent ions, which may contribute to structural integrity and osmotic regulation in jellyfish. Similar tissue specific variation in mineral composition has been reported, with the bell region containing higher mineral concentrations than the oral arms (Costa *et al*., 2019). These mineral rich fractions may have potential applications in food and nutraceutical formulations, although their mineral composition, bioavailabilty and safety require further evaluation.

This study is specifically novel in its focused examination of the body fluid, a typically discarded byproduct during jellyfish processing. While most research emphasises dried jellyfish meat, the characterisation of body fluid in this study revealed considerable nutritional and functional significance. The dry matter content of the fluid showed 59.4% inorganic and 31.79% protein composition, suggesting its potential as a concentrated source of bioavailable minerals and amino acids. This insight aligns with the growing interest in valorising marine processing waste under zero-waste and circular economy frameworks. Instead of being discarded, the jellyfish fluid could be transformed into mineral rich supplements, marine based broths, or bioactive ingredient solutions.

Gross energy content is a key parameter in nutritional evaluations, particularly when assessing the potential of marine organisms as food sources. Traditional bomb calorimetry tends to underestimate the energy value of gelatinous species due to their high moisture content and the presence of bound hydration water, which is not fully removed during standard drying procedures (Doyle *et al*., 2007). In this study, an adjusted calculation method that accounts for bound water yielded higher energy values for both the whole body (15.71 kJ g^{-1} dry matter) and the fluid (13.68 kJ g^{-1} dry matter). These values surpass those reported for species such as *Cyanea capillata*, offering a more accurate assessment of the nutritional contribution jellyfish can provide in diets or formulated feeds. This methodological refinement holds practical importance for future research on gelatinous marine organisms, where standard calorimetric approaches may not fully capture their energy potential.

The fatty acid profile of *R. hispidum* reinforces its potential as a health-supportive food source. The composition was analysed and is summarised in Table 2. Palmitic acid was the most prevalent fatty acid (1.553 mg 100 g^{-1}), followed by eicosapentaenoic acid (EPA) at 1.181 mg 100 g^{-1}, and stearic acid at 0.840 mg 100 g^{-1}. Arachidonic

Table 1: Data representing the proximate composition of the jellyfish body and fluid

Sample	Moisture (%) (Wet wt)	Protein (%) (Wet wt)	Ash (%) (Wet wt)	Fat (%) (Wet wt)	Carbohydrate (%) (Wet wt)	Revised gross energy (kJ g^{-1}) (Dry wt)
Whole body	96.3 ± 0.95[a]	2.03 ± 0.08[a]	1.15±0.32[a]	0.023 ± 0.01[a]	0.497+ 0.05[a]	15.71[a]
Body fluid	98.04±0.61[b]	1.10 ± 0.19[b]	1.02± 0.45[a]	0.013± 0.01[a]	Nil	13.68[b]

acid contributed 0.690 mg 100 g^{-1}, while pentadecylic acid (C15) was detected at the lowest concentration.

The total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) contents were 2.842±1.22 mg 100 g^{-1}, 0.597±0.35 mg 100 g^{-1}, and 4.361±1.44 mg 100 g^{-1} respectively. Differences in fatty acid composition are often influenced by factors such as age, processing techniques, and organism size (Wakabayashi *et al*., 2016). Palmitic acid was the dominant SFA, consistent with previous observations in other jellyfish species (Abdulla *et al*., 2015). EPA and arachidonic acid (ARA), both detected in appreciable quantities, contribute to the overall nutritional value of the species. These long-chain polyunsaturated fatty acids (PUFAs) are recogised for their roles in cardiovascular health, anti-inflammatory mechanisms, and cognitive functions. Desalted samples also showed the presence of ω-6 fatty acids, including linoleic and arachidonic acids, while *C. capillata* (sand jellyfish) has been found to contain ω-3 fatty acids such as cis-9,12,15-octadecatrienoic acid and cis-5,8,11,14,17-eicosapentaenoic acid (Kromfang *et al*., 2015). It was also observed that the PUFA concentration in desalted jellyfish samples was lower compared to that in fresh specimens, suggesting that desalting or associated processing may influence fatty acid retention.

Table 2. Data representing the fatty acid profile of whole jelly fish

Fatty acid	Composition (mg 100 g^{-1})
C12	0.049±0.07
C14	0.159±0.05
C15	0.044±0.24
C16	1.553±0.21
C16:1	0.172±0.16
C17	0.117±0.18
C18	0.840±0.43
C18:1	0.425±0.19
C18:2	0.163±0.22
C18:2	0.713±0.34
C18:3	0.450±0.28
C18:3	0.051±0.11
C20	0.080±0.04
C20:2	0.569±0.02
C20:4	0.690±0.17
C20:5	1.181±0.23
C22:6	0.544±0.07
SFA	2.842±1.22
MUFA	0.597±0.35
PUFA	4.361±1.44
n3/n6	1.421±0.94

The total PUFA content (4.361 mg 100 g^{-1}) and the ω-3/ω-6 ratio (1.421) observed in this study surpass the FAO/WHO (1994) recommended minimum of 0.1–0.2, indicating a favourable profile and potential for therapeutic applications. The ω-3/ω-6 ratio serves as a widely accepted index for assessing the nutritional quality of food sources, with higher values associated with improved health outcomes (Sreelakshmi *et al*., 2019). These results suggest that *R. hispidum* may have potential as a source of nutritionally relevant PUFAs and could be explored for incorporation into functional food products.

Antioxidant property of jellyfish body fluid

Jellyfish are an underexplored marine resource with strong potential as a sustainable source of bioactive compounds, particularly antioxidants. Their high protein content, with collagen comprising approximately 40–60% of the dry weight, highlights their suitability for nutraceutical applications (Khong *et al*., 2016). In the present study, the antioxidant activity of *R. hispidum* body fluid was assessed using ABTS, DPPH, and metal chelating assays. The body fluid exhibited 90.32±0.8% ABTS radical scavenging activity, 79.62±0.007% DPPH activity, and 57.97±2.02% metal chelating capacity (Fig. 1). The FRAP assay also demonstrated strong reducing potential, with an absorbance of 0.83 nm (Fig. 2).

The findings from this study are consistent with, and in some cases surpass, the antioxidant activities previously reported in other jellyfish species. For example, *C. tuberculata* and *Rhizostoma pulmo* have demonstrated strong antioxidant potential, attributed to their protein and phenolic content (Leone *et al*., 2015). Prieto *et al*. (2018) also reported antioxidant capacity varies across species, with *Rhizostoma luteum* showing the highest recorded activity at 32,598±9015 nmol TE per g dry weight. These comparisons highlight the distinct biochemical profile of *R. hispidum*, particularly the body fluid, which is often overlooked and discarded during processing. The antioxidant activity observed in *R. hispidum* fluid indicates the likely presence of low-molecular weight peptides and proteins. This is supported by findings from Domenico *et al*. (2019), who reported an inverse relationship between molecular weight and antioxidant activity in jellyfish protein hydrolysates. These results suggest that processing methods aimed at releasing smaller peptides may further improve the functional properties of jellyfish derived extracts.

These findings extend beyond basic compositional analysis and highlight the potential for developing high value functional foods and nutraceuticals. The strong antioxidant activity found in the naturally exuded jellyfish body fluid aligns with zero-waste principles and supports the sustainable use of marine resources. Given its protein rich and mineral dense profile, the body fluid of *R. hispidum* holds promise for formulation into marine based electrolyte beverages or rehydration supplements, especially in regions where marine functional foods are culturally and commercially significant, such as South-east Asia and Japan. Further research should focus on elucidating the mechanisms underlying these bioactivities, evaluating their stability in product formulations, and assessing their *in vivo* efficacy, thereby transforming an abundant and underutilised marine biomass into a source of functional and therapeutic ingredients.

Functional properties of jellyfish fluid

The jellyfish fluid collected was subjected to analysis of the functional properties of foaming capacity, foaming stability, emulsion activity index (EAI) and emulsion stability index (ESI) and the results are presented in Table 3.

Foaming properties

The naturally exuded body fluid of *R. hispidum* exhibited strong, concentration-dependent foaming properties across all evaluated parameters (Table 3). As the fluid concentration increased from

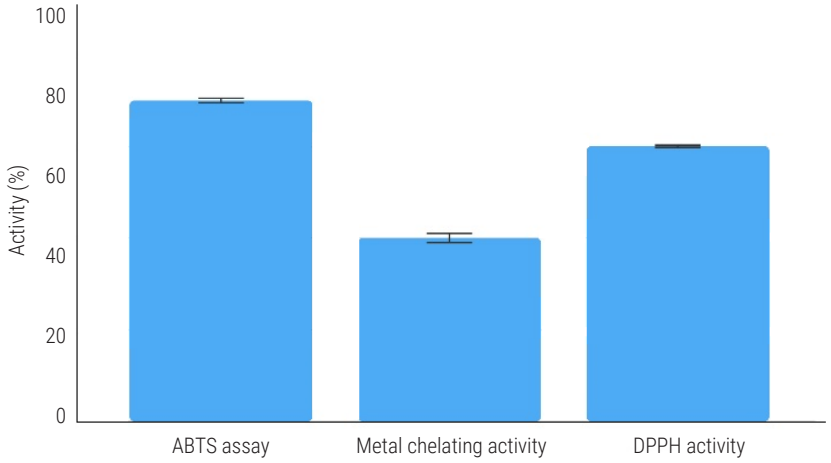

Fig. 1. ABTS radical scavenging activity, metal chelating activity and DPPH activity of the jellyfish body fluid

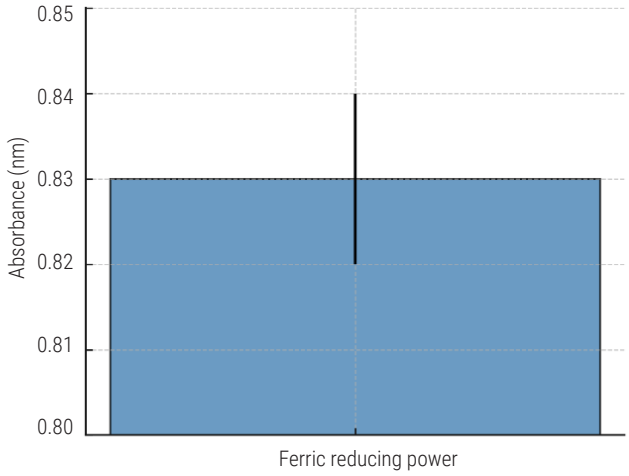

Fig. 2. Ferric reducing antioxidant power of the jellyfish body fluid

0.1 to 1%, a progressive enhancement was observed in both foaming capacity (FC) and foaming stability (FS). Specifically, the FC increased from 23.26±0.19% at a concentration of 0.1% to 30.40±0.33% at 0.5%, ultimately reaching a maximum of 33.16±0.24% at 1% concentration. A parallel trend was recorded for FS, which progressed from 19.06±0.15% at 0.1% concentration to 24.21±0.22% at 0.5%, achieving a peak stability value of 31.24±0.57% at 1% concentration.

Foam formation in proteinaceous solutions is driven by a sequence of dynamic molecular events, including the physical transportation, interfacial penetration, and structural rearrangement of surface active proteins or low molecular weight peptides at the air-water interface (Karami and Akbari-Adergani, 2019). The observed concentration dependent response occurs because higher fluid concentrations deliver a greater quantity of soluble amphiphilic peptides to the interface. These active molecules rapidly reduce air–water surface tension, promoting the incorporation of air bubbles during high speed homogenisation (Delhaize and Wierenga, 2022; Fameau *et al*., 2023). Furthermore, the higher availability of proteins facilitates cohesive intermolecular interactions and cross-linking across the air–water boundary. This produces flexible, continuous viscoelastic films capable of resisting bubble coalescence, film thinning, and drainage (Ho *et al*., 2024). Although electrostatic repulsions among similarly charged peptides can occasionally weaken interfacial cohesion in dilute systems, higher peptide concentrations mitigate these effects by establishing dense, continuous interfacial coverage (Elavarasan *et al*., 2014). These results indicate that *R. hispidum* body fluid contains functional proteinaceous constituents capable of acting as effective clean-label foaming agents in aerated food formulations.

Emulsion properties

The emulsifying activity index (EAI) and emulsion stability index (ESI) of *R. hispidum* body fluid were similarly governed by fluid concentration, exhibiting marked improvements as concentration levels were raised from 0.1 to 1% (Table 3). The EAI values demonstrated a sharp upward trajectory, rising from 7.07±0.25 $m^2 g^{-1}$ at 0.1% concentration to 13.50±0.30 $m^2 g^{-1}$ at 0.5%, and peaking

Table 3. Data representing the functional properties of jellyfish fluid

Fluid concentration (%)	Foaming capacity (%)	Foaming stability (%)	Emulsion activity index (EAI) (m^2/g)	Emulsion stability index (ESI) (min)
0.1%	23.26±0.19[a]	19.06±0.15[a]	7.07±0.25[a]	10.11±0.11[a]
0.5%	30.40±0.33[b]	24.21±0.22[b]	13.50±0.30[b]	14.06±0.27[b]
1%	33.16±0.24[c]	31.24±0.57[c]	15.38±0.43[c]	19.82±1.01[c]

Values are presented as Mean±SD of triplicate measurements (n=3). Different superscript letters (a, b, c) within the same column indicate statistically significant differences ($p<0.05$) determined by One way ANOVA followed by Duncan's multiple range test

at15.38±0.43 m^2 g^{-1} at 1% concentration. Concurrently, the ESI values increased from 10.11±0.11 min at 0.1% to 14.06±0.27 min at 0.50%, achieving maximum emulsion persistence of 19.82±1.01 min at the 1% fluid level.

Emulsifying activity reflects the capacity of surface active molecules to rapidly adsorb onto newly generated oil droplet surfaces during homogenisation, whereas stability reflects the ability of the protective layer to prevent droplet coalescence over time (Othmeni *et al*., 2024). At lower concentrations (0.1%), the reduced availability of surface active peptides results in incomplete surface coverage and lower steric hindrance, leaving dispersed oil droplets highly susceptible to flocculation and coalescence (Mirzaee *et al*., 2024). Conversely, at elevated concentrations (1%), the higher peptide density allows rapid migration, adsorption, and optimal alignment of hydrophobic and hydrophilic domains across the oil–water interface (Ricardo *et al*., 2021). This constructs robust, charged interfacial films around oil droplets, establishing electrostatic and steric barriers that effectively stabilise the oil-in-water emulsion (Elavarasan *et al*., 2014). Considering that exuded body fluid represents up to 15–20% of total jellyfish biomass by weight, valorising this fluid into functional bio-emulsifiers and foaming additives provides a viable approach for zero-waste marine bioprocessing.

Shelf life analysis of jellyfish stored at refrigerated condition

The biochemical stability of jellyfish during refrigerated storage was evaluated by monitoring key spoilage indicators such as total volatile base nitrogen (TVB-N), trimethylamine (TMA), and peroxide value (PV), over a 5-9 day period. These parameters are widely recognised for assessing freshness, spoilage progression, and oxidative changes in seafood products. TVB-N levels, which reflect the accumulation of volatile nitrogenous compounds such as ammonia, dimethylamine, and trimethylamine resulting from microbial metabolism and autolytic enzymatic activity, exhibited a steady rise throughout storage. Initial values measured 16.21±0.18 mg N 100 g^{-1} and exceeded the acceptable threshold of 30–35 mg N 100 g^{-1} (Castro *et al*., 2006) by the fifth day, reaching 37.4 ± 0.34 mg N 100 g^{-1} (Fig. 3a). This rapid increase indicates early onset of spoilage, aligning with previous observations in refrigerated jellyfish (Kromfang *et al*., 2015). The elevated TVB-N levels suggest significant proteolytic breakdown, highlighting the highly perishable nature of jellyfish and the critical need for immediate post-harvest handling or preservation.

Similarly, TMA concentrations produced by bacterial reduction of trimethylamine oxide (TMAO), rose from 6.54 mg N 100 g^{-1} to 28.6 mg N 100 g^{-1} by day 5 (Fig. 3b), also surpassing quality benchmarks. This increase reflects microbial activity and enzymatic degradation, contributing to the formation of off-odours. Compounds such as TMA, 1-penten-3-one, 2,3-pentanedione, and cis-4-heptenal are associated with undesirable fishy aromas that negatively impact consumer acceptance (Ganeko *et al*., 2008). Rathod *et al*. (2022) also attributed TMA accumulation to spoilage driven microbial and enzymatic pathways during storage. The data clearly show that both TVB-N and TMA levels exceeded regulatory and sensory limits within five days under chilled conditions, indicating a narrow window for safe handling and processing. These results reinforce the importance of applying preservation strategies such as vacuum packaging, acidification, or freezing, to extend shelf life and maintain product quality. Lipid oxidation, assessed through peroxide value (PV), further confirmed deterioration. PV, which quantifies hydroperoxides formed during the early stages of lipid degradation, showed a progressive increase during storage and exceeded acceptable limits by day 7 (Fig. 3c). Although jellyfish typically contain low lipid levels, any oxidation that occurs contributes to off-flavours and degrades overall quality. The rise in PV is consistent with findings in other seafood species, indicating that oxidative stability, while secondary to microbial spoilage, remains an important quality factor.

Collectively, these findings define the biochemical spoilage timeline of the jellyfish *R. hispidum* under refrigerated storage and demonstrate the urgency of implementing rapid post-harvest interventions. This information is essential for developing predictive shelf-life models and optimising industrial processing protocols aimed at utilising jellyfish as a commercially viable marine resource. This study presents *R. hispidum* as a valuable marine bioresource with strong potential for use in functional foods and nutraceutical formulations. For the first time, the naturally exuded body fluid of *R. hispidum* from the Indian coast was characterised, revealing significant antioxidant activity (ABTS, DPPH) and functional attributes such as emulsifying capacity (EAI, ESI) and foaming properties, features that have received limited attention in earlier studies, which primarily focused on dried jellyfish tissue. By incorporating revised energy calculation methods that account for bound water, the study addressed challenges commonly faced in evaluating the nutritional content of gelatinous organisms. While the high moisture content may limit direct applications, drying the material significantly concentrates its protein and mineral content while maintaining low fat and energy levels. Taken together, this study demonstrates that *R. hispidum*, particularly its underutilised body fluid, holds significant promise as a sustainable marine bioresource with potential applications in health promoting food systems. The detailed compositional characterisation and the incorporation of revised energy calculations provide a more accurate nutritional profile, forming a robust basis for the integration of jellyfish derived materials into functional food, nutraceutical, and circular bioeconomy initiatives.

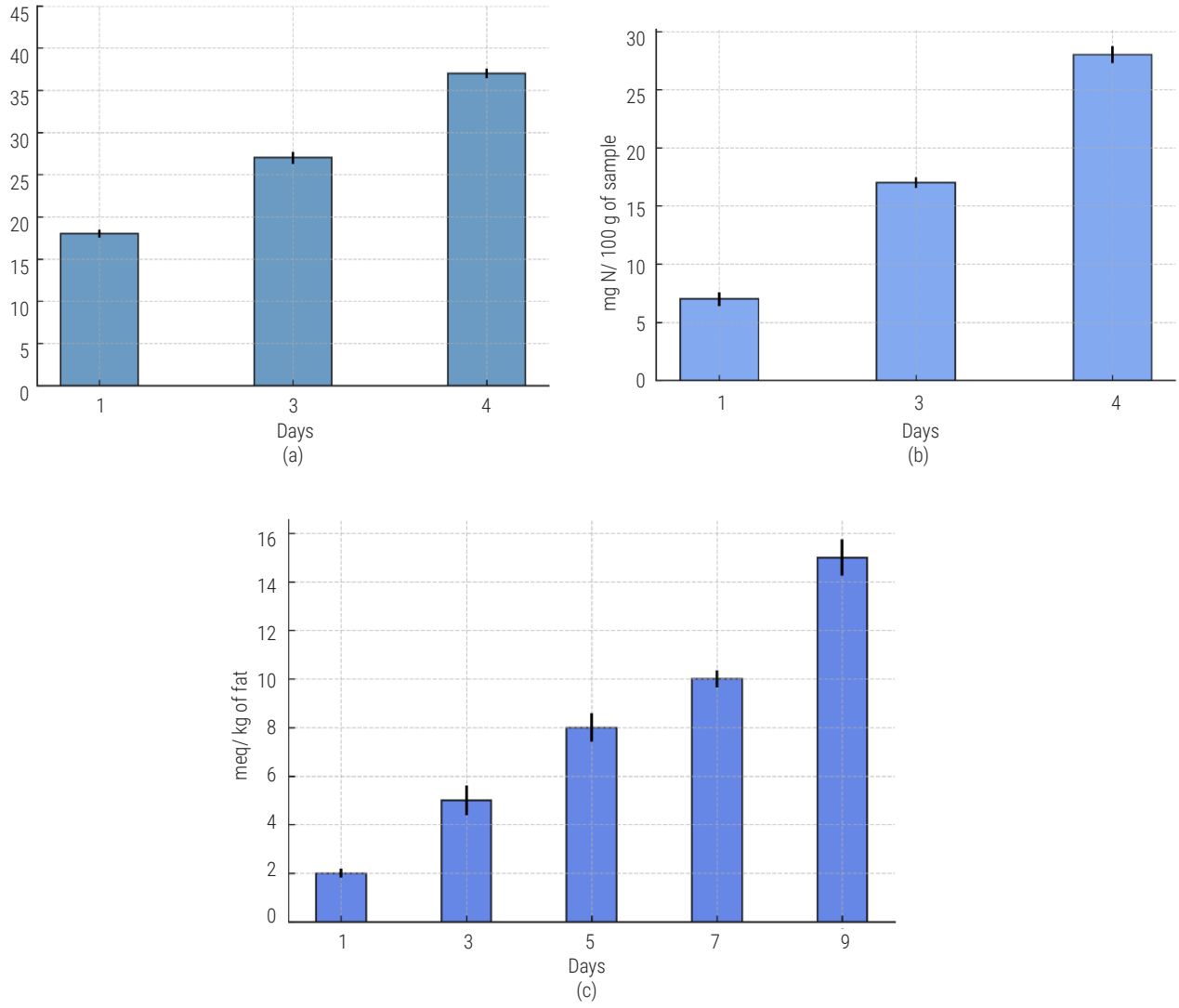

Fig. 3. (a) TVBN ; (b) TMA and (c) Peroxide values of jellyfish during storage

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