Journal of Food Science

ORIGINAL ARTICLE

Integrated Food Science

Whole Wheat Biscuits Fortified With Softened Whole Harpiosquillid Mantis Shrimp Powder: Characteristics, Nutritional Enhancement, and Acceptability

Mallikarjun Chanchi Prashanthkumar[1] | Umesh Patil[2] | Suriya Palamae[2] | Natchaphol Buamard[2] | Jirakrit Saetang[2] | Bin Zhang[3] | Soottawat Benjakul[2,4]

[1]Faculty of Agricultural Technology, Songkhla Rajabhat University, Khao Roop Chang, Mueang Songkla, Songkhla, Thailand | [2]International Center of Excellence in Seafood Science and Innovation, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand | [3]College of Food Science and Pharmacy, Zhejiang Ocean University, Zhoushan, Zhejiang, China | [4]Department of Food and Nutrition, Kyung Hee University, Seoul, Republic of Korea

Correspondence: Soottawat Benjakul (soottawat.b@psu.ac.th)

Received: 20 May 2026 | **Revised:** 6 October 2026 | **Accepted:** 20 October 2026

Keywords: calcium | fortification | Harpiosquillid mantis shrimps | hydroxymethylfurfural (HMF) | protein | whole wheat biscuits

ABSTRACT

Fortification of whole wheat biscuits with softened whole Harpiosquillid mantis shrimp (HMS) powder at different levels (5%, 10%, 15%, and 20%) was carried out. Physiochemical, amino acid, elemental composition, textural property, and consumer acceptance were determined. Rheology of dough and microstructure of biscuits were also examined. HMS powder fortification increased total protein, fats, ash, calcium, and magnesium contents, while reducing carbohydrate in comparison with the control biscuits. Fortification with upsurging concentrations of HMS powder altered dough rheology, as indicated by decreasing resistance to extension (R_{max}) and extensibility (E_{max}). This was associated with lower cutting force, higher spread ratios, and darker color of resulting biscuits. The biscuit fortified with 10% HMS powder was the most acceptable ($p < 0.05$). Both essential and nonessential amino acids of biscuits increased when powder at higher levels was incorporated. Microstructure analysis revealed that the HMS fortified biscuits had more pores in comparison with control biscuits. Energy-dispersive x-ray spectroscopy (EDX) evaluation showed the presence of calcium and magnesium in HMS fortified biscuits. Notably, 10% HMS fortification increased calcium by 12-fold and magnesium by 2-fold compared to control samples. Although addition of HMS powder increased browning of biscuits hydroxymethylfurfural (HMF) formation was reduced during baking. Thus, HMS powder is an effective ingredient for producing calcium and protein-rich biscuits.

Practical Applications

The current study demonstrates a sustainable approach to converting texture-softened, underutilized, consumer-rejected Harpiosquillid mantis shrimp into a high-value functional ingredient. Commercial bakers can substitute wheat flour with HMS powder to produce protein-enriched biscuits with significantly lower carbohydrate content, an appealing snack for diabetic patients. Fortification with HMS powder of 10% naturally enhances essential and nonessential amino acids, macro elements such as calcium by 12-fold and magnesium by 2-fold. Critically, the formulation maintains excellent consumer liking score while

Abbreviations: L^*, lightness; a^*, redness/greenness; b^*, yellowness/blueness; CFU, colony forming unit; EDX, energy-dispersive x-ray spectroscopy; E_{max}, extensibility; GC–MS/MS, gas chromatography–tandem mass spectrometry; HMF, hydroxymethylfurfural; HMS, Harpiosquillid mantis shrimp; HPLC, high-performance liquid chromatography; kDa, kilo Dalton; PCA, principal component analysis; PUFA, polyunsaturated fatty acids; R_{max}, resistance to extension; TVC, total viable count.

All rights reserved, including rights for text and data mining and training of artificial intelligence technologies or similar technologies.
© 2026 Institute of Food Technologists.

reducing formation of toxic hydroxymethylfurfural (HMF). This technology can be implemented in the seafood industry for the utilization of waste as functional ingredient.

1 | Introduction

The modern world is moving towards health-oriented food products with high nutritional value. As the demand for nutritious enriched food has increased, the development of fortified snacks has rapidly emerged and been introduced into the markets. Bakery products, for example, cookies and biscuits with a long shelf life have been in demand. Moreover, these bakery products are widely consumed across all age groups. In addition, the fortification of high-proteinaceous, macro- and micronutrients, antioxidants, and other functional components has been conducted in bakery products. Biscuit fortified with spiced shrimp (Kumar et al. 2023), protein concentrates from Pacific white shrimp waste (Chauhan et al. 2025), chitin and crayfish shell (Bai et al. 2023),*Piaractus brachypomus* fish protein (Hashem et al. 2023) and sea bass by-products (Honrdo et al. 2024) as well as microencapsulated shrimp oil rich in PUFA and astaxanthin (Takeungwongtrakul and Benjakul 2017) have been developed successfully. Furthermore, the seafood processing by-products and waste containing numerous nutraceuticals and active components are commonly discarded with exploitation. The increased interest in the uses of seafood waste or underutilized species with high nutritive value brings about the development of fortified products.

Harpiosquillid mantis shrimp (HMS) is a highly demanded crustacean (Chanchi Prashanthkumar, Fan et al. 2024). The textural softening occurred within 2–3 days of iced storage caused by trypsin existing inside the digestive tract (Chanchi Prashanthkumar, Temdee et al. 2024). Such a quality loss had led to rejection by the consumers. These autolyzed HMS samples contain highly nutritious ingredients such as proteins, biologically active peptides, and minerals such as calcium, magnesium, and so forth, whereas microbiological quality is still acceptable (<6 log colony forming unit [CFU]/g) (Chanchi Prashanthkumar, Fan et al. 2024). Autolyzed whole HMS had protein content up to 40%–45% and consisted of minerals and chitin, enzymes, and other nutritive components (Chanchi Prashanthkumar et al. 2023). Therefore, hydrolyzed HMS, which was not acceptable for texture, could be employed as the nutritive ingredients in biscuits to augment the nutritive values, and the exploitation of autolyzed HMS can be better exploited.

Among the bakery products, biscuit is the predominant product with dry and crunchy texture made up of wheat flour, sugar, and butter (Sinthusamran et al. 2019). Biscuits generally contain 7%–8% protein and lower mineral contents, which are essential to the human diet. To improve the nutritive value, proteinaceous and mineral-rich ingredients derived from underutilized seafoods, such as autolyzed HMS, could be another potential option to produce the fortified biscuits. Patil et al. (2025) added heme protein obtained from seabass gill to increase the iron and protein content in the cookies. Similarly, Sharma et al. (2025) used salmon fish frame hydrolysate to increase the protein level in the cookies. Basically, the type and amount of ingredients would impact the empirical rheology of the dough. Crucially, the properties of dough, such as resistance to stretch, elasticity, and gluten bonding, are affected with the addition of ingredients. Simultaneously, it directly affects the acceptability, mouthfeel, and structure of the biscuits. As the autolyzed whole HMS is loaded with protein and minerals with high nutritional quality, it could be used to increase the nutritional value and acceptability of the biscuits. Nonetheless, no information concerning the usage of softened whole HMS shrimp for fortification in biscuit exists. Thus, this study focused on the development of biscuits fortified with autolyzed HMS powder at varying concentrations. The characteristics, nutritive composition, rheology of dough, and consumer liking score of the obtained biscuits were also studied.

2 | Materials and Methods

2.1 | Chemicals

All analytical-grade chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ingredients for biscuit preparation were purchased from a local superstore in Hat Yai, Thailand.

2.2 | Collection of HMS

Fresh HMS were collected and packed in an insulated box from Satun dock, Thailand. After being transported to the laboratory within 2 h in iced water, the samples were cleaned using cold tap water and kept in crushed ice using HMS/ice ratio of 1:2 (w/w) for 3 days. The softened HMS obtained after storage for 3 days were used for this study. These samples had undesirable softened texture justified by the consumers but still had the total viable count (TVC) of 3 log CFU/g, which is less than 6 log CFU/g, a microbial limit (Ahmad et al. 2026).

2.3 | Preparation of HMS Powder

The softened whole HMS was treated with liquid nitrogen (−173°C) and ground using a high-speed grinder for 3 min. The obtained paste was completely frozen at −40°C and further lyophilized using a freeze-dryer at −45°C with absolute vacuum pressure of 0.001 hPa (Scanvac Model CoolSafe 55 freeze-dryer, CoolSafe, Lynge, Denmark). Lyophilized powder was subjected to high-speed grinding and sieved (mesh of 300 or diameter of 0.05 mm) to obtain the powder with particle size similar to that of whole wheat flour. The HMS powder was immediately used, and the rest of the portions were stored in ziplock bag at −40°C.

2.4 | Effect of HMS Powder on the Properties and Quality of Biscuits

2.4.1 | Preparation and Characterization of Whole Wheat Biscuits Fortified with HMS Powder

The biscuit dough preparation was done using a formulation containing 51.7 g whole wheat flour, 13.8 g butter, 6.4 g peanut butter, 2.6 g icing sugar, 1.0 g salt, 2.1 g baking powder, and 22.4 g water (total weight: 100 g). HMS powder was substituted for wheat flour at varying levels (0%–20%). First, the biscuits were prepared using all ingredients such as butter, peanut butter, icing sugar, salt, baking powder, and HMS powder, which were systematically mixed with the aid of a blender to obtain homogenous mixture. The water was then added regularly into the mixture. The dough was continuously kneaded, followed by covering with plastic film. After leaving (20 min, 25°C), the resultant dough was rolled out with a rolling pin to obtain the thickness of 0.4 cm and cut into circles with the aid of cutting mold. Prepared biscuits were placed in an oven (rotary baking oven, Uki Foods Technology Co. Ltd., Nonthaburi, Thailand) (180°C, 22 min). Thereafter, the biscuits were allowed to stand (25°C, 1 h). Biscuits were packed in the ziplock bags within 24 h before analyses.

2.4.2 | Analyses

2.4.2.1 | Proximate Composition and Energy Value. Proximate composition of biscuit samples was analyzed as per AOAC 1990 method. The energy value was calculated using conversion factors as follows: 16.7 kJ/g for protein and carbohydrate and 37.6 kJ/g for fat (Patil et al. 2025). To compute total energy, the energy from protein, carbohydrates, and fat was summed (Karnjanapratum and Benjakul 2018).

2.4.2.2 | Water Activity (a_w). The a_w of all biscuit samples was examined using the AquaLab 4TE (METER Group, Inc., Pullman, WA, USA).

2.4.2.3 | Dimension, Weight, and Baking Loss. The diameter (cm) and thickness (cm) of biscuits were determined using a vernier caliper at six different positions, and a mean was computed. The weight (g) of biscuits was measured using electronic precision balance (OHAUS PR series, OHAUS Corporation, Parsippany, NJ, USA) and averaged. The baking loss was determined using the following equation:

$$\text{Baking Loss}\,(\%) = \frac{(W_0 - W_1)}{W_0} \times 100$$

W_0: Weight of biscuit before baking; W_1: Weight of biscuit after baking.

2.4.2.4 | Color. The surface color of the biscuits was evaluated using a Hunter Lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). The L^*, a^*, b^*, and ΔE were recorded.

2.4.2.5 | Textural Analysis. To determine cutting force and fracturability of biscuits, a texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) was used, in which test speed of 10 mm/s and load cell of 50 kg were employed (Karnjanapratum and Benjakul 2018). A knife-edge equipped with a slotted insert blade set (heavy-duty platform [HDP]/BS, 60 mm × 20 mm) was adopted to simulate the biting behavior, in which the slotted insert was fixed on the HDP/90. The maximum force was required for breaking the biscuits and the distance at which breaking occurred was recorded. Ten samples were used for each treatment.

2.4.2.6 | Empirical Rheology of Dough. Empirical rheology of dough containing HMS powder at different levels was examined using texture profile analyzer equipped with an SMS/Kieffer Extensibility Rig (Stable Micro Systems Ltd., Godalming, United Kingdom). The dough samples were first placed into the Kieffer sample plate. After being equilibrated in the clamping device (20 min, 25°C, 90% RH), the setting was established under the following conditions: initial speed of 2 mm/s; test speed of 3.3mm/s, retaining speed of 10 mm/s, distance of 75 mm, and triggering force of 0.05 N. Finally, the extensibility (E_{max}) representing the distance (mm) where the dough began to disrupt, was recorded. The resistance to extension (R_{max}) was the force (N) required for dough rupture. Analyses were performed for 10 replicates.

2.4.2.7 | Consumer Acceptance Test. The consumer acceptance test of the control and all HMS powder-incorporated biscuits (5%, 10%, 15%, and 20%) was evaluated by 80 untrained panelists (40 females and 40 males with the age of 21–60 years). The participants involved students, staff, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, and outsiders, who were familiar with biscuits. The participants who were allergic to seafood were excluded from the study. Twenty participants per set were allowed for the sensory analysis with a time limit of 30 min. Thereafter, the rest (three sets) was continued for evaluation in the same manner. The participants rated liking score for appearance, color, odor, flavor, and taste, as well as overall liking, using a nine-point hedonic scale (1 = dislike very much, 5 = neither like nor dislike, and 9 = like extremely) (Meilgaard et al. 2007). The consumer hedonic approach is also consistent with the general purpose of ISO 11136:2014. Each sample with no TVC detected (data not shown) was placed on the white plate, which was labeled with random three-digit codes. The panelists were served with 1 whole biscuit of each treatment with a total of 5 biscuits. After each sample evaluation, panelists were asked to rinse their mouths with water, and wheat bread was provided to neutralize the bitterness or aftertaste (Meilgaard et al. 2024). The sensory evaluation was conducted in a sensory room consisting of 20 individual compartments. The temperature and moisture of the room were maintained at 25–26°C with the humidity of 45%–55%. The uniform artificial daylight was used in the room. The experiment was approved by the Ethical Review Committee for Research in Human Subjects, Research and Development Office of Prince of Songkla University, Hat Yai (Code: Ref.AQ084/2023).

2.5 | Characterization of the Selected Biscuits Enriched with HMS Powder

The biscuit added with HMS powder with highest liking score with satisfactory properties was characterized in comparison with the control biscuit.

2.5.1 | Amino Acid Composition

The amino acid composition of the selected biscuit samples was analyzed using a GC–MS/MS method (Patil et al. 2025). Samples (0.4 g) were hydrolyzed using 10 mL of 6 M HCl at 121–123°C for 3 h, except for tryptophan, in which the sample was hydrolyzed using 10 mL of 4.2 M NaOH. After hydrolysis, the mixtures were transferred to 20-mL volumetric flasks and diluted with 2 M NaOH. The samples were then filtered through Whatman No. 42 filter paper and a 0.45 µm syringe filter to remove any particulates. For derivatization, 100 µL of the filtered solution was mixed with 200 µL of BSTFA (with 1% TMCS) and 300 µL of pyridine. The mixture was heated at 70°C for 45 min. The derivatized samples were analyzed using GC–MS/MS with column Restek 5-MS 5% phenyl 95% dimethyl-polysiloxsane (30 mm, 0.25 mm, and 0.25 µm). Split ratio of 3.0:1, split flow of 3.0 mL/min and Helium gas flow of 1.2 mL/min were used. The oven temperature was increased with 15°C/min from 100°C to 300°C. MS was auto-tuned with EI scan mode 50–450 amu. The sampling rate was 2 with 3.6 scan/s, and a threshold limit of 150 and filament of 2.5 min were used. The MS temperature conditions were 230°C of source and quad with 150°C. The amino acids were quantified with the Triple Quadrupole Mass Analyzer detector (Agilent Technologies, Santa Clara, AC, USA).

2.5.2 | Scanning Electron Microscopy (SEM)

For the microstructure, the selected biscuit samples were mounted on individual bronze stubs, gold-sputter-coated, and visualized with a scanning electron microscope (Apreo, FEI, Brno, Czech Republic) at an acceleration voltage of 20 kV and a pressure of 5–10 Pa. A magnification of 30× was applied during the imaging process.

2.5.3 | SEM with Energy-Dispersive X-Ray (EDX)

SEM–EDX was used as per the method of Benjakul and Karnjanapratum (2018). A field emission scanning electron microscope (Apreo, FEI, Brno, Czech Republic) equipped with an EDX was employed. After being gold-coated and secondary electron mode at a 20 kV accelerating voltage was used for observation. Elemental analysis was performed on the cross section of the biscuits by EDX to verify the distribution of elements.

2.5.4 | Minerals and Heavy Metals Analysis

Inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 8000, Perkin Elmer Instrument, Waltham, USA) was adopted for the determination of Na, Ca, Mg, Cu, and Pb in biscuit samples as per the method of AOAC (2023) with the analytical number of 2015.01. For Hg, the direct method was adopted (AOAC 2023) with the analytical number of 988.11.

2.5.5 | Hydroxymethylfurfural (HMF) Analysis

HMF analysis was carried out as described by Zappala et al. (2005). Five grams of biscuit powder samples were ground and homogenized with 50 mL of distilled water at 15,000 rpm for 2 min. The homogenate was then filtered using Whatman paper No. 40 and then filtered through a 0.45 µm filter. The obtained filtrate was immediately injected into an HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with RP-18, 5 lm, 125·4 mm column. Mobile phase comprises water containing 1% acetic acid and 10% methanol; flow rate, 0.7 mL/min. Peak intensity was computed on the basis of a standard HMF (Sigma-Aldrich, Milan, Italy). The amount of HMF was determined using an external calibration curve, where the signal was detected at $\lambda = 285$ nm.

2.6 | Experimental Design and Statistical Analysis

A completely randomized design (CRD) was used for the whole study, except for consumer acceptance test, in which randomized complete block design (RCBD) was employed. The analyses were conducted in triplicate. The dimension, weight, and baking loss were conducted in six replicates. The color, texture, and empirical rheology were conducted in 10 replicates. The data were subjected to an analysis of variance (ANOVA). The mean comparison was done using Duncan's multiple-range test. The *t*-test was performed to compare the pairs (Steel and Torrie 1980). Results with p values less than 0.05 were considered statistically significant. The Statistical Package for Social Science (SPSS 23.0 for Windows, SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analysis.

Principal component analysis (PCA) biplots and hierarchical clustering analyses were employed to elucidate the multivariate relationships between sensory attributes such as appearance, color, odor, flavor, taste, and overall liking score. All multivariate analyses were performed using R software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria).

3 | Results and Discussion

3.1 | Proximate Composition of Biscuits Fortified with HMS Powder at Various Levels

Total protein content in the biscuit fortified with HMS powder notably increased with rising concentrations of HMS powders. The protein content in the biscuits statistically significantly rose from 10.79 g/100 g in the control to 16.70 g/100 g in the biscuits added with 20% HMS powder, indicating that higher protein concentration could be achieved with incorporation of HMS powder ($p < 0.05$). The different superscripts indicate the significant differences among the HMS powder incorporated biscuits. Chanchi Prashanthkumar et al. (2023) and Temdee et al. (2022) documented the protein content of 15–16 g/100 g in the meat of HMS. In addition, the augmented total protein content coincided with a decline in carbohydrate content. The HMS powder was substituted of wheat flour in the formulation. A similar trend of gradual decrease in the carbohydrate was reported by Patil et al. (2025), when heme proteins from gills of Asian seabass were fortified into whole wheat cookies. Furthermore, Sharma et al. (2025) also documented the significant decrease in the carbohydrate content with the incorporation of debittered salmon frame protein hydrolysates prepared using Maillard reactions and 2-butanol, respectively. HMS powder could, therefore, serve

TABLE 1 | Proximate composition, water activity (a_w), and total energy of whole wheat biscuits fortified without and with Harpiosquillid mantis shrimp (HMS) powder at different levels.

Proximate composition, a_w and total energy	HMS powder levels added (%)				
	Control	5	10	15	20
Protein (g/100 g)	10.79 ± 0.02^{e}	12.43 ± 0.12^{d}	13.42 ± 0.06^{c}	15.04 ± 0.06^{b}	16.70 ± 0.10^{a}
Fats (g/100 g)	26.50 ± 0.47^{d}	26.69 ± 0.09^{c}	26.90 ± 1.73^{abc}	27.04 ± 0.44^{ab}	27.32 ± 0.87^{a}
Carbohydrates (g/100 g)	55.95 ± 0.40^{a}	53.68 ± 0.03^{b}	51.70 ± 2.17^{c}	49.64 ± 0.38^{d}	46.87 ± 0.88^{e}
Moisture (g/100 g)	3.06 ± 0.09^{a}	2.52 ± 0.01^{b}	2.38 ± 0.05^{c}	2.20 ± 0.02^{d}	2.14 ± 0.03^{e}
Ash (g/100 g)	3.70 ± 0.18^{e}	4.67 ± 0.01^{d}	5.61 ± 0.33^{c}	6.07 ± 0.02^{b}	6.97 ± 0.11^{a}
a_w	0.275 ± 0.007^{a}	0.134 ± 0.002^{b}	0.109 ± 0.004^{c}	0.084 ± 0.001^{d}	0.072 ± 0.002^{e}
Total energy (kJ/g)[a]	21.15	21.11	21.03	21.01	20.93

Note: Values are expressed as mean ± SD ($n = 3$). Lowercase superscripts within the same row indicate significant differences ($p < 0.05$).

[a]The energy value was calculated using conversion factors of 16.7 kJ/g for protein and carbohydrate and 37.6 kJ/g for fat. Total energy (kJ/g) was determined by the sum of the energy values of protein, carbohydrate, and fat.

as potential source of proteins in effectively increasing the nutritional value of fortified biscuits.

The total fats in the biscuits slightly increased with increasing concentration HMS powder used for the fortification ($p > 0.05$). The increase in the fat content was not significant since the HMS contained only 2–3 g/100 g fat in its meat (Chanchi Prashanthkumar et al. 2023; Temdee et al. 2022). The overlapping of superscripts denotes nonsignificance. Notably, the total fat content in the control sample was around 26.50 g/100 g, whereas biscuits incorporated with 20% of HMS powder were around 27.32 g/100 g. High fat content found in biscuit samples were mainly from the butter and peanut butter used as ingredients. Overall, the fortification of HMS powder at high level (20%) slightly increased in fat content in the resulting biscuits.

In contrast, the moisture content of biscuits statistically significantly decreased when added with HMS powder, from 3.06 g/100 g in the control to 2.14 g/100 g in those containing 20% HMS powder (Table 1) ($p < 0.05$). The different superscripts indicate the significant differences among the HMS powder incorporated biscuits. The decrease in moisture with increasing HMS powder incorporation was plausibly due to weakening of the covalent bond of gluten network by HMS powder. The HMS powder plausibly interrupted the entrapment of water molecules in the dough, and water was evaporated due to high-temperature baking. The evaporation caused the decreased moisture content in the biscuits. Normally, the wheat flour has around 9–11 g/100 g of moisture, but the HMS powder consisted of chitin, a hydrophobic and insoluble in water. As a result, the water in flour was readily available for evaporation during baking. This could lower the moisture content in the biscuits with the addition of HMS powder. The hydrolyzed protein present in the HMS powder might have the exposed hydrophobic domains, which disrupted the interaction of water with the biscuit matrix. As a consequence, rapid evaporation took place during baking, and the moisture content became lower in the biscuits. Simultaneously, the lowered moisture negatively impacted the textural property of the biscuit by increasing the dryness and brittleness.

Ash content of biscuits fortified with HMS powder at varying levels was higher than that of control ($p < 0.05$). Among all samples, the control had the least ash content (3.70 g/100 g), whereas those comprising 5%, 10%, 15%, and 20% HMS powder showed progressively statistically significantly higher ash contents of 4.67, 5.61, 6.07, and 6.97 g/100 g, respectively ($p < 0.05$). The increased ash content could be the result of mineral in HMS powder, particularly from shells. The minerals, including essential elements such as calcium, magnesium, and sodium, present in the HMS shrimp shell, contributed to the mineral enrichment in resulting biscuits.

Overall, the addition of HMS powder provided more proteins, ash content, and fat content into the fortified biscuits. The augmentation in those ingredients gradually brought about a proportional decline in carbohydrate content. Notably, the reduction in carbohydrate amplified the nutritional profile of the biscuits for diabetic consumers by lowering the carbohydrate content. However, the total energy between the control biscuits and fortified biscuits had no significant difference among the samples. The control biscuits had a total energy of 21.15 kJ/g, whereas the biscuits added with 5%, 10%, 15%, and 20% of HMS had a total energy of 21.11, 21.03, 21.01, and 20.93 kJ/g, respectively ($p < 0.05$). There was no statistically significant change in the energy level obtained with the addition of HMS powder due to lower fat content, which yielded the highest energy among the major components.

3.2 | Water Activity (a_w)

The a_w of biscuits generally decreased with increasing levels of HMS powder added. The a_w decreased from 0.2752 in the control to 0.0719 in the biscuits incorporated with 20% HMS

powder. The results were in line with the slight decrease in the moisture content (Table 1) ($p < 0.05$). The different superscripts indicate the significant differences among the HMS powder incorporated biscuits The decreased a_w could extend the shelf life of the product and might have a positive effect on the texture of the biscuits, particularly crispiness. Storage stability of food is extremely governed by its a_w (Tapia et al. 2020). The a_w represents water molecules available for microbial, enzymatic, or chemical reactions, thus determining quality and stability of food during storage (Dimassi 2026). Bacteria are not able to proliferate at a_w of 0.80 or lower, whereas molds and yeasts can grow at a_w of 0.60 (Sharma et al. 2025). The a_w also affects lipid oxidation in food products, in which oxidation is enhanced at very high or very low a_w levels (Mora et al. 2025). Nonetheless, lipid autoxidation is lowered in a_w range of 0.30–0.50. The decreased interactions between fatty acids and reactive oxygen species become lower. Outside this range, increased oxidation takes place. The HMS incorporated biscuits had a_w between 0.0718 and 0.1339, which was lower than the control (0.2752). This could help prevent the microbial growth and chemical deterioration.

3.3 | Empirical Rheology of Dough

The empirical rheology of biscuit dough determines the interaction of gluten in the formulation of dough based on the textural profile analysis with SMS/Kieffer extensibility (Hackenberg et al. 2018). The rheological behavior of the dough covers elasticity and resistance to extension (M. Li et al. 2026). The resistance to extension is measured by the force required to rupture the dough, providing the information for investigating biscuit's volume and compactness. The results clearly demonstrated the altered rheological behavior of the dough with the addition of the HMS powder. A notable statistically significant reduction in resistance to extension R_{max} was found and extensibility E_{max} was obtained with higher levels HMS powder added. The resistance to extension of biscuits fortified with HMS powder at 5%, 10%, 15%, and 20% was reduced by 34.21%, 50.45%, 57.89%, and 75.12%, relative to that of the control (Table 2) ($p < 0.05$). Similarly, the reduction in extensibility was observed by 17.15%, 33.53%, 39.09%, and 69.70%, respectively, compared to that of the control. The rheological properties of biscuit dough are completely dependent on the structure and functionality of flour biopolymers, which were majorly influenced by the protein–starch interactions. Moreover, the starch present in the formulation not only acted as a filler in the protein phase but also interacted with the surrounding gluten, affecting the rheological behavior (Edwards et al. 2002). In addition, the mechanical treatment in the milling would change starch granule surface and directly influence the starch–starch and starch–protein interactions, resulting in the alteration of dough rheological behavior. Moreover, the chemical and functional characteristics of starch, as well as protein, directly affected the viscoelasticity of the dough matrix during kneading (Y. Li et al. 2026). HMS powder consisted of chitins, and minerals, which had direct influence on the rheology of dough. The incorporation of a mixture consisting of inorganic substances, mainly from shell of HMS could lower the protein interaction and reduce protein–protein interaction and network formation (Wang et al. 2022). Poor protein network is evidently observed by a decrease in R_{max} and also a lower E_{max} levels. As observed in the present study, the sharp decrease in R_{max} and E_{max} of dough incorporated with HMS powder at increasing levels indicated a poor protein network connectivity (Hackenberg et al. 2018). Shan et al. (2022) found that the incorporation of whey protein fibrils into wheat dough also resulted in the decreases in both R_{max} and E_{max}.

3.4 | Texture Properties of Biscuits Fortified with HMS Powder at Different Levels

The textural properties of biscuits expressed as cutting force and fracturability as influenced by addition of HMS powder at different levels are displayed in Table 2. The cutting force is known as the amount of force required by teeth to compress food, whereas fracturability is defined as capacity of food to break into pieces when bitten with teeth (Paula and Conti-Silva 2014). The control biscuits exhibited the highest cutting forces (5771.49 ± 526.11 g s) in comparison with all the other HMS fortified biscuits ($p < 0.05$). The cutting forces of biscuits gradually decreased with augmenting levels of HMS powder ($p < 0.05$). The biscuits fortified with 20% HMS powder required the lowest cutting force (2770.59 ± 294.54 g s), indicating the HMS powder made the biscuits softer, mainly by altering the structural integrity of the biscuits. Simultaneously, the decreases in R_{max} and E_{max} confirmed the interfering effect of HMS powder on dough formation. The lower interaction of starch–protein plausibly led to the weaker structural integrity during baking in biscuits. Without HMS powder, the control biscuits had a stronger protein network with higher interactions between gluten, causing a firmer and compact structure associated with stronger starch–starch, starch-muscle protein, and starch–gluten interactions. On the other hand, the starch–protein interaction and starch–gluten were lowered in the presence of HMS powder, thus reducing the hardness of the biscuits. Hence, the fortification of HMS powder at various levels in the biscuits resulted in smoother and brittle texture in comparison with the control biscuits. Similarly, the fracturability of control biscuits was 61.22 ± 0.40 mm, whereas the HMS powder fortified biscuits had a decline in fracturability. Patil et al. (2025) reported the decrease in fracutrability and hardness of cookies when heme proteins from Asian seabass gills were added up to 2%. Sharma et al. (2025) also documented the decreases in hardness and fracturability of cookies with the addition of salmon frame hydrolysate from 2% to 8% in comparison with the control cookies.

3.5 | Diameter, Thickness, Weight, and Baking Loss

Diameter, thickness, and weight of biscuits fortified without and with HMS powder at various levels are presented in Table 2 ($p > 0.05$). The biscuits containing 20% HMS powder had the highest diameter (4.91 ± 0.03) (Figure 1), whereas the control sample showed the lowest diameter (4.43 ± 0.05). The incorporation of HMS powder probably changed the consistency of the dough. The empirical rheology study confirmed such a change as indicated by the decreases in both R_{max} and E_{max} values. Notably, the protein content, starch–starch, starch–gluten, and hydration properties of affected the viscosity and structure of biscuits dough. In addition, the spreadability of the dough could be affected during the baking. The components present in the starch, such

TABLE 2 | Empirical rheology of dough, textural properties, dimensions, and color of whole wheat biscuits fortified without and with Harpiosquillid mantis shrimp (HMS) powder at different levels.

Parameters	Unit	Control	HMS levels added (%)			
			5	10	15	20
Resistance to extension	g	41.27 ± 1.21^{a}	27.15 ± 0.19^{b}	20.45 ± 2.17^{c}	17.38 ± 0.27^{d}	10.27 ± 0.40^{e}
Extensibility	mm	(−16.73) ± 0.80^{e}	(−13.86) ± 1.78^{d}	(−11.12) ± 1.66^{c}	(−10.19) ± 0.59^{b}	(−5.07) ± 0.96^{a}
Hardness	g	5771.49 ± 526.11^{a}	3767.19 ± 442.64^{bc}	3430.09 ± 351.22^{c}	3225.85 ± 456.47^{c}	2770.59 ± 294.54^{d}
Fracturability	mm	61.22 ± 0.40^{a}	61.62 ± 0.30^{a}	61.68 ± 1.39^{a}	61.07 ± 1.34^{a}	60.28 ± 0.95^{b}
Diameter	cm	4.43 ± 0.05^{e}	4.71 ± 0.05^{d}	4.83 ± 0.09^{bc}	4.86 ± 0.11^{ab}	4.91 ± 0.03^{a}
Thickness	mm	0.52 ± 0.02^{a}	0.53 ± 0.01^{a}	0.52 ± 0.01^{a}	0.53 ± 0.02^{a}	0.53 ± 0.02^{a}
Weight	g	7.72 ± 0.06^{a}	7.43 ± 0.01^{b}	7.31 ± 0.06^{c}	6.94 ± 0.05^{d}	6.47 ± 0.01^{e}
Baking loss	%	10.51 ± 0.05^{e}	11.72 ± 0.01^{d}	13.17 ± 0.05^{c}	14.24 ± 0.04^{b}	15.45 ± 0.0^{a}
Color	L^*	61.29 ± 0.43^{a}	48.79 ± 3.32^{b}	43.84 ± 0.41^{c}	41.50 ± 0.92^{d}	39.07 ± 0.63^{e}
	a^*	14.18 ± 0.36^{e}	16.00 ± 0.04^{d}	16.61 ± 0.51^{bc}	16.71 ± 0.19^{ab}	16.98 ± 0.08^{a}
	b^*	38.20 ± 0.77^{a}	33.22 ± 1.81^{b}	31.02 ± 0.23^{c}	28.54 ± 0.92^{d}	26.67 ± 0.66^{e}
	ΔE^*	—	13.68 ± 3.52^{d}	19.09 ± 0.77^{c}	22.17 ± 1.40^{b}	25.11 ± 1.26^{a}
Likeness score						
Appearance		7.66 ± 1.13^{a}	7.25 ± 1.03^{ab}	7.16 ± 1.09^{ab}	6.05 ± 0.82^{c}	5.19 ± 0.95^{d}
Color		7.17 ± 1.27^{a}	5.97 ± 1.48^{b}	5.99 ± 1.50^{b}	4.09 ± 1.36^{c}	3.47 ± 1.49^{d}
Odor		7.71 ± 1.13^{a}	6.70 ± 0.86^{b}	6.68 ± 0.84^{b}	4.45 ± 0.94^{c}	2.73 ± 1.29^{d}
Flavor		7.41 ± 1.31^{a}	7.18 ± 0.84^{b}	7.16 ± 0.88^{b}	4.21 ± 1.32^{c}	2.00 ± 1.70^{d}
Taste		7.42 ± 1.14^{a}	6.94 ± 1.36^{b}	6.98 ± 1.36^{b}	4.46 ± 1.16^{c}	1.73 ± 0.45^{d}
Overall		7.41 ± 0.93^{a}	7.18 ± 1.28^{b}	7.24 ± 1.27^{ab}	4.21 ± 1.13^{c}	2.23 ± 0.47^{d}

Note: Values are expressed as mean ± SD ($n = 10$). For likeness, score values are expressed as mean ± SD ($n = 80$). Lowercase superscripts within the same row indicate significant differences ($p < 0.05$).



FIGURE 1 | The appearance of whole wheat biscuits fortified without and with HMS powder at different levels. (A) Surface of cookies and (B) cross section of cookies. HMS, Harpiosquillid mantis shrimp.

as amylose, amylopectin, and so forth, have a direct impact on the structure of the biscuits. The degree of branching and chain length of the starch and gluten present in the formulation determine the degree of association and rigidity of gel network (Xu and Kuang 2024). The amylose and amylopectin in the starch play a key role in capturing the air and carbon dioxide during baking process, causing the expansion of the dough, crispiness, and puffiness in the final product. This phenomenon

also determined the expansion of biscuit as reflected by the increased diameter or thickness. Protein interacts within the dough matrix, thus forming the strong and dense networks (Zhang et al. 2026). Moreover, less protein network leads to collapsed or poorer structure, causing spreadability of biscuits. Apart from proteins, HMS powder still consisted of chitin and inorganic compounds. As a result, the interaction within dough matrix was weaker, causing the expansion of air with ease. As a result, HMS powder fortified biscuit were slightly larger in diameter. However, Patil et al. (2025) and Sharma et al. (2025) found that the diameter of cookies fortified with heme proteins and salmon frame hydrolysate, respectively, slightly decreased, compared to that of control sample.

The thickness of samples was similar, regardless of HMS powder addition, with the values ranging from 0.52 ± 0.01 to 0.53 ± 0.02 cm. Therefore, the incorporation of HMS powder showed no substantial effect on thickness of the biscuits. However, the percent spread was proportional to the level of HMS powder. As the HMS powder concentration increased, the spread increased (Table 2). During baking, the biscuit network could be formed, and air expansion or water evaporation of dough with higher spreadability might be higher. As a result, the thickness among all samples was similar, although the spreadability was slightly different.

The weight of biscuits ranged from 6.47 to 7.72 g. The control biscuits had an average weight of 7.72 ± 0.06, which was statistically significantly higher than the HMS powder fortified biscuits. The difference in the weight suggested that HMS powder containing mineral and chitin to some levels could lower water retention, whereas the wheat dough could be formed and trapped the water inside the network more effectively. The hydrophobic domain of HMS powder also lowered the hydrogen bond formation with water present in dough matrix. The interruption might cause the loss of water in the dough matrix, resulting in more availability of water and evaporated during high temperatures of baking. This contributed to the loss of biscuit weight. The results coincided with the decreases in the moisture and a_w of HMS fortified biscuits (Table 1).

Baking loss is an indicator of moisture release during baking. The baking loss ranged from 10.51% to 15.45% (Table 2). The control samples had the least baking loss of 10.51% ($p < 0.05$). HMS powder-incorporated biscuits had the increase in baking loss when the HMS concentration upsurged ($p < 0.05$). The biscuits added with 5%, 10%, 15%, and 20% HMS powder had baking loss of 11.72%, 13.17%, 14.24%, and 15.45%, respectively. The loss was associated with the escape of gas along with water vapor during the baking process. The hydrolyzed proteins present in HMS powder had short-chain peptides that could not form a strong structural network and lost water-retaining capabilities. In addition, the inorganic elements present might have altered structure in the biscuits. Moreover, the inorganic elements had poor water-binding capacity. Collectively, the addition of higher levels of HMS enhanced baking loss of biscuits. Generally, starch–protein interaction, gluten content, the size, and structure of starch granules can influence water-binding capacities. Ammar et al. (2021) found that the addition of whey proteins reduced the baking loss by retaining water in gluten-free sponge cakes.

3.6 | Color

The surface color of whole wheat biscuits fortified with HMS powder at varying levels in comparison with the control biscuits is tabulated in Table 2 ($p < 0.05$). The control biscuit possessed the highest L^* value (61.29 ± 0.43), in comparison to HMS powder fortified biscuits. With the augmentation in concentration of HMS powder added, the L^* value decreased significantly ($p < 0.05$). The different superscripts indicate the significant differences among the HMS powder incorporated biscuits. The decline in L^* indicated darker color when the levels of HMS powder increased. Notably, the decreased L^* could be a result of the formation of the Maillard reaction between the reducing sugar formed under high temperature and amino groups in proteins, without gluten or muscle protein from HMS.

The a^* value representing redness values, ranged from 14.18 ± 0.36 to 16.98 ± 0.08. The a^* values of biscuits upsurged with increasing HMS powder levels ($p < 0.05$). Addition of HMS powder increased the redness of biscuits, due to the presence of carotenoprotein, which probably incorporated the reddish tint to some extent as seen in the 20% HMS incorporated biscuits.

The b^* value representing the yellowness declined rapidly with the augmentation of HMS powder fortification. Among all the biscuits, the control biscuits had the highest b^* values (38.20 ± 0.77), and notably the biscuits with 20% of HMS powder exhibited the lowest b^* values (26.67 ± 0.66). The intrinsic pigment with orange reddish color of HMS powder could be developed as induced by heat after baking along with Maillard reaction. These carotenoids present in the shrimps partially contributed to the color of the biscuit. The pigments present in the HMS powder overshadowed the yellowness, plausibly leading to significant lower b^* value.

Color difference (ΔE^*) indicates a significant difference among the samples. The increase in ΔE^* values augmented with the upsurging concentration of HMS powder, confirming the changes in color of biscuit as affected by HMS powder addition.

3.7 | Consumer Liking Score, PCA Biplot, and Cluster Analysis

Consumer liking score of biscuits fortified with HMS powder at different levels are displayed in Figure S1. Color, appearance, odor, texture, flavor, taste, and overall liking scores between the biscuit added with HMS powder at 5% and 10% were similar ($p > 0.05$). Nevertheless, further addition of HMS powder above 10% resulted in a significant reduction in liking scores for all quality attributes. The lowest liking scores were observed for biscuits supplemented with 20% HMS powder. The decrease in liking score was more likely associated with darker color developed and strong flavor of HMS. Notably, ingredients, such as butter, peanut butter, sugar, and salt, could not mask the typical characteristics of HMS added into the biscuits. Furthermore, the texture of the HMS powder incorporated biscuits was getting slightly soft with the increase in HMS concentrations. The preference on the hardness and softness was dependent on individual choices in the consumption context. Overall, the addition of 10% HMS into the

biscuits had no negative effects on liking score. Conversely, the biscuits fortified with 10% HMS powder showed the highest liking scores and were further subjected to the principal component and cluster analysis for confirmation of values.

The PCA and cluster analysis on liking score of biscuits (Figure S3A,B) provided further insight into the liking score differentiation of whole wheat biscuits fortified with varying levels of HMS powder (control, 5%, 10%, 15%, and 20%) (Figure 1). It revealed that the first two principal components (PC1 and PC2) accounted for 79.63% of total variance, with PC1 and PC2 explaining 70.24% and 9.39%, respectively. This indicated a distinct separation of sample groups based on sensory attribute perceptions. The consumer liking score loading vectors demonstrate that overall acceptability taste, flavor, and odor loaded negatively on the PC1 axis. Color and appearance vectors showed distinct directional variance along PC2. This clearly indicated that consumer preference attributes such as color, taste, flavor, and overall liking were the key drivers in distinguishing low-fortified levels over higher fortification groups. However, the PCA Biplot clearly separated the low-fortified biscuits from higher fortified biscuits along PC1. Control, 5% and 10% HMS fortified biscuits clustered closely together on the negative side of PC1, directly overlapping with vectors for overall acceptability, taste, flavor, and odor. The result indicated highly favorable and comparable consumer liking score among these formulations. In contrast, the biscuits fortified with 15% and 20% HMS powder shifted significantly towards the positive side of PC1, forming distinct groups associated with lower liking scores.

Hierarchical cluster analysis (Figure S3B) further supported the PCA results by dividing the consumer liking score evaluation into two primary cluster lineages at a high Euclidean distance. The first major cluster comprised control, 5% and 10% HMS powder fortified biscuits, demonstrating that sensory panel evaluation pattern for biscuits remained closely aligned up to a 10% fortification level. However, the second major cluster comprised the 15% and 20% HMS powder treatments, which reflected a significant shift in consumer liking score and reduced acceptability from the panel for higher concentration levels of HMS powder fortified biscuits.

Overall, these findings demonstrated the effects of HMS powder fortification on sensory attributes of the biscuits. It also confirmed that fortification up to 10% of HMS powder successfully maintained the sensory attributes in comparison to control biscuits. Thus, biscuits fortified with 10% of HMS powder were selected for further analyses.

3.8 | Characteristics of Biscuits Without and With Fortification of HMS Powder at the Selected Level

3.8.1 | Amino Acid Composition

The amino acid profiles of HMS powder, the control biscuits, and biscuits fortified with 10% HMS powder are shown in Table 3. The amounts of amino acids in all three samples showed slightly higher nonessential amino acids in comparison with essential amino acids. The dominant amino acid present in all three samples was glutamic acid/glutamine, cystine, arginine, histidine, lysine, glycine, leucine, and aspartic acid/asparagine. Glutamic acid and aspartic acid are known to contribute to the umami taste in the product. Similarly, as the HMS powder was obtained in the hydrolyzed form, arginine, histidine, lysine, and leucine likely contributed to the bitter taste in the biscuit samples (Harmon et al. 2024). Furthermore, glycine contributes to the sweet flavor of the biscuits (Sasanam et al. 2021), thus increasing the liking score. The fortification of 10% HMS powder resulted in the increasing levels of essential and nonessential amino acids of the biscuits. Hydroxylysine was only detected in the HMS powder and fortified biscuits, confirming the fortification of HMS powder in the biscuit. Hydroxyproline was detected in all three samples, and a slight increase in the quantity was found in fortified biscuits. Basically, wheat gluten was rich in glutamic acid, proline, and leucine (Rombouts et al. 2009). HMS meat had high contents of glutamic acid, arginine, cystine, glycine, and histidine. Thus, the addition of 10% HMS powder enhanced the contents of both essential and nonessential amino acids, increasing the overall nutritional quality of the biscuits. The developed biscuits could serve as useful snacks with higher nutritional value.

3.8.2 | Microstructure

The microstructures from the cross-section of biscuits without and with the incorporation of 10% HMS powder are illustrated in Figure 2. The control biscuits had a continuous and compact matrix with fewer voids. The pores were uniformly distributed throughout the matrix after the process of baking. The uniform pores were the result of a high level of interaction in the dough matrix. The homogeneity of the dough matrix indicated the effective incorporation of ingredients during the process of kneading and baking, which was effective in retaining the structural integrity. Notably, the gluten network through starch–starch interaction, particularly amylose–amylopectin interactions, helped in retaining the compact nature of the biscuits (Scott and Awika 2023; Hu et al. 2023). The results were in accordance with empirical rheology, texture, and the moisture content.

However, the biscuits fortified with 10% HMS powder showed a discontinuous matrix and possessed slightly larger voids in comparison with the control biscuits. The pores were unevenly distributed throughout the biscuit matrix. The larger voids were formed by the enlargement of gases produced during the process of baking. The pore was the result of poor interaction of starch–protein and starch–starch in the biscuit network. Even though 10% HMS powder was used, the protein could not form a complete interaction with starch due to the interruption by the chitin and other inorganic elements present in the matrix. However, the biscuit with 10% HMS powder fortified had the balanced texture, brittleness, taste, and mouth feel, determining the consumers' acceptability.

3.8.3 | Distribution and Content of Elements

The distribution of the elements and their content in the biscuits without and with 10% HMS powder were evaluated by SEM–EDX of the cross-section (Figure 2A,B). The elemental distribution and mapping of the control biscuits and those added with 10%

TABLE 3 | Amino acid content of Harpiosquillid mantis shrimp (HMS) powder and whole wheat biscuits fortified without and with 10% HMS powder.

Amino acid	Content (g/100 g)		
	HMS powder	Control	10% HMS added biscuit
Essential amino acids			
L-arginine	4.29	0.86	0.90
Histidine	3.68	0.44	0.48
Isoleucine	1.19	0.3	0.37
Leucine	2.49	0.8	0.94
Lysine	3.31	0.34	0.4
Methionine	0.65	0.13	0.2
Phenylalanine	1.35	0.5	0.57
Threonine	1.84	0.37	0.46
Valine	1.37	0.38	0.45
Total essential amino acids	20.17	4.12	4.77
Non-essential amino acids			
L-alanine	2.15	0.38	0.5
Aspartic acid/Asparagine	3.02	0.64	0.82
Tryptophan	0.34	0.26	0.25
Cystine	4.29	0.79	0.71
Glutamic acid/Glutamine	5.12	1.75	1.79
Glycine	3.86	0.54	0.64
Hydroxylysine	0.15	<0.01	0.01
Hydroxyproline	0.11	0.02	0.03
Proline	1.64	1.13	1.17
Serine	1.3	0.52	0.61
Tyrosine	1.09	0.19	0.25
Total non-essential amino acids	23.06	6.22	6.78

HMS powder indicated that carbon and oxygen were the most abundant elements found in both samples. Along with organic elements, the inorganic elements, such as Na, P, K, Cl, and S, were found in both samples. On the basis of the SEM–EDX, carbon (64.40%), oxygen (33.10%), Na (0.80%), Cl (0.80%), P (0.50%), K (0.30%), and S (0.10%) were found in the control biscuits, whereas, carbon (73.00%), oxygen (23.00%), Na (0.60%), Cl (1.40%), P (0.70%), K (0.60%), Ca (0.30%), S (0.30%), and Mg (0.10%) were found in the 10%HMS biscuits. The higher amounts of inorganic elements were related with a higher amount of ash content present in the 10% HMS biscuits. Ca and Mg were mainly detected in samples incorporated with 10% HMS powder. Notably, the amount of Ca, K, P, S, and Mg was higher in the fortified HMS biscuits in comparison with control samples, confirming the fortification was successful in enhancing micro and macro elements required for human consumption. The results indicated that Ca and Mg contents increased in the whole wheat biscuits with the addition of 10% HMS powder. Further analysis on ICP-OES analysis confirmed the exact contents of both Ca and Mg in the fortified HMS biscuits.

It was observed that biscuits fortified with 10% HMS had an increase in sodium by 1.1-fold, calcium by 12-fold, and magnesium by 2-fold (Table 4). The increase in sodium content was a result of HMS powder added into the biscuit. The drastic increase in calcium demonstrated that the fortified HMS biscuits could serve as calcium-enriched snacks. Similarly, fortified biscuits showed an increase in magnesium, which can serve as a source of a macro element. However, Cu was below 10 mg/kg. Among the heavy metals, lead was not detected in either sample. Mercury was detected in the 10% HMS fortified biscuits, which is too low to cause toxicity. The mercury found could be plausibly contaminated from the environment where HMS lived. Thus, marked increases in Ca and Mg contents in the biscuits could be achieved by the fortification of 10% HMS powder. As per WHO's Recommended Nutrient Intakes (RNIs) for Ca is 1000 mg for children and adults. Hence, the consumption of HMS fortified biscuits would meet the daily requirement of a normal human being. Similar products, such as crackers, butter cake, and fermented pork with high calcium content, were reported by Benjakul and Karnjanapratum (2018), Salem and Habiba (2013),

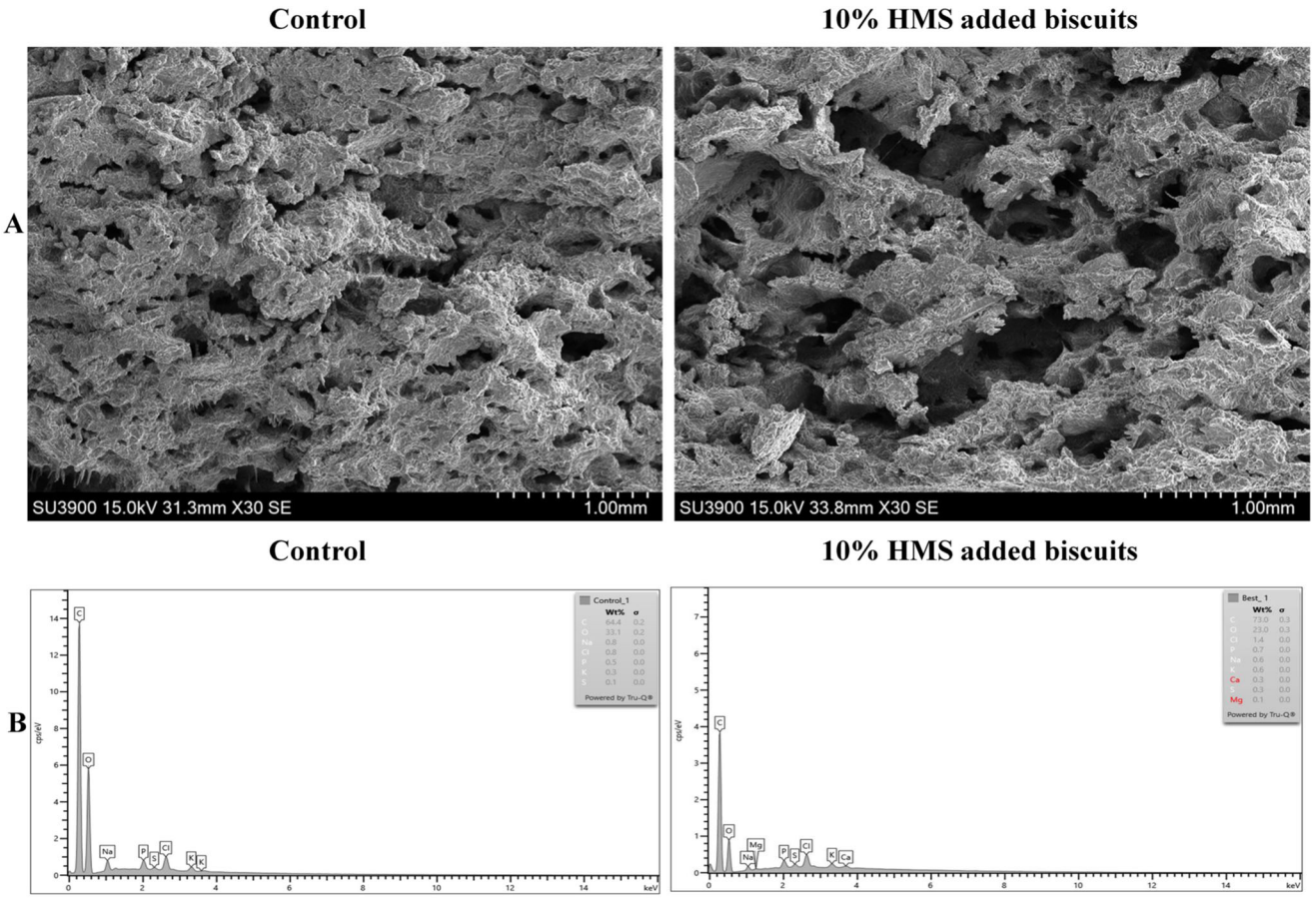

FIGURE 2 | Scanning electron microscopic images (A) and EDX spectra of a cross-section (B) of whole wheat cookie fortified without (A) and with 10% HMS powder (B). Magnification: 30X. HMS, Harpiosquillid mantis shrimp.

TABLE 4 | Minerals, heavy metals and hydroxymethylfurfural (HMF) content in whole wheat biscuits without and with 10% Harpiosquillid mantis shrimp (HMS) powder.

Minerals, heavy metals and HMF contents (mg/kg)	Control	10% HMS
Sodium (Na)	9009 ± 140^{b}	9994 ± 100^{a}
Calcium (Ca)	547.0 ± 1.3^{b}	6545 ± 87^{a}
Copper (Cu)	<10.00	<10.00
Magnesium (Mg)	830.2 ± 9.9^{b}	1669 ± 3^{a}
Lead (Pb)	ND	ND
Mercury (Hg)	ND	<0.0050
Hydroxymethylfurfural (HMF)	98.8	48.18

Note: Lowercase superscripts within the same row indicate significant differences ($p < 0.05$).

and Daengprok et al. (2002). Overall, the biscuits fortified with 10% HMS powder could serve as mineral-enriched snacks.

3.8.4 | HMF Content

In general, HMF is formed in bakery products, especially in biscuits. The high temperatures during the baking process led to from HMF via Maillard reaction and caramelization (Hu, Wang, Huang et al. 2022; Hu, Wang, Shen et al. 2022; Dong et al. 2022). Usually, HMF formation is influenced by the baking temperature, baking time, and ingredients used in the formulation (de Borba et al. 2023). HMF is a toxic chemical compound associated with cancer and anaphylactic reactions (Greilberger et al. 2025). HMF content in the biscuit typically ranges between 0.5 and 182.5 mg/kg (Roldan et al. 2026). The higher levels of HMF are found in whole grain products and products containing higher levels of sugar. In the current study, the HMF content

in the control biscuits was 98.8 mg/kg, which was higher than that of 10% HMS-fortified biscuits (48.18 mg/kg). This result indicated that the fortification with HMS powder prevented the formation of HMF during baking. Ca or Mg might interfere with the glycation process, thereby lowering the HMF formation. In addition, the presence of salts, especially containing the Ca^{2+} ions stabilizes the zwitterionic form of amino acids by complex formation, thereby blocking nucleophilic attack on carbonyl compounds and formation of HMF (Taş et al. 2023). Zou et al. (2015) reported that cysteine and glycine reduced the formation of HMF. The interaction between HMF and amine group during Maillard reaction also occurred (Zou et al. 2015). As HMF contains a carbonyl group and two double bonds (α,β-unsaturated carbonyl compounds), cations, such as Ca^{2+}, could interact with those carbonyl groups through electrostatic attraction. Hence, the glycation between carbonyl groups and amino groups could be impeded, thus lowering the formation of HMF in the biscuits. Thus, the fortification with HMS powder not only enhanced the nutritive value of the fortified biscuits but also prevented the formation of toxic substances such as HMF (Hu, Wang, Huang et al. 2022). Overall, the fortification of biscuits with HMS powder enhanced protein content, amino acids, macro elements, and prevented the formation of HMF content.

4 | Conclusion

The fortification of whole wheat biscuits with HMS powder successfully improved the nutritional profile of whole wheat biscuits particularly in terms of proteins, amino acids, calcium, and magnesium and lowered the HMF content. Nevertheless, the addition of HMS powder led to a softer texture and a darker appearance, especially when increasing concentrations were employed. The consumer liking score revealed that the fortification with 10% of HMS provided the increased acceptability. HMS powder also prevented the development of HMF in the resulting biscuits. Ultimately, the rejected autolyzed HMS could be exploited as a functional ingredient, offering a sustainable approach to produce health-oriented products for diverse consumer groups.

Author Contributions

Mallikarjun Chanchi Prashanthkumar: conceptualization, investigation, methodology, writing – original draft, software, formal analysis, data curation, visualization. **Umesh Patil**: methodology, writing – review and editing. **Suriya Palamae**: methodology, writing – review and editing. **Natchaphol Buamard**: writing – review and editing. **Jirakrit Saetang**: writing – review and editing. **Bin Zhang**: writing – review and editing. **Soottawat Benjakul**: funding acquisition, validation, visualization, project administration, supervision, resources, writing – review and editing.

Acknowledgments

Authors would like to thank International Center of Excellence in Seafood Science and Innovation for providing the facilities to carry out this research.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

References

A. International. 2023. "Official Methods of Analysis of AOAC International." (G. W. Latimer Jr., Ed.; 22nd ed.). *AOAC International*. Oxford University Press. https://doi.org/10.1093/9780197610145.001.0001.

Ahmad, A. S., S. Palamae, B. Zhang, J. T. Kim, H. Hong, and S. Benjakul. 2026. "Single and Combined Emerging Preservation Technologies Affect Quality and Microbiota of Refrigerated Pacific White Shrimp." *Food Control* 188: 112254. https://doi.org/10.1016/j.foodcont.2026.112254.

Ammar, I., H. Gharsallah, A. B. Brahim, et al. 2021. "Optimization of Gluten-Free Sponge Cake Fortified With Whey Protein Concentrate Using Mixture Design Methodology." *Food Chemistry* 343: 128457. https://doi.org/10.1016/j.foodchem.2020.128457.

AOAC International. 1990. *Official Methods of Analysis of the Association of Official Analytical Chemists*. AOAC International.

Bai, C., J. Zhu, G. Xiong, et al. 2023. "Fortification of Puffed Biscuits With Chitin and Crayfish Shell: Effect on Physicochemical Property and Starch Digestion." *Frontiers in Nutrition* 10: 1107488. https://doi.org/10.3389/fnut.2023.1107488.

Benjakul, S., and S. Karnjanapratum. 2018. "Characteristics and Nutritional Value of Whole Wheat Cracker Fortified With Tuna Bone Bio-Calcium Powder." *Food Chemistry* 259: 181–187. https://doi.org/10.1016/j.foodchem.2018.03.124.

de Borba, V. S., A. C. Lemos, M. H. P. Rodrigues, M. B. R. Cerqueira, and E. Badiale-Furlong. 2023. "Acrylamide and Hydroxymethylfurfural in Cakes: An Approach to Reduce the Formation of Processing Contaminants in Sweet Bakery Products." *Food Research International* 165: 112518. https://doi.org/10.1016/j.foodres.2023.112518.

Chanchi Prashanthkumar, M., X. Fan, Q. Zhao, U. Patil, J. Saetang, and S. Benjakul. 2024. "Effects of Decapitation in Combination With Pulsed Electric Field and Chitooligosaccharide Pretreatment on Freshness and Physicochemical Properties of Muscle in Harpiosquillid Mantis Shrimp (*Harpiosquilla raphidea*) During Storage." *International Journal of Food Science and Technology* 59, no. 9: 6443–6453. https://doi.org/10.1111/ijfs.17389.

Chanchi Prashanthkumar, M., W. Temdee, A. Mittal, et al. 2024. "Impact of Prior Pulsed Electric Field and Chitooligosaccharide Treatment on Trypsin Activity and Quality Changes in Whole and Beheaded Harpiosquillid Mantis Shrimp During Storage in Iced Water." *Foods* 13, no. 1: 28. https://doi.org/10.3390/foods13010028.

Chanchi Prashanthkumar, M., W. Temdee, W. Suyapoh, et al. 2023. "Effects of Proteases on Textural Softening and Changes in Physical Property of Harpiosquillid Mantis Shrimp (*Harpiosquilla raphidea*) During the Iced Storage." *International Journal of Food Science & Technology* 58, no. 12: 6372–6385. https://doi.org/10.1111/ijfs.16745.

Chauhan, V., S. Ahalavat, A. Singh, A. Singh, S. Verma, and S. K. Maurya. 2025. "Characterization of Biscuits Fortified With Shrimp (*Litopenaeus vannamei*) Waste Protein Concentrate." *Indian Journal of Ecology* 52, no. 4: 821–828. https://doi.org/10.55362/IJE/2025/4580.

Daengprok, W., W. Garnjanagoonchorn, and Y. Mine. 2002. "Fermented Pork Sausage Fortified With Commercial or Hen Eggshell Calcium Lactate." *Meat Science* 62, no. 2: 199–204. https://doi.org/10.1016/S0309-1740(01)00247-9.

Dimassi, O. 2026. "Water Activity as a Central Determinant of Tahini Quality, Safety, and Shelf Stability." *Journal of Food Science* 91, no. 1: e70810. https://doi.org/10.1111/1750-3841.70810.

Dong, L., C. Qiu, F. Wei, Z. Yu, Y. Zhang, and S. Wang. 2022. "The Effect of Microwave Baking Conditions on the Quality of Biscuits and the Control of Thermal Processing Hazards in the Maillard Reaction." *Frontiers in Nutrition* 9: 825365. https://doi.org/10.3389/fnut.2022.825365.

Edwards, N. M., and J. E. Dexter, and M. G. Scanlon. 2002. "Starch Participation in Durum Dough Linear Viscoelastic Properties." *Cereal Chemistry* 79, no. 6: 850–856. https://doi.org/10.1094/CCHEM.2002.79.6.850.

Greilberger, J., G. Feigl, M. Greilberger, S. Bystrianska, and M. Greilberger. 2025. "5-Hydroxymethylfurfural: A Particularly Harmful Molecule Inducing Toxic Lipids and Proteins?" *Molecules* 30, no. 19: 3897. https://doi.org/10.3390/molecules30193897.

Hackenberg, S., M. Jekle, and T. Becker. 2018. "Mechanical Wheat Flour Modification and Its Effect on Protein Network Structure and Dough Rheology." *Food Chemistry* 248: 296–303. https://doi.org/10.1016/j.foodchem.2017.12.054.

Harmon, C. P., O. M. Ahmed, and P. A. Breslin. 2024. "Amino Acid Bitterness: Characterization and Suppression." *Journal of Agricultural and Food Chemistry* 72, no. 41: 22753–22765. https://doi.org/10.1021/acs.jafc.4c05281.

Hashem, A. M., S. D. Sakhare, and T. G. Kudre. 2023. "Effect of *Piaractus brachypomus* Fish Protein Hydrolysate on Physicochemical, Sensory, and Storage Properties of Cookies." *Biocatalysis and Agricultural Biotechnology* 51: 102761. https://doi.org/10.1016/j.bcab.2023.102761.

Honrado, A., P. Ardila, P. Leciñena, J. A. Beltrán, and J. Calanche. 2024. "Nutritional Enrichment of Cereal Foods: Feasibility Study and Characterisation of Biscuits Fortified With Seabass by-products." *International Journal of Food Science and Technology* 59, no. 8: 5376–5388. https://doi.org/10.1111/ijfs.17230.

Hu, H., Y. Wang, Y. Huang, et al. 2022. "Natural Antioxidants and Hydrocolloids as a Mitigation Strategy to Inhibit Advanced Glycation End Products (AGEs) and 5-Hydroxymethylfurfural (HMF) in Butter Cookies." *Foods* 11, no. 5: 657. https://doi.org/10.3390/foods11050657.

Hu, H., Y. Wang, M. Shen, et al. 2022. "Effects of Baking Factors and Recipes on the Quality of Butter Cookies and the Formation of Advanced Glycation End Products (AGEs) and 5-Hydroxymethylfurfural (HMF)." *Current Research in Food Science* 5: 940–948. https://doi.org/10.1016/j.crfs.2022.05.012.

Hu, X., L. Cheng, Y. Hong, Z. Li, C. Li, and Z. Gu. 2023. "An Extensive Review: How Starch and Gluten Impact Dough Machinability and Resultant Bread Qualities." *Critical Reviews in Food Science and Nutrition* 63, no. 13: 1930–1941. https://doi.org/10.1080/10408398.2021.1969535.

Karnjanapratum, S., and S. Benjakul. 2018. "Coconut Oil-Based Cookies Fortified With Bio-Calcium: Characteristics and Nutritional Compositions." *Carpathian Journal of Food Science and Technology* 10, no. 4: 79–89. http://chimie-biologie.ubm.ro/carpathian_journal/index.html.

Kumar, A., V. K. Sreejith, C. O. Mohan, et al. 2023. "Nutritional, Textural and Sensory Attributes of Spiced Shrimp Fortified Biscuits." *Fishery Technology* 60: 37–43. https://www.researchgate.net/publication/368510363_Nutritional_Textural_and_Sensory_Attributes_of_Spiced_Shrimp_Fortified_Biscuits.

Li, M., Y. Cui, C. Liu, X. Zheng, and Y. Lu. 2026. "Influence of Gluten Quality and Mixing Time on the Rheological and Structural Properties of Starch-Gluten Dough." *Journal of Food Engineering* 402: 112677. https://doi.org/10.1016/j.jfoodeng.2025.112677.

Li, Y., Y. Chen, H. Liu, et al. 2026. "Improving Properties of Dough and Bread Through the Synergistic Effect of Chitosan and Fermented Whey." *Journal of the Science of Food and Agriculture* 106, no. 6: 3833–3842. https://doi.org/10.1002/jsfa.70474.

Meilgaard, M. C., G. V. Civille, and B. T. Carr. 2007. *Sensory Evaluation Techniques*. 4th ed. CRC Press.

Mora, R. L., S. P. Vanare, and R. B. Pegg. 2025. "Mechanisms, Causes, and Solutions: A Comprehensive Review of Lipid Oxidation in Low-Moisture Packaged Snacks." *European Journal of Lipid Science and Technology* 127, no. 9: e70044. https://doi.org/10.1002/ejlt.70044.

Patil, U., M. C. Prashanthkumar, K. Sharma, et al. 2025. "Development of Whole Wheat Functional Cookies Fortified With Natural Iron-Rich Hemeproteins From Asian Seabass Gills: Nutritional, Textural, and Sensory Properties." *Food Bioscience* 66: 106276. https://doi.org/10.1016/j.fbio.2025.106276.

Paula, A. M., and A. C. Conti-Silva. 2014. "Texture Profile and Correlation Between Sensory and Instrumental Analyses on Extruded Snacks." *Journal of Food Engineering* 121: 9–14. https://doi.org/10.1016/j.jfoodeng.2013.08.007.

Roldán, M., M. Mesias, and F. J. Morales. 2026. "Balancing Nutrition and Safety: Acrylamide and HMF in Biscuits From Different Cereal Flours." *Food Chemistry: X* 34: 103579. https://doi.org/10.1016/j.fochx.2026.103579.

Rombouts, I., L. Lamberts, I. Celus, B. Lagrain, K. Brijs, and J. A. Delcour. 2009. "Wheat Gluten Amino Acid Composition Analysis by High-Performance Anion-Exchange Chromatography With Integrated Pulsed Amperometric Detection." *Journal of Chromatography A* 1216, no. 29: 5557–5562. https://doi.org/10.1016/j.chroma.2009.05.066.

Salem, I., and R. Habiba. 2013. "Chemical and Rheological Characteristics of Butter Cake as Affected by Date Seed Powder Addition." *Suez Canal University Journal of Food Sciences* 1, no. 1: 13–18. https://journals.ekb.eg/article_6671_5ad0afc38960e05514283d76b8fec368.pdf.

Sasanam, S., V. Rungsardthong, B. Thumthanaruk, et al. 2021. "Properties and Volatile Profile of Process Flavorings Prepared From D-Xylose With Glycine, Alanine or Valine by Direct Extrusion Method." *Food Bioscience* 44: 101371. https://doi.org/10.1016/j.fbio.2021.101371.

Scott, G., and J. M. Awika. 2023. "Effect of Protein–Starch Interactions on Starch Retrogradation and Implications for Food Product Quality." *Comprehensive Reviews in Food Science and Food Safety* 22, no. 3: 2081–2111. https://doi.org/10.1111/1541-4337.13141.

Shan, S., D. Chen, E. Federici, O. G. Jones, and O. H. Campanella. 2022. "The Effects of Whey Protein Fibrils on the Linear and Non-Linear Rheological Properties of a Gluten-Free Dough." *Frontiers in Nutrition* 9: 909877. https://doi.org/10.3389/fnut.2022.909877.

Sharma, K., M. C. Prashanthkumar, U. Patil, L. Ma, and S. Benjakul. 2025. "Characteristics and Nutritional Value of Cookies Fortified With Debittered Salmon (*Salmo salar*) Frame Protein Hydrolysate." *Journal of Agriculture and Food Research* 19: 101732. https://doi.org/10.1016/j.jafr.2025.101732.

Sinthusamran, S., S. Benjakul, K. Kijroongrojana, and T. Prodpran. 2019. "Chemical, Physical, Rheological and Sensory Properties of Biscuit Fortified With Protein Hydrolysate From Cephalothorax of Pacific White Shrimp." *Journal of Food Science and Technology* 56, no. 3: 1145–1154. https://doi.org/10.1007/s13197-019-03575-2.

Steel, R. G. D., and J. H. Torrie. 1980. *Principles and Procedures of Statistics: a Biometrical Approach*. MacGraw-Hill Book Company.

Taş, N. G., T. Kocadağlı, D. P. Balagiannis, V. Gökmen, and J. K. Parker. 2023. "Effect of Salts on the Formation of Acrylamide, 5-Hydroxymethylfurfural and Flavour Compounds in a Crust-Like Glucose/Wheat Flour Dough System During Heating." *Food Chemistry* 410: 135358. https://doi.org/10.1016/j.foodchem.2022.135358.

Takeungwongtrakul, S., and S. Benjakul. 2017. "Biscuits Fortified With Micro-encapsulated Shrimp Oil: Characteristics and Storage Stability." *Journal of Food Science and Technology* 54, no. 5: 1126–1136. https://doi.org/10.1007/s13197-017-2545-4.

Tapia, M. S., S. M. Alzamora, and J. Chirife. 2020. Effects of Water Activity (a_w) on Microbial Stability as a Hurdle in Food Preservation. In *Water Activity in Foods: Fundamentals and Applications*, edited by G. V. Barbosa-Cánovas, A. J. Fontana Jr., S. J. Schmidt, and T. P. Labuza, 323–355. Wiley. https://doi.org/10.1002/9781118765982.ch14.

Temdee, W., A. Singh, and S. Benjakul. 2022. "Rapid Quality Deterioration of Harpiosquillid Mantis Shrimp (*Harpiosquilla raphidea*) During Iced Storage." *Journal of Food Science and Technology* 59, no. 5: 1812–1822. https://doi.org/10.1007/s13197-021-05192-4.

Wang, X., P. Peng, R. Appels, L. Tian, and X. Zou. 2022. "Macromolecular Networks Interactions in Wheat Flour Dough Matrices During Sequential Thermal-Mechanical Treatment." *Food Chemistry* 366: 130543. https://doi.org/10.1016/j.foodchem.2021.130543.

Xu, K., and J. Kuang. 2024. "Rheological, Thermal, and Structural Properties of Heat-induced Gluten Gel: Effects of Starch With Varying Degrees of Debranching." *International Journal of Biological Macromolecules* 272: 132678. https://doi.org/10.1016/j.ijbiomac.2024.132678.

Zappala, M., B. Fallico, E. Arena, and A. Verzera. 2005. "Methods for the Determination of HMF in Honey: A Comparison." *Food Control* 16, no. 3: 273–277. https://doi.org/10.1016/j.foodcont.2004.03.006.

Zhang, L., F. Guo, K. Bian, T. Zhang, et al. 2026. "Modulation of Gluten Network Structure, Dough Rheology, and Chinese Steamed Bread Quality by Wheat Bran Albumin Through Molecular Interactions With Gluten Proteins." *Food Chemistry* 527: 150637. https://doi.org/10.1016/j.foodchem.2026.150637.

Zou, Y., C. Huang, K. Pei, et al. 2015. "Cysteine Alone or in Combination With Glycine Simultaneously Reduced the Contents of Acrylamide and Hydroxymethylfurfural." *LWT—Food Science and Technology* 63, no. 1: 275–280. https://doi.org/10.1016/j.lwt.2015.03.104.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Figure: jfds71622-sup-0001-FigureS1–S3.docx