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Original Research Article

Taurine Content and Bioactivity of Hydrolysate, Isolate, and Crude Taurine from *Hippocampus* sp. for Enhancing Physical Stamina

Rahman Karnila[1]*, Harifa Syah Putra[2], Muhammad Zakiyul Fikri[1], Putriana Sari Sirait[1], Dian Iriani[1], Zahtamal[3], Sharifudin Md Shaarani[4], Amelia Rike Triana[1], Naurah Aathirah[1]

[1] *Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Riau University, Pekanbaru, Indonesia*
[2] *Department of Marine Science, Faculty of Fisheries and Marine Science, Riau University, Pekanbaru, Indonesia*
[3] *Department of Public Health, Faculty of Medicine, Riau University, Pekanbaru, Indonesia*
[4] *Department of Food Biotechnology Programme, Faculty of Science and Technology, Sains Islam Malaysia University, Negeri Sembilan, Malaysia.*

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ABSTRACT

Taurine ($C_2H_7NO_3S$) is a sulfur-containing amino acid that plays a crucial role in osmotic regulation, antioxidant activity, and physical stamina enhancement while seahorses (*Hippocampus* sp.) are recognized as potential taurine source, however the detailed systematic comparisons of the antioxidant activity of its different protein fractions remain limited. This study evaluated the optimal seahorse protein fractions (hydrolysate, isolate, and crude taurine) as a natural taurine source with regards to antioxidant activity for stamina enhancement. The protein hydrolysate was produced via enzymatic hydrolysis using alcalase (1%, 1.5%, and 2%), the protein isolate through pH adjustment (2.5, 3.0, and 3.5), and the crude taurine by ultrasonication (30, 45, and 60 min). Each fraction was analyzed for yield, chemical composition, and antioxidant activity. The optimal treatment methods were evaluated using Duncan and PCA. The results demonstrated that seahorse flesh dominated the biomass (94.63%), with protein contents of 55.64% (flour) and 63.18% (concentrate). Crude taurine extracted at 30 min yielded the highest taurine content (9.56 mg/g) and exhibited superior antioxidant activity (IC_{50} DPPH = 2.01 mg/mL; IC_{50} hydroxyl = 4.15 mg/mL), significantly outperforming hydrolysate and isolate. Significant correlations between taurine content and antioxidant activity (R^2 = 0.51–0.57) confirmed taurine as the primary bioactive component. PCA analysis revealed distinct separation of crude taurine from other fractions based on taurine content and bioactivity. These findings established *Hippocampus* sp. as a as a promising natural source of crude taurine with potent antioxidant properties having functional food applications in physical stamina enhancement, warranting further *in vivo* validation.

Keywords: Seahorse, Taurine, Protein Hydrolysate, Protein Isolate, Crude Taurine, Antioxidant, Physical Stamina.

Introduction

In recent years, the amino acid taurine ($C_2H_7NO_3S$) has gained recognition due to its numerous benefits in physiological functions, including the enhancement of stamina and physical performance. Taurine acts as a neurotransmitter in osmotic processes and androgen hormone production. Consequently, taurine serves as an osmoregulatory agent[1], antioxidant[2], cardiovascular health promoter[3], antithrombotic agent in enhancing sexual and renal function[4], as well as a neuroprotective compound[5]. Various studies have demonstrated that synthetic taurine supplementation can improve physical performance and energy metabolism in experimental rats[6]. However, taurine cannot be easily synthesized due to the extremely low activity of cysteine sulfinic acid decarboxylase (CSAD) –the rate-limiting enzyme in taurine biosynthesis in human[7,8].

*Corresponding author. E mail: rahman.karnila@lecturer.unri.ac.id
Tel: 081318406022

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Therefore, humans obtain most of their taurine from dietary sources. Moreover, synthetic drugs may cause severe side effects, high costs, addiction, and disruption of the body's metabolic system[9]. Consequently, natural materials offer an alternative, with ongoing development of their potential and efficacy for health benefits. Among marine sources, seahorses serve as a natural source for the extraction of taurine. Indonesia ranks as the third-largest seahorse catch producer (500 individuals/kg) after the Philippines and India[12]. Seahorses are commonly found near temperate and tropical coastal areas, and most seahorses are traded as bycatch[10,11,12]. Therefore, seahorses are abundant, with 792 individuals recorded in market surveys, particularly the genus *Hippocampus comes* (family Syngnathidae), which is the most prevalent at 51.5% among 46 species[12]. Seahorses (*Hippocampus* sp.) are widely recognized in natural medicine and traditional Chinese medicine for their health benefits. Dried seahorses are incorporated whole into beverages or consumed in powdered form to treat various ailments such as respiratory diseases and arteriosclerosis[13]. Although limited scientific research supports the nutritional benefits of seahorses, they are believed to possess medicinal properties in promoting human health. Several studies have reported that seahorses contain phenolic compounds such as paeonol with neuroinflammatory activity[14], fatty acids and essential amino acids that maintain health and support various body functions[15,16], antioxidants[16], and anticancer properties[17-19]. Seahorses are suspected to contain taurine related to immune function, particularly in its free amino acid form. Therefore, taurine offers a solution as a functional food source for improving physical stamina.
Physical stamina among Indonesian youth is significantly affected by nutritional status, with malnutrition leading to decreased physical

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endurance and impaired development. The prevalence of poor physical stamina and energy in Indonesia is approximately 9.2% among adolescents aged 13-15 years[20]. Chronic malnutrition can cause impaired physical development, reduced stamina, and increased fatigue[21,22]. This condition is particularly vulnerable in children, who are 4.661 times more likely to experience stunting, which directly affects their physical capabilities[23,24]. The use of expensive chemical drugs with their side effects also limits access to affordable interventions for improving stamina and health for those with limited economic means[24]. This presents a challenge for researchers to provide solutions to physical stamina problems, particularly in Indonesia. Therefore, the utilization of seahorse-derived taurine represents a promising approach for physical stamina enhancement.

The novelty of this research lies in the comprehensive comparison of three distinct seahorse protein fractions hydrolysate, isolate, and crude taurine as natural sources for stamina enhancement. While previous studies have demonstrated taurine's benefits for physical performance, no systematic investigation has compared these protein forms regarding taurine content and antioxidant bioactivity in the context of stamina improvement. This study employed enzymatic hydrolysis, pH-based isolation, and ultrasonication extraction methods to identify the optimal fraction with the highest taurine yield and bioactivity. Furthermore, this research provides novel insights into the correlation between taurine content and antioxidant capacity across different protein fractions, establishing a scientific basis for developing seahorse-based functional foods for physical stamina enhancement.

Therefore, this study aimed to: (1) extract and characterize three protein fractions from seahorse, (2) determine the optimal extraction conditions for maximum taurine yield and antioxidant activity, and (3) evaluate the correlation between taurine content and antioxidant bioactivity to identify the most promising fraction for stamina-enhancing functional food applications

Materials and Methods

Materials and equipment

The primary material used in this study was seahorse *(Hippocampus* sp.) obtained from Senggarang Village (0°55'12.0"N 104°26'24.0"E), Tanjung Pinang City, Riau Islands, Indonesia (Figure 1). Other materials included alcalase enzyme (≥2.4 U/g, Solarbio, Beijing, China, CAS No. 9014-01-1), BSA (≥98% purity, Sigma-Aldrich, St. Louis, MO, USA), H_2SO_4 (95%, analytical grade, Merck KGaA, Darmstadt, Germany), 96% ethanol, 96% methanol, hexane (≥99.8%, HPLC grade, Merck KGaA, Darmstadt, Germany), TCA (Merck, Germany), phenolphthalein indicator (Merck, Germany), Kjeldahl Tab catalyst (Merck, Germany), 45% NaOH, 20% NaOH, boric acid (H_3BO_3) (Merck, Germany), and HNO_3 (65%, analytical grade, Merck KGaA, Darmstadt, Germany). The equipment utilized in this study included digital scales (Kova, model KV-300, China**)**, incubator (Binder GmbH, model BD 56, Tuttlingen, Germany), measuring cylinder, beaker, spatula, Erlenmeyer flask (Pyrex, Corning Inc., NY, USA), hot plate (Thermo Scientific, model Cimarec, Waltham, MA, USA), porcelain cup (OEM, China), filter paper (Whatman No. 1, GE Healthcare, UK), desiccator (Duran Group, Wertheim, Germany), UV-Vis spectrophotometer (Optima SP-300, Tokyo, Japan), ultrasonicator (Wiggens, model WUC-D22H, Berlin, Germany), blender (Philips, model HR2115, Amsterdam, Netherlands), centrifuge (Gemmy Industrial Corp., model PLC-03E, serial no. 2012040, Taiwan), and freeze dryer (Buchi, model BK FD10P, Flawil, Switzerland).

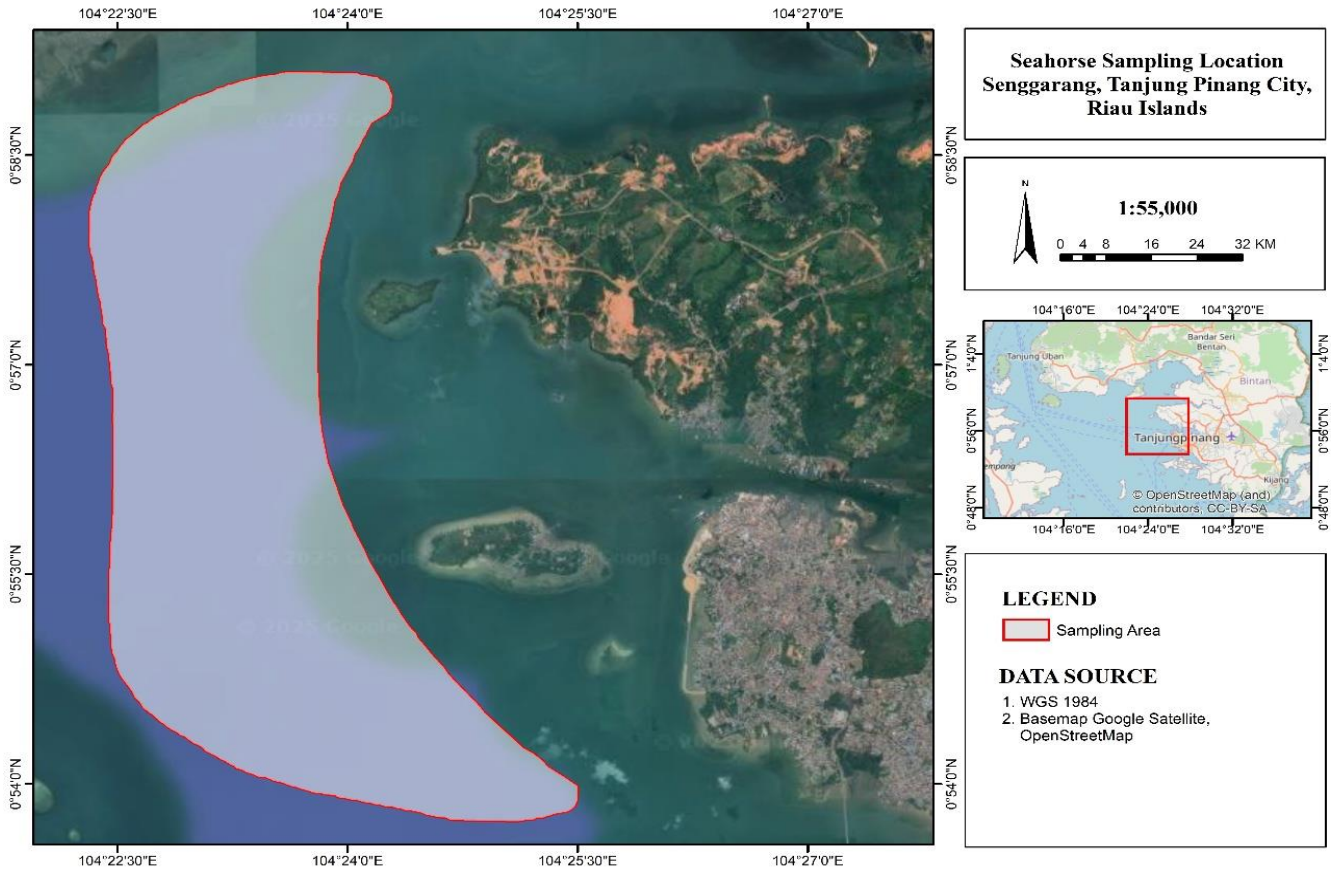

Figure 1: Shows the geographical location of seahorse sampling site in Senggarang Village, Tanjung Pinang City, Riau Islands, Indonesia.

Study design and setting
This research employed an experimental design to obtain the optimal protein hydrolysate, protein isolate, and crude taurine from seahorses obtained through enzymatic and physicochemical processes. The study was conducted in sequential stages: 1) preparation of protein hydrolysate through hydrolysis of protein concentrate using alcalase enzyme at varying concentrations (1%, 1.5%, and 2%); 2) protein isolation through pH adjustment (2.5, 3, and 3.5); and 3) extraction of crude taurine through ultrasonication of seahorse flour at different durations (30, 45, and 60 min). All experimental procedures were designed to evaluate the morphometric, chemical composition, yield, and antioxidant activities (DPPH and hydroxyl radical scavenging) of these fractions. The optimal fraction from each treatment method was analyzed for taurine content. Subsequently, their taurine content were correlated to DPPH and hydroxyl free radical scavenging activity evaluated using Duncan's Multiple Range Test (DMRT), Pearson correlation, linear regression, and Principal Component Analysis (PCA)

Seahorse preparation
Seahorse samples were obtained from local fishermen in Senggarang, Tanjung Pinang City, Riau Islands. Morphometric measurements were conducted following procedures described in previous studies[25,26] after the samples were sun-dried for 2 days. Subsequently, 1.2 kg of seahorses with an average weight of 16.71 g/individual were selected and transported to the Fisheries Product Chemistry Laboratory, Riau University, Pekanbaru, Indonesia. The flesh and by-products were separated and weighed individually. The flesh was ground using a blender and passed through an 80-mesh sieve to obtain a seahorse flour. The yield and chemical composition were analyzed according to established methods[27,28] for further testing.

Extraction of Seahorse Protein Concentrate
The preparation of protein concentrate was adapted from the method reported in previous research[29] with minor modifications. A total of 100 g of seahorse flour was weighed and placed into an Erlenmeyer flask, then extracted using acetone as solvent at a ratio of 1:4 (w/v) and stored in a refrigerator for 24 hours (4°C). After the extraction process, the supernatant (liquid phase) was separated from the precipitate (residue) through centrifugation at 9,000 rpm for 15 minutes using a centrifuge (Gemmy PLC-03E, Taiwan). The obtained precipitate was subsequently freeze-dried at –50 °C for 24 hours using a freeze dryer (Buchi BK FD10P, Switzerland). The resulting concentrate was packaged in sample bottles wrapped with aluminum foil and stored in a cool room at 4°C until further use.

Protein Concentrate Hydrolysis
The preparation of protein hydrolysate was adapted from the method reported in previous research[30] with minor modifications. A total of 4 g of seahorse protein concentrate was weighed and placed into an Erlenmeyer flask, then suspended with 100 mL of distilled water and homogenized using a magnetic stirrer at 40 °C for 15 minutes. Subsequently, alcalase enzyme was added at concentrations of 1%, 1.5%, and 2% (w/w), followed by hydrolysis at 37 °C and pH 7.5 for 24 hours in an incubator (Binder BD 56, Germany). The enzymatic reaction was terminated through inactivation at 85 °C for 15 min using a hot plate (Thermo Scientific Cimarec, USA). The solution obtained from the hydrolysis was then centrifuged at 9,000 rpm for 15 minutes, and the obtained supernatant was evaporated at 40°C until the solvent is evaporated, this followed by freeze-drying at –50 °C for 24 hours. The resulting hydrolysate was packaged in sample bottles wrapped with aluminum foil and stored in a cool room at 4°C until used for subsequent experiments. The hydrolysate was also evaluated for its degree of hydrolysis (DH%)[26].

Protein Concentrate Isolation
The preparation of protein isolate was adapted from the method reported in previous research[31] with minor modifications. Seahorse protein concentrate was mixed with distilled water at a ratio of 1:15 (w/v), then the pH of the mixture was gradually adjusted to pH 11 using 35% NaOH with the assistance of a magnetic stirrer. The suspension was heated at 40 °C for 30 minutes, then centrifuged at 9,000 rpm for 15 minutes. The obtained supernatant was then gradually readjusted to pH 2.5, 3.0, and 3.5 using 1 M HCl, followed by re-centrifugation (same speed and duration). The formed precipitate was collected and dried using a freeze-dryer at –50 °C for 24 hours. The obtained protein isolate was packaged in sample bottles wrapped with aluminum foil and stored in a cool room at 4°C until further use.

Crude Taurine Extraction
The preparation of crude taurine was adapted from the method reported in previous research[32] with minor modifications. Seahorse flour samples were mixed with ethanol at a ratio of 1:20 (w/v), then extracted using an ultrasonication method (Wiggens WUC-D22H, Germany) with time variations of 30, 45, and 60 min at 100 W power and 40 kHz frequency. After treatment, the mixture was centrifuged at 10,000 rpm for 30 minutes, and the obtained supernatant was filtered using Whatman No. 1 filter paper. The filtrate was subsequently fractionated by evaporation at 40 °C for 30 minutes, then rinsed with distilled water (1:1 v/v) and the solution pH was adjusted to 3 using 1 M HCl.
A total of 5 mL of this solution was precipitated by adding 5 mL of ethanol, then the formed precipitate was separated and rinsed with 5 mL distilled water, followed by a second rinse with 3 mL ethanol. The solution was then stored at 4 °C in a refrigerator until white crystalline precipitate is formed. The formed crystals were filtered using Whatman No. 1 filter paper and dried using a freeze-dryer at –50 °C for 24 hours. The obtained crude taurine was packaged in sample bottles wrapped in aluminum foil and stored in a cool room at 4 °C until further use.

Determination of Chemical Composition
Seahorse flour and protein concentrate were analyzed for protein, fat, carbohydrate, moisture, ash, and fiber content according to AOAC methods[28]. Protein content was determined by the Kjeldahl method (AOAC 2001.11), fat content by Soxhlet extraction (AOAC 920.39), moisture content by oven drying (AOAC 925.10), ash content by muffle furnace incineration (AOAC 923.03), and carbohydrate content by difference. Seahorse flour was also tested for mineral content (phosphate, calcium, potassium, magnesium, sodium, iron, zinc) following standard procedures[28] using Atomic Absorption Spectrophotometry (AAS)

Determination of DPPH and Hydroxyl Free Radical Scavenging Activity
DPPH free radical scavenging activity was determined based on the method described in previous research[33]. Testing was performed using a microplate reader (BioTek Epoch 2, Winooski, VT, USA). The best hydrolysate, isolate, and crude taurine samples were serially diluted using the two-fold dilution method in a 96-well microplate with methanol as the solvent, resulting in a final concentration range of 100–3.125% (v/v) with a volume of 50 µL each. Each well was then supplemented with 80 µL DPPH solution (100 µg/mL) and incubated for 30 min under dark conditions at room temperature (25°C). The absorbance of the mixture was measured at a wavelength of 520 nm. DPPH radical scavenging activity was calculated using equation 1:

$$\text{Inhibition (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100 \quad \text{Equation 1}$$

Equation 2 was used to determine the IC_{50} value (50% inhibitory concentration) of each tested sample by setting the y value as 50 and solving for x as IC_{50}.

$$y = a + bx \quad \text{Equation 2}$$

Subsequently, hydroxyl radical scavenging activity was determined based on the method described in previous research[34] with minor modifications. Seahorse protein fraction samples were serially diluted using the two-fold dilution method in a 96-well microplate using 0.1 M sodium phosphate buffer (pH 7.4), resulting in final concentrations of 100–3.125% (v/v) with a volume of 50 µL. Separately, 3 mM 1,10-phenanthroline solution was dissolved in 0.1 M sodium phosphate

buffer (pH 7.4), 3 mM $FeSO_4$ was dissolved in distilled water, and 0.01% hydrogen peroxide solution was prepared separately. Each well containing 50 µL of protein fraction solution (buffer as control) was supplemented with 50 µL of 1,10-phenanthroline solution and 50 µL $FeSO_4$. The Fenton reaction was initiated by adding 50 µL of hydrogen peroxide solution, then the mixture was covered and incubated at 37 °C for 1 hour with shaking. Absorbance was measured using a spectrophotometer (Optima SP-300, Japan) at a wavelength of 536 nm every 10 min during incubation. A blank solution consisting of buffer (50 µL), 1,10-phenanthroline (50 µL), and $FeSO_4$ (50 µL) without hydrogen peroxide addition was used. Hydroxyl radical scavenging activity was calculated using the reaction rate equation (ΔA/min) as follows (equation 3):

Hydroxyl radical scavenging activity (%) =

$$\frac{(\Delta A/min)\ sample - (\Delta A/min)\ control}{(\Delta A/min)\ blank - (\Delta A/min)\ control} \times 100 \qquad \text{Equation 3}$$

Determination of Taurine Content

Taurine quantification was performed using a validated two-step analytical approach following the procedure described in previous research[35], with modifications. A calibration curve was established using taurine standards at seven concentration levels (1.01, 2.52, 4.04, 5.55, 7.06, 8.58, and 10.09 mg/L). Standards were prepared by diluting stock solution (100.90 mg/L) with distilled water to achieve final volumes of 10.00 mL. Standards were analyzed using an HPLC system (Shimadzu LC-20AT, Kyoto, Japan) equipped with a fluorescence detector (excitation 330 nm, emission 530 nm) and a reversed-phase C18 column (250 mm × 4.6 mm, 5 µm particle size). Each standard was injected in triplicate with an injection volume of 10 µL and a run time of 30 minutes. Taurine was detected at retention times 12.865 minutes (Figure 2).

The calibration curve demonstrated excellent linearity over the concentration range of 1.01–10.09 mg/L, with the regression equation y = 5,464,533.67x + 1,005,397.77 (R^2 = 0.9988, where y represents peak area and x represents taurine concentration in mg/L). The high coefficient of determination (R^2 = 0.9988) indicated strong linear correlation and reliability of the method for taurine quantification.

Subsequently, taurine concentration in seahorse flour extracts from each treatment method was determined using a UV-Vis spectrophotometer (Optima SP-300, Tokyo, Japan) at 340 nm. Samples were appropriately diluted with distilled water to ensure absorbance readings fell within the linear range of the calibration curve. Absorbance measurements were performed in triplicate using quartz cuvettes with 1 cm path length. Taurine concentration was calculated using the regression equation derived from the HPLC calibration curve. Taurine yield was expressed as milligrams per gram of sample (mg/g), calculated according to equation 4:

$$\text{Taurine yield } \left(\frac{mg}{g}\right) = \frac{m}{M} \qquad \text{Equation 4}$$

where *m* represents the mass of taurine (mg), and *M* denotes the mass of seahorse flour (g).

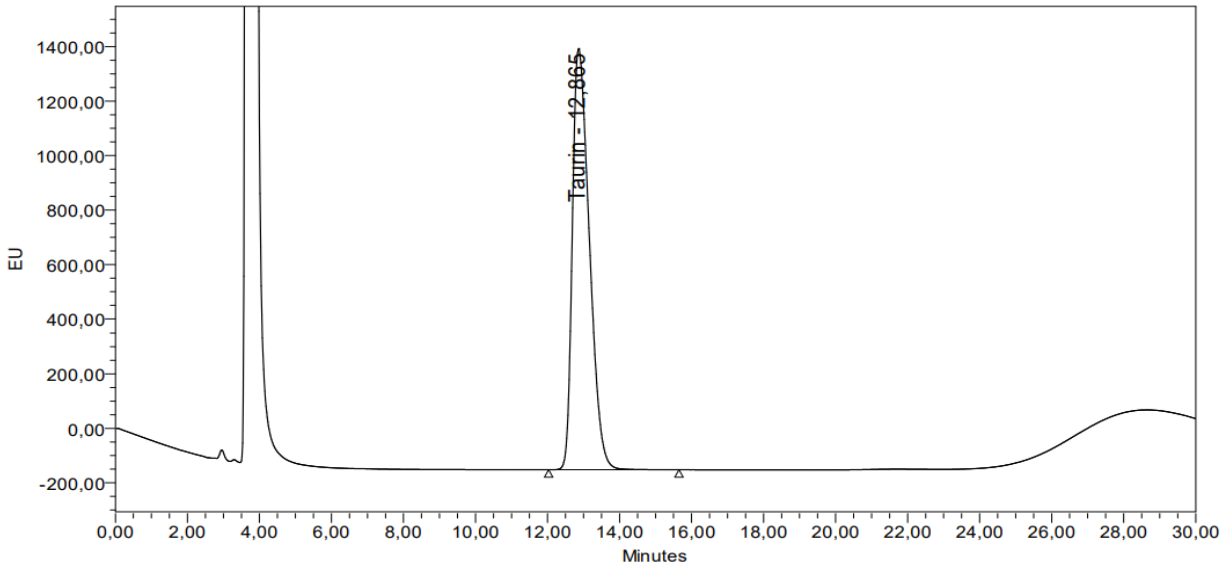

Figure 2: Representative chromatogram of taurine standard. All measurements were performed in triplicate.

Data analysis

Seahorse morphometrics, yield, and proportion, chemical composition of the seahorse flour and concentrate, and the amino acid content of the optimum hydrolysate, isolate, and crude taurine were analyzed descriptively. Subsequently, the experimental design used in this study was a non-factorial Completely Randomized Design (CRD) with the 3 treatment methods i.e. hydrolysate (enzyme concentration variations of 1%, 1.5%, and 2%), isolate (pH variations of 2.5, 3, 3.5), and crude taurine (time variations of 30, 45, 60 min) with 3 replications (mean ± SD). Furthermore, the optimum results from hydrolysate, isolate, and crude taurine were re-analyzed, and significant differences between the treatment methods) were tested at $p<0.01$ and $p<0.001$using Duncan's Multiple Range Test (DMRT) with the assistance of Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Pearson correlation and simple linear regression were used to explore the relationship between DPPH (IC_{50}), hydroxyl (IC_{50}), and taurine content. The normalized data were then analyzed using Principal Component Analysis (PCA) to visualize multivariate patterns and identify variables that contributed most in the treatment, including bar plots of mean ± SD with regression scatter plots and PCA biplots.

Results and Discussion

Morphology and Body Proportion of Seahorse

The seahorse (*Hippocampus* sp.) used in this study had an average weight of 16.71 g and length of 15.08 cm (Table 1). Generally, adult seahorses in Southeast Asian waters measure 14–17 cm in length[1], thus the samples used can be categorized as adult individuals. This size is also relatively larger compared to *Hippocampus bargibanti*, which averages 8–11 cm in length[2]. The morphology and proportions of the seahorse used are presented in Figure 3 and Table 1. Seahorses exhibit distinctive morphology characterized by a horse-like head, elongated tubular snout, and body protected by bony rings.

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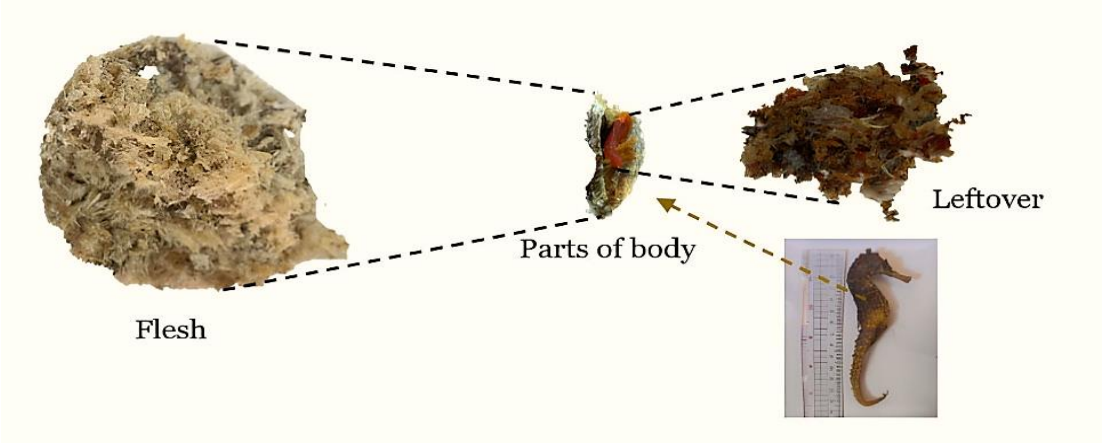

Figure 3: Seahorse preparation

Table 1: Morphometric characteristics and body composition of seahorses (*Hippocampus* sp.)

Morphometrics	Value
Body length (cm)	15.08 ± 0.34
Abdomen width (cm)	4.88 ± 0.62
Head width (cm)	2.33 ± 0.29
Number of Pectoral fins	16 ± 0.00
Number of Dorsal fins	17 ± 0.00
Whole body weight (g)	16.71 ± 1.52
Body component	Weight (g)
Flesh	15.69 ± 1.28
Leftover	0.89 ± 0.46
Total	16.58 ± 1.74

This structure makes the body appear rigid, yet remains flexible in the tail region. The prehensile tail functions to grasp substrates such as seagrass or soft corals as an adaptation to water currents. A single dorsal fin is located on the dorsal section, while paired pectoral fins are positioned on the sides of the head and play an important role in maintaining balance and body movement. Morphometric measurements showed body length of 15.08 cm, an abdomen width of 4.88 cm, and a head width of 2.33 cm. The number of pectoral fins was recorded at 16, while dorsal fins numbered 17. Individual whole weight reached 16.71 g, indicating medium size within the normal range for this species[3]. The body proportion of seahorse consisted of flesh and leftover at a ratio of 18:1 (w/w). Flesh was the dominant component, reaching 94.63% of total body weight, while leftover accounted for only 5.37%. The dominance of seahorse flesh biomass (94.63%) emphasizes the higher body conversion efficiency of seahorse compared to hard-shelled organisms such as crabs or shrimp, making it more prospective as a functional food ingredient[4,5].

Chemical Composition of Seahorse Flour and Protein Concentrate

The yield of seahorse flour was calculated at 95.24% (Table 2), based on the percentage of dried product obtained after lyophilization and milling, while protein concentrate yield was the percentage obtained after protein concentrate lyophilization..

Table 2: Chemical composition and yield of seahorse flour and protein concentrate

Proximate composition	Seahorse flour	Protein concentrate
Moisture (%wb)	9.27 ± 0.25	8.40 ± 0.01
Ash (%db)	27.05 ± 0.73	27.56 ± 1.11
Fat (%db)	3.16 ± 0.15	0.75 ± 0.03
Protein (%db)	55.64 ± 0.63	63.18 ± 0.24
Carbohydrate (%db)	4.88 ± 1.39	8.51 ± 1.38
Mineral content		
Phosphate (%)	0.21 ± 0.02	-
Calcium (%)	0.04 ± 0.01	-
Potassium (%)	0.33 ± 0.02	-
Magnesium (%)	0.11 ± 0.02	-
Sodium (%)	0.14 ± 0.01	-
Iron (ppm)	82.60 ± 0.55	-
Zinc (ppm)	106.23 ± 0.08	-
Yield (%)	95.24 ± 0.45	90.20 ± 0.12

- = not determined; wb= wet basis; db= dry basis

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Only 4.76% weight loss occurred due to moisture content and the milling process. Proximate analysis showed that seahorse flour contained 9.27% moisture, 27.05% ash, 3.16% fat, 55.64% protein, and 4.88% carbohydrate, as well as minerals including phosphate (0.21%), calcium (0.04%), potassium (0.33%), magnesium (0.11%), sodium (0.14%), iron (82.60 ppm), and zinc (106.23 ppm). Protein concentrate contained 8.40% moisture, 27.56% ash, 0.75% fat, 63.18% protein, and 8.51% carbohydrate The proximate chemical composition of seahorse flour was characterized by higher moisture and fat content, while protein concentrate was characterized by higher protein and carbohydrate content. The protein content in flour (55.64%) and protein concentrate (63.18%) in this study is considered high and within a competitive range compared to other marine biota such as sea cucumber (60–70%). This value also exceeds several plant protein products such as legumes (15–50.4%), thereby strengthening the prospect of seahorse as a functional protein raw material[26]. The differences in seahorse chemical composition are also caused by differences in function and extraction processes. Therefore, this high protein contents can be isolated to enhance its functional properties and implications for physical stamina in the forms of hydrolysate, isolate, and crude taurine.

Scaled-Up hydrolysates, isolate, and crude-taurin bioactivities

The yield from the seahorse protein hydrolysate and isolate groups were calculated as the percentage of product obtained after lyophilization and milling of protein concentrate, while crude taurine yield was the percentage obtained after lyophilization and milling of seahorse flour. The results showed the highest hydrolysate yield at 2% enzyme concentration of 45.18%, but was not significant at $p>0.01$. The highest isolate yield was at pH 3.5 of 14.21%, but not different from pH 3 at 13.95% ($p<0.01$), and the highest crude taurine yield at 30 min extraction time was 8.7% ($p<0.01$) (Figure 4). Subsequently, the analysis showed that each treatment method had significant differences in DPPH free radical scavenging (Figure 5).

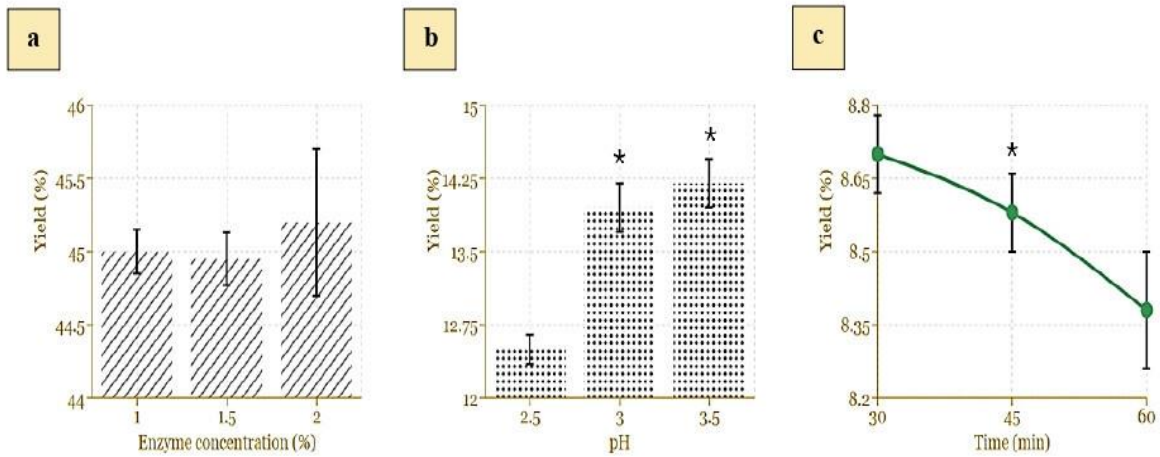

Figure 4: Effect of (a) enzyme concentration in hydrolysate production, (b) pH effect in isolate production, and (c) extraction time effect in crude taurine production on yield; * significant difference ($p<0.01$).

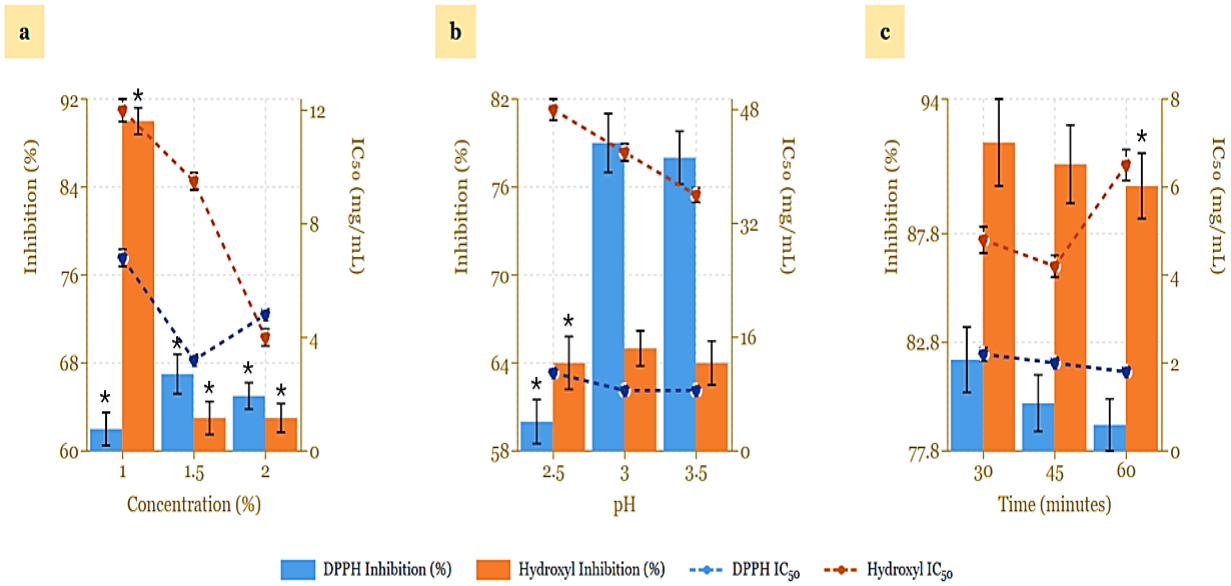

Figure 5: Effect of (a) enzyme concentration in hydrolysate production, (b) pH effect in isolate production, and (c) extraction time effect in crude taurine production on DPPH and hydroxyl radical scavenging presented in boxplot form; * significant difference ($p<0.01$).

In hydrolysate, 1.5% enzyme concentration provided the optimum activity with DPPH inhibition value of 86.86% and IC_{50} of 3.42 mg/mL compared to 1% concentration (IC_{50} 7.01 mg/mL) or 2% (IC_{50} 3.88 mg/mL) ($p<0.01$). However, a different trend was observed in hydroxyl free radical scavenging where 2% enzyme concentration provided the optimum activity with hydroxyl inhibition value of 83.24% and IC_{50} of 1.48 mg/mL, followed by 1.5% concentration (IC_{50} 9.29 mg/mL) and 1% concentration (IC_{50} 11.75 mg/mL) ($p<0.01$).

The optimum 1.5% enzyme concentration in the hydrolysate production is thought to produce bioactive peptides with medium chain length (2-20 amino acid residues) that effectively interact with free radicals through hydrogen donation and electron transfer mechanisms[36,37], but did not significantly increase DPPH activity. However, over-hydrolysis at 2% concentration may produce peptides that are too small (dipeptides or tripeptides) that lose important conformational structures for antioxidant activity, although still showing good performance in hydroxyl radical scavenging (IC_{50} 1.48 mg/mL). The different trends between DPPH and hydroxyl tests indicate that shorter peptides at 2% enzyme concentration have better mobility and accessibility to hydroxyl radicals and are more reactive, while peptides with medium molecular weight (1.5% enzyme concentration) are more effective at scavenging the more stable DPPH radicals that require more complex structural interactions[34,40]

In the protein isolate, pH of 3 showed the most optimum results with DPPH inhibition of 78.22% and IC_{50} of 9.55 mg/mL,This was followed by pH 3.5 (IC_{50} 10.17 mg/mL), which was significantly different from pH 2.5 (IC_{50} 31.47 mg/mL; $p<0.01$). The same trend was observed in hydroxyl radical test where pH value of 3 provided the best activity with hydroxyl inhibition value of 66.21% and IC_{50} of 39.41 mg/mL, which was not significantly different from pH 3.5 (IC_{50} 37.27 mg/mL) but significantly different ($p<0.01$) from pH 2.5 (IC_{50} 46.67 mg/mL).

pH value of 3 proved to be optimum, and presumed to be near the isoelectric point of seahorse protein, facilitating optimal protein precipitation while maintaining tertiary and quaternary structures which is important for antioxidant activity[41]. Excessive acidic pH (pH 2.5) can damage peptide stability and cause excessive denaturation and partial peptide hydrolysis, altering the conformation of amino and thiol groups responsible for radical scavenging activity, thereby significantly reducing antioxidant capacity[42,43]. This condition aligns with recent findings that protein structural stability at pH near the isoelectric point is crucial for antioxidant bioactivity through preservation of aromatic amino acid residues (Tyr, Trp, Phe) and sulfur residues (Cys, Met) that act as electron donors[44,45].

In the crude taurine, 30 min extraction time gave the highest activity with 88.42% DPPH inhibition and IC_{50} of 2.01 mg/mL, which was significantly different from 45 min (i.e. IC_{50} of 2.35 mg/mL) and 60 min (IC_{50} of 2.37 mg/mL) at $p<0.01$. The same trend was observed in the hydroxyl radical test where 30 min gave the optimum activity with hydroxyl inhibition value of 92.56% and IC_{50} of 4.15 mg/mL, which was not significantly different from 45 min (IC_{50} of 4.02 mg/mL) but significantly different ($p<0.01$)from 60 min (IC_{50} of 6.91 mg/mL).

Crude taurine with 30 min extraction time is superior because taurine is presumed to be extracted with more optimal structural conformation, which directly neutralizes hydroxyl radicals through electron donation mechanisms from sulfonate ($-SO_3^-$) and amino ($-NH_2$) groups[46,47]. Longer extraction time (60 min) is thought to cause partial thermal degradation and taurine oxidation, reducing bioavailability and direct reactivity toward reactive oxygen species[48,49]. Free taurine has greater bioavailability and reactivity compared to protein-bound forms, enabling more efficient radical scavenging through direct interaction without requiring prior enzymatic digestion[50,57].

Thus, the optimum treatment (hydrolysate at 1.5% enzyme concentration, protein isolate at pH value of 3, crude taurine at 30 min) were re-evaluated for taurine content and its correlation with antioxidant activity. This was to determine the most influential factor regarding implications for physical stamina.

Taurine Content and Antioxidant Activity of the optimal values of the protein hydrolysate, protein Isolate, and Crude Taurine

ANOVA results showed that the various treatments had significant effect on taurine content ($p<0.001$) (Table 3), with the treatments contributing 99.85% of total variation. Duncan's post-hoc test confirmed that all groups were significantly different ($p<0.001$). Crude taurine produced the highest taurine content of 9.56 mg/g, followed by hydrolysate at 4.83 mg/g, and isolate had the lowest taurine content at 3.15 mg/g (Table 4).

Table 4: Duncan's analysis for taurine yield from the optimal protein fraction treatment methods

Treatment	Taurine ± SD (mg/g)	Grouping
Crude taurine[x]	9.56 ± 0.19	a
Protein Hydrolysate[y]	4.83 ± 0.12	b
Protein isolate[z]	3.15 ± 0.04	c

[x] the best treatment was 30 minutes extraction time; [y] the best treatment was 1.5% enzyme concentration; [z] the best treatment was pH 3; values in the column followed by different letters (a, b, c) indicate significant differences at the significance level of $p<0.001$.

Table 3: Analysis of Variance results for taurine yield from the optimal hydrolysate, isolate, and crude taurine treatments methods

Source of variance	Sum of square	Df	Mean square	F value	P value
Treatment	66.337	2	33.169	1949.82	<0.001***
Residual	0.102	6	0.017		
Total	66.439	8			

$R^2 = 0.9985$ indicates that 99.85% of the variation in taurine content is explained by treatment type. ***$p<0.001$ indicates highly significant differences among treatments.

Taurine is a sulfur-containing amino acid that plays an important role in osmotic regulation, antioxidant activity, cardiovascular protection, and has potential to support physical stamina enhancement. Overall, ANOVA analysis showed that crude taurine had a highly significant effect, followed by hydrolysate and isolate ($p<0.001$), where the taurine content in crude taurine was higher at 9.56 mg/g compared to hydrolysate at 4.83 mg/g (DH 81.76% ± 0.08) and isolate at 3.15 mg/g (Table 4). The taurine content in this study is higher than beef liver reported by previous studies at 3.22-7.12 mg/g[56,35]. It is even higher than red algae (*Porphyra yezoensis*) reported in previous studies at 8.6-12.1[35] mg/g, but comparable to soft coral reported at 8.9-10.3 mg/g[57]. These results prove that crude taurine provides the highest bioactivity compared to hydrolysate or isolate, characterized by high taurine content and strong antioxidant activity. This aligns with the observed lower IC_{50} values (IC_{50} DPPH 2.01 mg/mL and hydroxyl 4.15 mg/mL) of crude taurine compared to the hydrolysate or isolate (Figure 5). These results confirm that crude taurine had the highest antioxidant capacity, followed by hydrolysate, while the isolate was the lowest. The IC_{50} value of crude taurine (<5 mg/mL) falls within the category of very strong antioxidant activity. Conversely, isolate showed the lowest activity, while hydrolysate was at intermediate activity. Based on

established criteria for potent antioxidants[58,59], this indicates high effectiveness in functional applications.

Pearson correlation analysis showed significant relationships for all three treatment's (hydrolysate, isolate, and crude taurine) n taurine content and IC_{50} values of DPPH and hydroxyl free radical scavenging (Figure 6). The correlation of taurine content with DPPH free radicals was $R^2 = 0.510$, the correlation of taurine content with hydroxyl radicals was $R^2 = 0.568$, and the correlation between DPPH and hydroxyl radical scavenging was $R^2 = 0.9986$ ($p<0.01$), indicating that higher taurine content results in stronger antioxidant activity, although still influenced by other factors presumed to be the type and content of other amino acids contained in different fractions[44].

A moderate positive correlation was observed between taurine content and DPPH free radical scavenging activity ($R^2 = 0.510$, $p<0.01$), while a slightly stronger correlation was found in hydroxyl radical scavenging activity ($R^2 = 0.568$, $p<0.01$). These moderate correlation coefficient values indicate that taurine makes a substantial contribution to antioxidant activity, although other bioactive compounds present in the samples also play important roles. The moderate R^2 values (0.510 and 0.568) indicate that approximately 51% and 57% of the variation in DPPH and hydroxyl radical scavenging activity respectively, can be explained by taurine content. The remaining variation (approximately 43-49%) can be attributed to other factors, including the presence and concentration of other amino acids, peptides with varying chain lengths, and synergistic or antagonistic interactions among these components[52,53]. This is particularly evident in hydrolysate and isolate, where complex mixtures of bioactive peptides and amino acids collectively contribute to overall antioxidant capacity beyond taurine contribution alone.

Interestingly, a very strong correlation ($R^2=0.9986$, $p<0.01$) was observed between DPPH and hydroxyl radical scavenging activities, showing excellent consistency between both test methods in evaluating antioxidant capacity. This high correlation validates the reliability of antioxidant testing and confirms that samples with strong DPPH radical scavenging activity also demonstrate optimal hydroxyl radical scavenging capacity. Thus, the very high positive correlation between DPPH and hydroxyl radical scavenging demonstrates the consistency of both methods in evaluating antioxidant activity.

PCA analysis also showed clear separation of hydrolysate, isolate, and crude taurine, where crude taurine clustered on PC1 due to the contribution of high taurine content and stronger antioxidant activity. The distribution of hydrolysate, crude taurine, isolate, and protein concentrate was visualized, with PC1 and PC2 explaining 88.4% and 11.6% of total variance, respectively (Figure 6). These results were also supported by PCA analysis showing clear separation between fractions, with crude taurine clustering on PC1 due to the contribution of high taurine content and strong antioxidant activity (low IC_{50} values).

Therefore, taurine content is a key factor distinguishing the antioxidant activity of the three fractions. The negative relationship between taurine content and IC_{50} values in both DPPH and hydroxyl tests confirms that taurine plays a direct role in antioxidant activity.

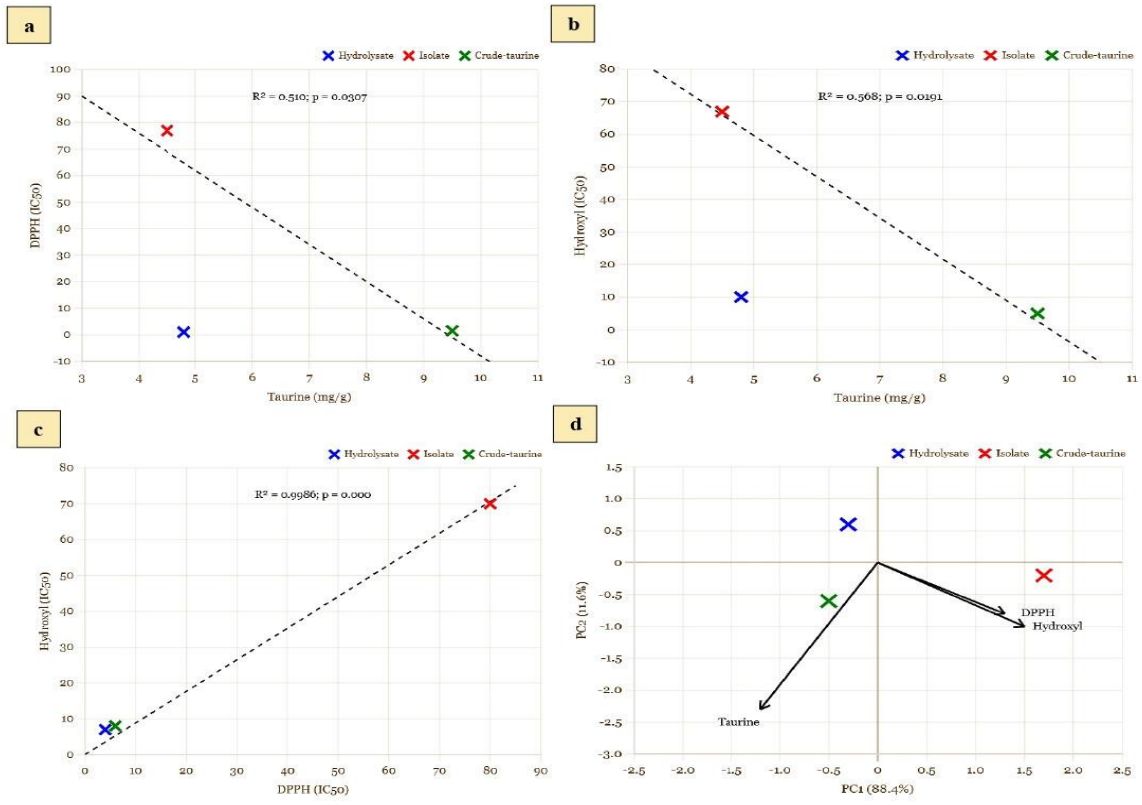

Figure 6: Correlation analysis and multivariate comparison of taurine content and antioxidant activity in optimal seahorse protein fractions. The optimal treatments evaluated were: hydrolysate (1.5% alcalase), isolate (pH 3.0), and crude taurine (30 min ultrasonication). (a) Correlation between taurine content and DPPH (IC_{50}); (b) Correlation between taurine content and hydroxyl (IC_{50}); (c) Correlation between DPPH and hydroxyl (IC_{50}); (d) Principal Component Analysis (PCA) biplot showing distribution of hydrolysate, isolate, and crude taurine.

These findings strengthen the hypothesis that taurine can function as the main bioactive molecule in supporting redox balance and metabolic health[3,5,32,46-51]. Although hydrolysate also shows intermediate activity, its contribution cannot be ignored. Enzymatic hydrolysis is known to produce short-sized peptides with bioactive activity, including antioxidants[60,61]. Thus, both crude taurine and hydrolysate have potential for development, although crude taurine is more prominent in antioxidant activity.

Conclusion

The findings of this study demonstrate that seahorse is not only a valuable protein source but also a rich natural source of bioactive taurine with significant antioxidant properties. The superior performance of crude taurine, characterized by the highest taurine content (9.56 mg/g) and strongest antioxidant activity (IC_{50} DPPH = 2.01 mg/mL; IC_{50} hydroxyl = 4.15 mg/mL), positions it as the most promising fraction for development as a functional food ingredient. The moderate correlations between taurine content and antioxidant activity (R^2 = 0.51–0.57) suggest that while taurine is the primary bioactive component, synergistic effects with other bioactive peptides and amino acids may contribute to the overall antioxidant capacity. These results provide a scientific foundation for utilizing seahorse-derived taurine in stamina-enhancing functional foods. However, further research is needed to explore the physiological mechanisms through in vivo studies, particularly examining the effects on physical endurance, exercise performance, and metabolic responses. Additionally, bioavailability studies and clinical trials are warranted to validate the practical applications of seahorse-derived taurine in human nutrition and sports supplementation. Future prospects include developing commercial products such as energy drinks and stamina supplements with optimized formulations, advancing green extraction technologies to improve taurine yield and sustainability, investigating molecular mechanisms underlying its physiological effects, establishing sustainable aquaculture systems for ethical sourcing, and standardizing analytical methods for regulatory compliance. This research paves the way for innovative marine bioactive-based products that can benefit athletes, active individuals, and the general population seeking enhanced stamina and antioxidant protection

Conflict of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Wang L, Xie Z, Wu MC, Chen Y, Wang X, Li X, Liu F. The role of taurine through endoplasmic reticulum in physiology and pathology. Biochem Pharmacol. 2024; 226: 116386. https://doi.org/10.1016/j.bcp.2024.116386
2. Maskur MA, Prihanto AA. Taurine from aquatic resources: extraction and functional properties. In: Prihanto AA, Trowulan E, Maskur M, Nurdiani R, Bahari A, Firdaus M. Aquatics products as functional food sources. 2023; 22-40. https://doi.org/10.11594/futscipress22m
3. Tzang C, Lin W, Lin L, Lin T, Chang K, Wu W, Ozcakar L. Insights into the cardiovascular benefits of taurine: a systematic review and meta analysis. Nutr J. 2024; 23(1): 93. https://doi.org/10.1186/s12937-024-00996-4
4. Luo H, Geng CJ, Miao SM, Wang LH, Li Q. Taurine attenuates the damage of lupus nephritis mouse via inactivation of the NF-κB pathway. Ann Palliat Med. 2021; 10(1): 310-318. https://doi.org/10.21037/apm-20-2265
5. Yachou Y, El Idrissi A. Taurine role in neuroprotection. In: Oliveira MRD. Natural molecules in neuroprotection and neurotoxicity. Academic Press. 2024: 677-696. https://doi.org/10.1016/B978-0-443-23763-8.00065-8
6. Duttagupta S, Roy NK, Dey G. Efficacy of amino acids in sports nutrition review of clinical evidences. Food Res Int. 2024; 187: 114311. https://doi.org/10.1016/j.foodres.2024.114311
7. Tramonti A, Contestabile R, Florio R, Nardella C, Barile A, Salvo MLD. A novel, easy assay method for human cysteine sulfinic acid decarboxylase. Life (Basel). 2021; 11(5): 438. https://doi.org/10.3390/life11050438
8. Vitvitsky V, Garg SK, Banerjee R. Taurine biosynthesis by neurons and astrocytes. J Biol Chem. 2011; 286(37): 32002-32010. https://doi.org/10.1074/jbc.M111.253344
9. Sepulveda-Lizcando L, Arenas-Villamizar VV, Jaimes-Duarte EB, Gracia-Pacheco H, Paredes CS, Bermudez V, Rivera-Porras D. Metabolic adverse effects of psychotropic drug therapy: a systematic review. Eur J Investig Health Psychol Educ. 2023; 13(8): 1505-1520. https://doi.org/10.3390/ejihpe13080109
10. Silveira RB, Vidal MD, Silva JRSS. Magnitude of bycatch of Hippocampus patagonicus, an endangered species, in trawl fisheries in Southeast and South Brazil. Front Mar Sci. 2023; 10: 1151029. https://doi.org/10.3389/fmars.2023.1151029
11. Koning S, Hoeksema BW. Diversity of seahorse species (*Hippocampus* spp.) in the international aquarium trade. Diversity. 2021; 13(187): 1-37. https://doi.org/10.3390/d13050187
12. Boehm JT, Bovee E, Harris SE, Eddins K, Akahoho I, Foster M, Pell SK, Hickerson MJ, Amato G, DeSalle R, Waldman J. The United States dried seahorse trade: a comparison of traditional Chinese medicine and e-commerce curio markets using molecular identification. PLoS One. 2023; 18(10): e0292309. https://doi.org/10.1371/journal.pone.0292309
13. Vincent AC, Foster SJ, Koldewey HJ. Conservation and management of seahorses and other Syngnathidae. J Fish Biol. 2011; 78(6): 1681-1724. https://doi.org/10.1111/j.1095-8649.2011.02958.x
14. Latif S, Choi SH, Gyawali A, Hyeon SJ, Kang YS, Ryu H. Antioxidant and neuroprotective effects of paeonol against oxidative stress and altered carrier-mediated transport system on NSC-34 cell lines. Antioxidants (Basel). 2022; 11(7): 1392. https://doi.org/10.3390/antiox11071392
15. Lin Q, Lin J, Wang C. Biochemical composition of the wild and cultured seahorses, *Hippocampus kuda bleeker* and *Hippocampus trimaculatus leach*. Aquac Res. 2009; 40(6): 710-719. https://doi.org/10.1111/j.1365-2109.2008.02150.x
16. Yang F, Yang Y, Xiao D, Kim P, Lee J, Jeon Y-J, Wang L. Anti-Photoaging Effects of Antioxidant Peptide from Seahorse (Hippocampus abdominalis) in In Vivo and In Vitro Models. Mar Drugs. 2024; 22(10): 471. https://doi.org/10.3390/md22100471
17. Xu Y, Ryu S, Lee YK, Lee HJ. Brassicasterol from edible aquacultural *Hippocampus abdominalis* exerts an anti-cancer effect by dual-targeting AKT and AR signaling in prostate cancer. Biomedicines. 2020; 8(9): 370. https://doi.org/10.3390/biomedicines8090370
18. Ghasemi A, Ghavimi R, Momenzadeh N, Hajian S, Mohammadi M. Characterization of antitumor activity of a synthetic moronecidin-like peptide computationally predicted from the tiger tail seahorse *Hippocampus comes* in tumor-bearing mice. Int J Pept Res Ther. 2021; 27: 2391-2401. https://doi.org/10.1007/s10989-021-10264-w

19. Cabral AE, Rey F, Domingues MR, Cabral M, Planas M, Palma J, Calado R. Fatty acid profiles of cultured *Hippocampus trunk muscles* and potential nutritional value. Front Mar Sci. 2023; 10: 1120744. https://doi.org/10.3389/fmars.2023.1120744
20. Sari RI, Sartika RAD. Factors associated with nutritional status of adolescents age 12-15 years in Indonesia (secondary data analysis of RISKESDAS 2007). KnE Life Sci. 2019; 4(10): 359-366. https://doi.org/10.18502/kls.v4i10.3806
21. Aisyah RD, Suparni S, Subowo E. Empowerment of SMART Stunting Cadres (prevention and detection in prospective brides, pregnant women, and toddlers). Community Dev J. 2024; 5(3): 434-445. https://doi.org/10.33024/cdj.v5i3.16014
22. Kustanto A, Rachmat O, Setyadi S. The prevalence of stunting in Indonesia: an examination of the health, socioeconomic status, and environmental determinants. J Iran Med Counc. 2025; 8(1): 67-79. https://doi.org/10.58209/jimc.8.1.67
23. Nurbaiti N, Safitri SI, Sari YI. The relationship between dietary diversity and the risk of stunting in young children in Indonesia. Int J Health Med Res. 2024; 3(6): 368-373. https://doi.org/10.62711/ijhmr.v3i6.259
24. Sanggelorang Y, Sebayang FAA, Malonda NSH, Rumayar AA. Insights into childhood malnutrition: an analysis on food vulnerability and stunting using 2021 Indonesian nutritional status survey data. Media Gizi Indonesia. 2024; 19(3): 282-290. https://doi.org/10.20473/mgi.v19i3.282-290
25. Mundijo T, Suyatna FD, Wibowo AE, Supriyono A, Midoen YH. Characterization of seahorse (*Hippocampus comes* L.) extract originated from cultured and nature from Pesawaran, Lampung, Indonesia. J Adv Vet Anim Res. 2022; 9(4): 610-616. https://doi.org/10.5455/javar.2022.i630
26. Guo Z, Lin D, Guo J, Zhang T, Zheng B, Zheng Y, Cheng K, Zeng H. In vitro antioxidant activity and in vivo anti-fatigue effect of seahorse (*Hippocampus*) peptides. Molecules. 2017; 22(3): 482. https://doi.org/10.3390/molecules22030482
27. Karnila R, Diharmi A, Putra HS, Iriani D, Hasan B, Avissa ME, Syahada M. Identification of protein types and molecular weight of mangrove snail (*Telescopium telescopium*) isolate at different pH. Trop J Nat Prod Res. 2025; 9(8): 3551-3555. https://doi.org/10.26538/tjnpr/v9i8.17
28. AOAC. Official methods of analysis of AOAC International. 18th ed. Washington DC: AOAC International; 2005.
29. Kumoro AC, Wardhani DH, Kusworo TD, Djaeni M, Ping TC, Azis YMF. Fish protein concentrate for human consumption: a review of its preparation by solvent extraction methods and potential for food applications. Ann Agric Sci. 2022; 67(1): 42-59. https://doi.org/10.1016/j.aoas.2022.05.003
30. Vazquez JA, Sotelo CG, Sanz N, Perez-Martin RI, Rodriguez-Amado I, Valcarcel J. Valorization of aquaculture by-products of salmonids to produce enzymatic hydrolysates: process optimization, chemical characterization and evaluation of bioactives. Mar Drugs. 2019; 17(12): 676. https://doi.org/10.3390/md17120676
31. Voron'ko NG. Properties of protein isolates from marine hydrobionts obtained by isoelectric solubilisation/precipitation: influence of temperature and processing time. Int J Mol Sci. 2022; 23(22): 14221. https://doi.org/10.3390/ijms232214221
32. Wang F, Guo XY, Zhang DN, Wu Y, Wu T, Chen ZG. Ultrasound-assisted extraction and purification of taurine from the red algae Porphyra yezoensis. Ultrason Sonochem. 2015; 24: 36-42. https://doi.org/10.1016/j.ultsonch.2014.12.005
33. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004; 26(2): 211-219.
34. Girgih AT, Udenigwe CC, Aluko RE. In vitro antioxidant properties of hemp seed (*Cannabis sativa* L.) protein hydrolysate fractions. J Am Oil Chem Soc. 2011; 88: 381-389. https://doi.org/10.1007/s11746-010-1686-7
35. Guo Z, Ge X, Han L, Yang L, Yu QL. Ultrasound-assisted extraction and purification of taurine from bovine liver. J Food Compos Anal. 2020; 90: 103495. https://doi.org/10.1016/j.jfca.2020.103495
36. Chakrabarti S, Guha S, Majumder K. Food-derived bioactive peptides in human health: challenges and opportunities. Nutrients. 2018; 10(11): 1738. https://doi.org/10.3390/nu10111738
37. Ashaolu TJ. Immune-boosting functional foods and their mechanisms: a critical evaluation of probiotics and prebiotics. Biomed Pharmacother. 2020; 130: 110625. https://doi.org/10.1016/j.biopha.2020.110625
38. Sila A, Bougatef A, Balti R, Martinez-Alvarez O, Nedjar-Arroume N, Moncef N, Bougatef A. Biochemical and antioxidant properties of peptidic fraction of carotenoproteins generated from shrimp by-products by enzymatic hydrolysis. Food Chem. 2014; 148: 445-452. https://doi.org/10.1016/j.foodchem.2013.10.096
39. Harnedy PA, FitzGerald RJ. Extraction and enrichment of protein from red and green macroalgae. In: Stengel DB, Connan S. Natural products from marine algae. New York: Humana Press; 2015: 103-108. https://doi.org/10.1007/978-1-4939-2684-8_6
40. Zhu Y, Lao F, Pan X, Wu J. Food protein-derived antioxidant peptides: molecular mechanism, stability and bioavailability. Biomolecules. 2022; 12(11): 1622. https://doi.org/10.3390/biom12111622
41. Karnjanapratum S, Benjakul S. Cryoprotective and antioxidative effects of gelatin hydrolysate from unicorn leatherjacket skin. Int J Refrig. 2015; 49: 69-78. https://doi.org/10.1016/j.ijrefrig.2014.10.011
42. Chai L, Shi W, Tan Y, Che X, Lu J, Bai B, Zhang C. Effect of pH-shifted compound heating treatment on the structure and properties of walnut protein isolate. Foods. 2025; 14(10): 1754. https://doi.org/10.3390/foods14101754
43. Liu Y, Li Y, Ke Y, Li C, Zhang Z, Wu Y, Zhu X, Yang Y. Effects of pH on the structure, antioxidant activity and emulsifying properties of egg white protein. Food Hydrocoll. 2023; 134: 108012. https://doi.org/10.1016 /j.foodhyd.2022.108012
44. Wang X, Yu H, Xing R, Li P. Characterization, preparation, and purification of marine bioactive peptides. Biomed Res Int. 2017; 2017: 9746720. https://doi.org/10.1155/2017/9746720
45. Ren Y, Huang L, Zhang Y, Li H, Zhao D, Cao J, Liu X. Application of molecular modification techniques for the improvement of the functional properties of egg white protein. Foods. 2024; 13(2): 224. https://doi.org/10.3390/foods13020224
46. Schaffer S, Kim HW, Azuma J. Mechanisms underlying taurine protection against ROS-induced cell damage. Adv Exp Med Biol. 2010; 643: 305-316. https://doi.org/10.1007/978-0-387-75681-3_31
47. De Luca A, Pierno S, Camerino DC. Taurine: the appeal of a safe amino acid for skeletal muscle disorders. J Transl Med. 2015; 13: 243. https://doi.org/10.1186/s12967-015-0610-1
48. Jong CJ, Ito T, Mozaffari M, Azuma J, Schaffer S. Effect of β-alanine treatment on mitochondrial taurine level and 5-taurinomethyluridine content. J Biomed Sci. 2010; 17(Suppl 1): S25. https://doi.org/10.1186/1423-0127-17-S1-S25
49. Bae M, Ahmed K, Yim JE. Beneficial effects of taurine on metabolic parameters in animals and humans. J Obes Metab Syndr. 2022; 31(1): 37-45. https://doi.org/10.7570/jomes21088

50. Hansen SH, Andersen ML, Cornett C, Gradinaru R, Grunnet N. A role for taurine in mitochondrial function. J Biomed Sci. 2010; 17(Suppl 1): S23. https://doi.org/10.1186/1423-0127-17-S1-S23
51. Xu M, Yao J, Shi Y, Yin H, Lu Y, Feng Z. Taurine supplementation might benefit exercise through antioxidant and anti-inflammatory mechanisms. Appl Sci. 2023; 13(3): 1648. https://doi.org/10.3390/app13031648
52. Sarmadi BH, Ismail A. Antioxidative peptides from food proteins: a review. Peptides. 2010; 31(10): 1949-1956. https://doi.org/10.1016/j.peptides.2010.06.020
53. Chalamaiah M, Yu W, Wu J. Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: a review. Food Chem. 2018; 245: 205-222. https://doi.org/10.1016/j.foodchem.2017.10.087
54. Jong CJ, Azuma J, Schaffer S. Mechanism underlying the antioxidant activity of taurine: prevention of mitochondrial oxidant production. Amino Acids. 2012; 42(6): 2223-2232. https://doi.org/10.1007/s00726-011-0962-7
55. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci USA. 2006; 103(8): 2653-2658. https://doi.org/10.1073/pnas.0511154103
56. Ma G, Sun N, Yu Q, Han L, Wu L, Cao H, Han G. Characterization of separation and purification technology and identification of taurine from the bovine liver. Food Anal Methods. 2018; 11: 415-425. https://doi.org/10.1007/s12161-017-1005-y
57. Chen WC, Kuo YH, Cheng CY, Hsu MJ, Lin PY, Lin YC, Wang MY, Kao SH, Lin CC. Isolation and identification of bioactive peptides from soft coral (*Sarcophyton glaucum*) with antioxidant and anti-inflammatory properties. Mar Drugs. 2022; 20(3): 186. https://doi.org/10.3390/md20030186
58. Lee HG, Nagahawatta DP, Yang F, Jayawardhana HHACK, Liyanage NM, Lee DS, Lee JM, Yim MJ, Ko SC, Kim JY, An H, Baek K, Jeon YJ, Kim HS. Antioxidant potential of hydrolysate-derived seahorse (*Hippocampus abdominalis*) peptide: protective effects against AAPH-induced oxidative damage in vitro and in vivo. Food Chem. 2023; 407: 135113. https://doi.org/10.1016/j.foodchem.2022.135113
59. Xu Y, Yang Y, Ma CM, Bian X, Liu XF, Wang Y, Chen FL, Wang B, Zhang G, Zhang N. Characterization of the structure, antioxidant activity and hypoglycemic activity of soy (*Glycine max* L.) protein hydrolysates. Food Res Int. 2023; 173(Pt 2): 113473. https://doi.org/10.1016/j.foodres.2023.113473
60. Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.
61. Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl Altern Med. 2014; 11(3):221-227.
62. Tadesse SA, Emire SA. Production and processing of antioxidant bioactive peptides: a driving force for the functional food market. Heliyon. 2020; 6(8): e04765. https://doi.org/10.1016/j.heliyon.2020.e04765
63. Gonzalez-Osuna MF, Bernal-Mercado AT, Wong-Corral FJ, Ezquerra-Brauer JM, Soto-Valdez H, Castillo A, Rodriguez-Figueroa JC, Del-Toro-Sanchez CL. Bioactive peptides and protein hydrolysates used in meat and meat products' preservation a review. ACS Food Sci Technol. 2024; 4(5): 1003-1016. https://doi.org/10.1021/acsfoodscitech.3c00664