



Research article

Astaxanthin-enriched shrimp shell oil as natural preservative in *Notopterus chitala* fish balls

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Abstract

Importance of the work: Shrimp oil enriched with astaxanthin, derived from black tiger shrimp (*Penaeus monodon* Fabricius, 1798) shells, possesses strong antioxidant and antimicrobial properties. Its application as a natural preservative could enhance the quality and shelf life of fish-based products, offering a sustainable alternative to synthetic additives.

Objectives: To evaluate the effects of astaxanthin-rich shrimp oil on antioxidant and antimicrobial activities, as well as its influence on the physicochemical, textural, sensory and microbiological properties of *Notopterus chitala* (Hamilton, 1822) fish balls during refrigerated storage.

Materials and Methods: Shrimp oil was extracted from shrimp shells and analyzed for astaxanthin content and antioxidant activity (IC₅₀). The oil was incorporated into fish ball formulations at concentrations of 0–3.5%. Physicochemical parameters (pH, peroxide value, TBARS, sulfhydryl content), texture (gel strength, water-holding capacity), color, sensory attributes and microbial stability (total viable count) were monitored over 12 days of chilled storage.

Results: The shrimp oil contained 142 µg/g astaxanthin and demonstrated strong radical-scavenging activity (half maximal inhibitory concentration = 25.12 µg/mL) and antimicrobial activity against *Staphylococcus aureus*. Incorporation at 2.0–2.5% improved the gel strength, water-holding capacity and sensory acceptability. During storage, 2.5–3.5% supplementation effectively reduced pH decline, lipid and protein oxidation and microbial growth, showing preservative effects comparable to the synthetic antioxidant butylated hydroxytoluene.

Main finding: Astaxanthin-enriched shrimp oil, effectively enhanced the oxidative stability, inhibited microbial growth and maintained the physicochemical, textural and sensory qualities of *N. chitala* fish balls during refrigerated storage. At optimal incorporation levels, the preservative performance of the astaxanthin-enriched shrimp oil was comparable to that of synthetic antioxidants, highlighting its potential as a natural and sustainable alternative for seafood preservation.

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Introduction

Astaxanthin (3,3'-dihydroxy- β,β -carotene-4,4'-dione) is a potent ketocarotenoid with an antioxidant capacity reported to be over 500 times stronger than vitamin E (Higuera-Ciapara et al., 2006; Hu et al., 2019). Beyond its antioxidant effects, it has been widely investigated for its anti-inflammatory and anti-ageing properties (Silva et al., 2021; Seo et al., 2021). However, more than 90% of the global astaxanthin supply is produced synthetically, a process that is expensive and poses environmental concerns (Hu et al., 2019; Zhang et al., 2020).

Natural sources of astaxanthin include shrimp, krill and microalgae, among which shrimp shells are particularly noteworthy as a rich but underutilized source with high antioxidant potential (Panagiotakopoulos and Nasopoulou, 2024; Tran et al., 2025). Shrimp shells are a major by-product of the seafood industry and often are discarded, leading to the loss of valuable bioactive compounds such as astaxanthin (Abuzar et al., 2023; Fotodimas et al., 2024). Studies have demonstrated that astaxanthin-enriched shrimp oil, obtained from shrimp shells, can serve as a sustainable natural antioxidant for food applications (Denise et al., 2021; Barbara Stachowiak et al., 2021; Zhu et al., 2022). This oil exhibits strong antioxidant and antimicrobial activities, effectively inhibiting lipid oxidation and microbial growth, thereby helping to preserve food quality and extend shelf life (Silva et al., 2021; Zhu et al., 2022; Panagiotakopoulos and Nasopoulou, 2024; Cholidis et al., 2024). Such properties make it a promising natural preservative, particularly for perishable seafood-based products such as fish balls.

In Vietnam, *Notopterus chitala* (Hamilton, 1822) is a freshwater fish, commonly known as 'Ca Nang Hai' and valued for its low cost, high nutritional quality and firm texture, making it ideal for fish ball production (Jafri, et al., 1964; Zakaria-Ismail, 1994; Khansa et al., 2023). However, fish balls, like other comminuted fish products, are highly vulnerable to lipid oxidation and microbial spoilage due to their high moisture content and disrupted tissue structure (Seo et al., 2021; Zhu et al., 2022; Hu et al., 2025). Currently, synthetic preservatives such as butylated hydroxytoluene (BHT) and sodium benzoate are used widely to maintain the quality and extend the shelf life of such products. Nevertheless, growing consumer concerns about health risks—including allergies, carcinogenicity and associations with chronic diseases—have driven the search for natural and safer alternatives (Stratton et al., 2000; Schrenk et al., 2020). Although natural antioxidants have been applied

in various fish and surimi products, no study has yet evaluated shrimp shell-derived astaxanthin oil in preserving *N. chitala* fish balls, highlighting a clear research gap.

Therefore, the current study investigated the potential of astaxanthin-enriched shrimp oil, derived from *Penaeus monodon* Fabricius, 1798 shells, as a natural preservative in fish balls made from *N. chitala*. Unlike other studies focusing on marine or surimi-based products, this work: 1) targets a freshwater species; 2) compares the product with a synthetic preservative (BHT); and 3) upcycles shrimp shell by-products into a value-added ingredient. Collectively, these contributions should advance the understanding of natural antioxidant applications in fish-based products, while supporting sustainable food preservation and seafood industry circularity.

Materials and Methods

Astaxanthin extraction

Astaxanthin was extracted from dried *P. monodon* shells using a modified method of Tran et al. (2025). The shells were washed, dried at 60°C to constant weight and ground into fine powder. A 5 g portion of the powder was mixed with 20% (v/v) ethanol and subjected to ultrasound-assisted extraction (UAE) at 50°C and 300 W for 45 min under continuous agitation. Then, the extract was centrifuged at 6,000×g for 15 min and the solvent was removed to obtain shrimp oil enriched with astaxanthin. UAE was chosen because it enhanced cell disruption and mass transfer, enabling high recovery with reduced solvent use and a shorter processing time, whereas supercritical CO₂ extraction provides high-purity astaxanthin but requires high pressure and costly equipment (Panagiotakopoulos and Nasopoulou, 2024; Seeger et al., 2025). Furthermore, while enzymatic extraction is environmentally friendly, it is time- and cost-intensive (Zhao et al., 2019). Thus, UAE offered a practical balance between efficiency, scalability and applicability for food-related purposes.

Raw material and fish ball formulation

Fresh *N. chitala* (locally known as 'Ca Nang Hai') were procured from Phu Tan District, An Giang Province, Vietnam. The fish were filleted, deboned and skinned, followed by washing three times with chilled water (5°C) at a fish-to-water ratio of 1:3 for 10 min per cycle. This washing step was

effective at removing blood and sarcoplasmic proteins, thereby enriching the myofibrillar proteins essential for gel formation (Hoque et al., 2021). The washed meat was processed using a meat grinder. The proximate composition of the minced fish meat was: moisture 78.30%; protein 17.50 g/100 g; lipid 2.21 g/100 g; and ash 1.92% (Table 1).

Table 1 Proximate composition of *Notopterus chitala* meat (mean $\pm$ SD, $n = 3$)

Component	Content
Moisture (%)	78.30 $\pm$ 1.19
Protein (g/100g)	17.50 $\pm$ 1.05
Oil (g/100g)	2.21 $\pm$ 0.08
Total ash (%)	1.92 $\pm$ 0.06

The effects of astaxanthin-enriched shrimp shell oil were evaluated on the physicochemical and structural properties of *N. chitala* fish balls based on five formulations, with astaxanthin-enriched shrimp oil on a % weight per weight basis at 0% (F0, control), 0.5% (F0.5), 1.5% (F1.5), 2.5% (F2.5) and 3.5% (F3.5), (F0–F3.5) (Table 2). The amount of fish meat was fixed at 77.5% in all formulations to ensure comparable protein content and to maintain a consistent textural baseline among treatments. As the oil content increased, the proportion of chilled water was proportionally reduced to maintain the

total formulation at 100%. This adjustment was made to prevent excessive moisture and to ensure emulsion stability, as partial substitution of water with oil is a common approach in emulsion-type products to balance protein-lipid interactions and textural integrity (Fan et al., 2022). Other ingredients were kept constant across all treatments, specifically tapioca starch (10%), salt (1.5%) and sugar (1%). Accordingly, the total astaxanthin content in the fish balls increased from 70.56 μ g/100 g (0.5% oil) to 493.92 μ g/100 g (3.5% oil), as shown in Table 3. The formulation method was adapted from Hoque et al. (2021).

In addition, a positive control (FBHT) containing 0.02% butylated hydroxytoluene (BHT) as a synthetic antioxidant was prepared following the procedure of Trong et al. (2025). All ingredients were homogenized using a bowl cutter mixer (Bosch; Germany) under chilled conditions ($\leq 10^{\circ}\text{C}$) to prevent protein denaturation. Each emulsified mixture was molded into spherical fish balls (approximately 25 mm in diameter) and cooked in a water bath at 90°C for 15 min. After cooking, the samples were cooled to room temperature ($25\pm 2^{\circ}\text{C}$) and stored at 4°C . Analyses of pH, peroxide value, thiobarbituric acid reactive substances (TBARS), total sulfhydryl content and total viable count (TVC) were performed on days 4, 8 and 12 to evaluate the functional and oxidative stability of the products.

Table 2 Fish ball formulations (% weight per weight basis)

Ingredient	F0	F0.5	F1.5	F2.5	F3.5
Fish meat (%)	77.5	77.5	77.5	77.5	77.5
Tapioca starch (%)	10	10	10	10	10
Salt (%)	1.5	1.5	1.5	1.5	1.5
Sugar (%)	1	1	1	1	1
Shrimp oil-enriched astaxanthin (%)	0	0.5	1.5	2.5	3.5
Chilled water (%)	10	9.5	8.5	7.5	6.5
Total	100	100	100	100	100

Fish balls were formulated with astaxanthin-enriched shrimp oil at 0% (F0, control), 0.5% (F0.5), 1.5% (F1.5), 2.5% (F2.5) and 3.5% (F3.5).

Table 3 pH and functional properties (mean $\pm$ SD, $n = 3$) of fish balls formulated with different levels of astaxanthin-enriched shrimp oil: 0% (F0, control), 0.5% (F0.5), 1.5% (F1.5), 2.5% (F2.5), and 3.5% (F3.5).

Sample	Shrimp oil (%)	Protein (%)	Astaxanthin (μ g/100 g fish ball)	Gel strength (g.cm)	WHC (%)	pH	L	a	b	WI (%)
F3.5	3.50	12.95 $\pm$ 0.05	493.92 $\pm$ 0.92 ^a	3005 $\pm$ 22 ^b	72.51 $\pm$ 0.35 ^b	6.61 $\pm$ 0.02	75.87 $\pm$ 1.03 ^a	0.7 $\pm$ 0.03 ^a	3.1 $\pm$ 0.05 ^a	76.08 $\pm$ 0.24 ^a
F2.5	2.50	13.13 $\pm$ 0.05	352.80 $\pm$ 0.09 ^b	3220 $\pm$ 28 ^a	75.15 $\pm$ 0.40 ^{ab}	6.61 $\pm$ 0.02	75.64 $\pm$ 1.15 ^a	0.5 $\pm$ 0.03 ^b	2.8 $\pm$ 0.06 ^b	75.80 $\pm$ 0.21 ^a
F1.5	1.50	13.30 $\pm$ 0.05	211.68 $\pm$ 0.08 ^b	3310 $\pm$ 24 ^a	76.77 $\pm$ 0.31 ^a	6.60 $\pm$ 0.03	74.34 $\pm$ 1.03 ^{ab}	0.3 $\pm$ 0.03 ^b	1.9 $\pm$ 0.04 ^c	74.42 $\pm$ 0.23 ^{ab}
F0.5	0.50	13.48 $\pm$ 0.05	70.56 $\pm$ 0.06 ^c	3325 $\pm$ 26 ^a	77.78 $\pm$ 0.27 ^a	6.59 $\pm$ 0.02	74.05 $\pm$ 1.09 ^{ab}	0.2 $\pm$ 0.04 ^c	0.9 $\pm$ 0.04 ^d	74.06 $\pm$ 0.22 ^{ab}
F0	0.00	13.56 $\pm$ 0.05	0.00 $\pm$ 0.00 ^d	3380 $\pm$ 20 ^a	77.87 $\pm$ 0.33 ^a	6.59 $\pm$ 0.02	73.97 $\pm$ 0.94 ^b	-0.1 $\pm$ 0.04 ^d	0.4 $\pm$ 0.03 ^c	73.97 $\pm$ 0.20 ^{ab}
FBHT	-	13.56 $\pm$ 0.05	0.00 $\pm$ 0.00 ^d	3372 $\pm$ 25 ^a	77.73 $\pm$ 0.28 ^a	6.60 $\pm$ 0.02	73.11 $\pm$ 0.98 ^b	-0.2 $\pm$ 0.02 ^d	0.4 $\pm$ 0.03 ^c	73.11 $\pm$ 0.24 ^{ab}

FBHT = formulation containing the synthetic antioxidant BHT; WI = whiteness index; WHC = water holding capacity; L, a, b = CIE water-holding capacity; L*, a*, and b* = CIE color parameters representing lightness, redness, and yellowness, respectively.

FBHT contains 0.02% BHT (0.02g BHT per 100 g fish ball), whereas the F0 sample (control) contained neither BHT or shrimp oil.

Values in the same column with different superscript letters are significantly different ($p < 0.05$).

Functional analyses

The functional attributes of the fish ball samples were evaluated systematically to assess their physical stability and overall product quality. The water-holding capacity (WHC) was determined following the procedure of Fan et al. (Fan et al., 2022), in which 5 g of homogenized sample was centrifuged at $5000 \times g$ for 15 min at 4°C . The retained water was calculated as a percentage of the initial sample weight, providing an indication of the product's ability to retain moisture under processing stress. Textural integrity was assessed based on a strength measurement using a CTX texture analyzer (Brookfield, USA) equipped with a TA-AACC36 cylindrical probe. In accordance with the method described by Hong et al. (Hong et al., 2023; Nguyen et al., 2024), cube-shaped samples (25 mm per side) were compressed to 50% of their original height at a constant speed of 1 mm/s and the maximum compression force (g) was recorded as an indicator of gel strength. Surface color characteristics, based on L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness), were measured using a CR-400 colorimeter (Konica Minolta, Japan). Then, the whiteness index (WI) was calculated using Eq. (1) adapted from Jeyakumari et al. (2015):

$$WI = 100 - \sqrt{(L - 100)^2 + a^2 + b^2} \quad (1)$$

Determination of astaxanthin content and antibacterial properties of shrimp oil

The astaxanthin in the shrimp oil was quantified using first-order derivative ultraviolet-visible light spectrophotometry to enhance selectivity and to minimize interference. The oil was diluted with an ethyl acetate-to-ethanol ratio (1:1, volume per volume, v/v), centrifuged at $5,000 \times g$ and the clear supernatant was analyzed at 432 nm, characteristic of astaxanthin (Liu et al., 2011). Calibration was performed using standard astaxanthin (2.0–8.0 $\mu\text{g/mL}$).

The antibacterial activity of the shrimp oil extract enriched with astaxanthin (containing approximately 0.142 mg/mL of astaxanthin) was evaluated using the disk diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). For the disk diffusion assay, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) cultures (1×10^8 colony-forming units, CFU/mL) were evenly spread on Mueller-Hinton agar (MHA) plates (Olchowik-Grabarek et al., 2022;

Nguyen et al., 2024). Sterile filter paper discs (6 mm in diameter) were impregnated with 5 μL of the shrimp oil extract (65 mg/mL, equivalent to approximately 9.2 μg astaxanthin) and placed onto the inoculated agar surface. Sodium benzoate (50 μg) and sterile distilled water were used as the positive and negative controls, respectively. Plates were incubated at 37°C for 24 hr. The inhibition zone diameter was calculated by subtracting the disc diameter from the total diameter of the inhibition zone. The MIC was determined by diluting the shrimp oil extract in tryptic soy broth to final concentrations in the range 3.125–100 mg/mL (equivalent to approximately 0.44–14.2 $\mu\text{g/mL}$ astaxanthin). Each tube contained 10 mL of the test solution inoculated with bacterial suspension at a final concentration of 1×10^6 CFU/mL. After incubation at 37°C for 24 hr, the MIC was recorded as the lowest concentration at which no visible turbidity was observed.

To determine the MBC, 50 μL of culture from tubes showing no visible growth was streaked onto MHA plates and incubated at 37°C for another 24 hr. MBC was defined as the lowest concentration at which no colony growth was detected. The MBC-to-MIC ratio was used to assess the antibacterial nature of the extract, where a ratio ≤ 4 indicated bactericidal activity and a ratio > 4 indicated bacteriostatic activity.

Chemical and microbiological analyses

The chemical stability was evaluated based on measuring the pH of fish ball samples using a digital pH meter, following the method of Hultmann et al. (2012). Lipid oxidation was assessed based on the peroxide value (PV) and thiobarbituric acid reactive substances (TBARs), determined according to Moosavi-Nasab et al. (2018) and You et al. (2022), respectively. Protein oxidation was evaluated based on quantifying the total sulfhydryl content (TSC), following the protocols of Vossen and De Smet (2015) and Wu et al. (2022). Total volatile basic nitrogen (TVB-N), an indicator of protein degradation, was measured using the steam distillation method described by Goulas and Kontominas (2005) and the results were expressed as milligrams of N per 100 g of sample.

Microbiological quality was assessed based on the total viable count (TVC), following the pour plate technique on plate count agar as outlined by Nguyen et al. (2024). Plates were incubated at 30°C for 48 hr and microbial loads were reported in log CFU per gram. A threshold of 6 log CFU/g was used as the microbiological acceptability limit (Clinical and Laboratory Standards Institute, 2015).

Sensory evaluation of fish ball products

The sensory properties of the fish ball samples were evaluated using a consumer acceptance test based on a standardized 7-point hedonic scale, with minor modifications to the method described by Moosavi-Nasab et al. (2018). In total, 30 volunteer panelists participated in the assessment (20 females and 10 males, aged 20–22 yr), who were affiliated with the Ho Chi Minh City University of Industry and Trade (Vietnam). This method facilitated the quantification of consumer preferences and provided insight into the organoleptic quality of each formulation under controlled preparation and serving conditions.

Prior to evaluation, the food samples were equilibrated to ambient temperature ($25\pm1^{\circ}\text{C}$) and reheated in a domestic microwave oven (OX-78TS; Oxone; Indonesia) at 100°C for 3 min to ensure a consistent serving temperature. Each panelist assessed five sensory attributes: color, aroma, taste, texture and overall acceptability. Each attribute was rated on a structured 7-point hedonic scale, ranging from 1 ("dislike very much") to 7 ("like very much").

Statistical analysis

The experimental results were expressed using the mean $\pm$ SD of three repeats and data were analyzed using one-way analysis of variance, followed by Duncan's multiple-range test. All statistical analyses were processed using the Design-Expert software (version 11.0; Stat-Ease Inc., USA). All tests were considered significant at $p < 0.05$.

Results and Discussion

Antioxidant and antimicrobial activities of shrimp oil-enriched astaxanthin extracts

The shrimp oil extracted from black tiger shrimp shells contained $142\text{ }\mu\text{g/g}$ astaxanthin, a concentration similar to that reported in other crustacean by-products (Dong et al., 2014; Ambati et al., 2014), but substantially lower than the approximately 28.9 mg/g ($28,900\text{ }\mu\text{g/g}$) reported by Chintong et al. (2019) and 4.8 mg/g ($4,800\text{ }\mu\text{g/g}$) reported by Hamdi et al. (2022). These differences could be attributed to variations in the extraction method, the raw material form (oil versus whole shell), shrimp species and pretreatment procedures, highlighting the critical influence of the processing conditions on the astaxanthin yield.

Based on the DPPH assay assessment, the antioxidant potential of the shrimp oil was $12.50\text{ mg Trolox equivalents (TE)/g}$, slightly lower than for synthetic BHT (14.65 mg TE/g), while the IC_{50} value of $25.12\text{ }\mu\text{g/mL}$ indicated moderate radical-scavenging activity comparable to that of BHT ($22.30\text{ }\mu\text{g/mL}$). Although Chintong et al. (2019) reported stronger scavenging activity for shell extracts (IC_{50} of approximately $17.5\text{ }\mu\text{g/mL}$ for DPPH and approximately $7.7\text{ }\mu\text{g/mL}$ for ABTS), the current results demonstrated that even at a moderate astaxanthin concentration, the shrimp oil exhibited substantial antioxidant function. This suggests that not only the astaxanthin content but also its lipid matrix and co-extracted bioactives, such as tocopherols and phospholipids, contribute synergistically to radical scavenging, enhancing the overall antioxidant efficiency.

In parallel, the shrimp oil displayed broad-spectrum antimicrobial activity. It inhibited *Escherichia coli* (Gram-negative) with a 12.30 mm zone, an MIC of $15\text{ }\mu\text{g/mL}$ and an MBC of $35\text{ }\mu\text{g/mL}$ and *Staphylococcus aureus* (Gram-positive) with a larger zone of 14.11 mm , an MIC of $8.50\text{ }\mu\text{g/mL}$ and an MBC of $14.50\text{ }\mu\text{g/mL}$ (Table 4). The greater sensitivity of Gram-positive bacteria was consistent with their lack of a protective lipopolysaccharide outer membrane, allowing easier disruption by lipid-soluble bioactives (Liang et al., 2022; Lin et al., 2023). The MBC-to-MIC ratios for both strains were below 4, confirming a bactericidal effect (Clinical and Laboratory Standards Institute, 2015; Nguyen et al., 2024). Notably, *S. aureus* is a common foodborne pathogen and its strong inhibition by shrimp oil highlights the relevance of this natural preservative in enhancing the microbial safety of fish-based products. Furthermore, the inhibition zones observed were larger than those reported for microalgal astaxanthin extracts, such as *Haematococcus pluvialis* (Shah et al., 2016), demonstrating the superior antimicrobial efficacy of shrimp oil.

Collectively, based on these results, the shrimp oil enriched with astaxanthin functioned as a dual-purpose natural preservative. Its antioxidant and bactericidal activities likely arose from the synergistic interaction of astaxanthin with other lipid-soluble bioactives, enhancing both radical-scavenging and antimicrobial functions. The oil's comparable or superior performance to synthetic BHT in several assays underscored its potential application in improving oxidative stability and microbial safety in food systems.

Table 4 Antioxidant and antimicrobial properties of shrimp oil enriched with astaxanthin (mean $\pm$ SD, n = 3).

Parameter	Value	Bacterial strain	Zone (mm)		MIC ($\mu\text{g/mL}$)		MBC ($\mu\text{g/mL}$)	
			Shrimp oil	BHT	Shrimp oil	BHT	Shrimp oil	BHT
Astaxanthin content ($\mu\text{g/mL}$ oil)	142 $\pm$ 1.14	<i>E. coli</i>	12.30 $\pm$ 0.22	14.30 $\pm$ 0.54	15.00 $\pm$ 1.23	16.58 $\pm$ 1.24	35.00 $\pm$ 1.40	36.42 $\pm$ 1.35
DPPH _{shrimp oil} (mg TE/g)	12.50 $\pm$ 0.80	<i>S. aureus</i>	14.11 $\pm$ 0.14	15.12 $\pm$ 0.24	8.50 $\pm$ 0.91	9.45 $\pm$ 0.93	14.50 $\pm$ 0.8	15.68 $\pm$ 1.01
IC ₅₀ (Astaxanthin, $\mu\text{g/mL}$)	25.12 $\pm$ 1.50							
DPPH _{BHT} (mg TE/g)	14.65 $\pm$ 0.79							
IC ₅₀ (BHT, $\mu\text{g/mL}$)	22.30 $\pm$ 1.50							

Values are expressed as mean $\pm$ SD (n = 3). DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; IC₅₀: concentration required to inhibit 50% of DPPH radicals; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; BHT: butylated hydroxytoluene.

Influence of oil enriched astaxanthin on the physicochemical and gel properties of *N. chitala* fish balls

The incorporation of the shrimp oil rich in astaxanthin had a significant impact on both the structural and sensory properties of *N. chitala* fish balls, with effects closely dependent on the level of supplementation (Table 3). Generally, as the shrimp oil content increased, the protein gel network was weakened, as reflected by declines in the gel strength (from 3,380 g·cm in the control to 3,005 g·cm at 3.5% oil) and the WHC (from 77.87% to 72.51%). Such reductions could be attributed to the integration of lipids into the myofibrillar protein matrix, disrupting protein-protein interactions, reducing hydrophobic bonding and limiting disulfide cross-linking and ultimately, diminishing gel-forming capacity (Niu et al., 2020; Wang et al., 2024). Small decreases in the protein content further supported the contribution of lipid-protein interactions to the modified gel structure (Lin et al., 2020; Lv et al., 2024).

In contrast, shrimp oil supplementation enhanced color development due to astaxanthin deposition. The highest oil level (F3.5%) produced the greatest astaxanthin content (31.56 $\mu\text{g}/100\text{ g}$), generating a vivid reddish-orange color with elevated a^* and b^* values and an L^* of 75.87 (Table 3). The lower supplementation levels (0.5–1.5%) maintained higher gel strength and WHC but produced paler coloration (L^* values 73.97–74.34), indicating a clear trade-off between textural integrity and visual appeal. Furthermore, this balance was reflected in the sensory evaluation, where the F3.5% group, despite its enhanced color, received the lowest overall acceptability score (5.35), due to its softer texture and more pronounced oil aroma (Fig. 1). Conversely, the F2.5% group achieved the highest score (6.08), indicating that moderate supplementation provided the most favorable combination of texture, water retention, color and flavor. Lower supplementation levels retained firmer gels but lacked distinctive sensory appeal, while both the control and BHT-treated groups preserved the gel structure but were considered less visually attractive.

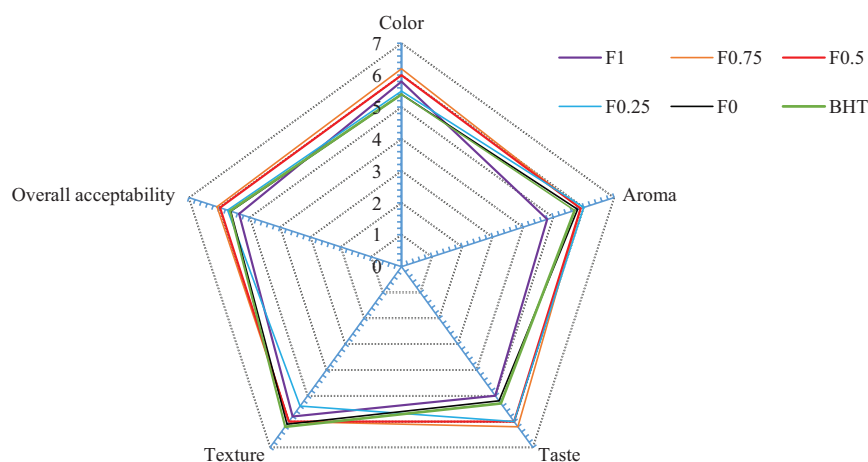


Fig. 1 Sensory spider chart of fish balls formulated with different levels of astaxanthin-enriched shrimp oil and compared with the control and BHT treatments. Sensory attributes were scored using a 7-point hedonic scale (1 = very poor, 7 = excellent). Fish balls were prepared with astaxanthin-enriched shrimp oil at 0% (F0, control), 0.5% (F0.5), 1.5% (F1.5), 2.5% (F2.5), and 3.5% (F3.5). BHT = 0.02% butylated hydroxytoluene.

These results were consistent with other findings, demonstrating that lipid incorporation could influence surimi gel functionality and quality (Niu et al., 2020; Wang et al., 2024). Collectively, the data highlighted the strong interdependence between the physicochemical properties and consumer perception. An optimal supplementation level of 2.0–2.5% shrimp oil not only would supply sufficient astaxanthin (23.67 µg/100 g) to enhance the color and flavor but would also maintain the gel integrity and WHC at satisfactory levels, representing the most effective strategy to improve both the structural quality and sensory appeal of the fish ball products. However, notably, the sensory evaluation panel was relatively small and homogenous ($n = 30$ young university students), which may limit the generalizability of the findings. Future studies with larger and more diverse consumer panels are recommended to validate these results.

Quality evolution of fish balls under cold storage

The addition of shrimp oil enriched with astaxanthin significantly influenced the overall quality and storage stability of the fish balls during storage for 12 d at 4°C, as reflected by the interrelated changes in the pH, WHC, gel strength and WI (Table 5).

At day 0, the pH values were similar across all formulations (6.59–6.61), indicating that initial acidity was unaffected by the amount of shrimp oil. During storage, the pH gradually decreased in all samples, with the control (F0) exhibiting the most pronounced drop to 5.63 by day 12. This pH decline corresponded closely with reductions in the WHC and gel strength, highlighting the interdependence between biochemical changes and structural integrity. However, the shrimp oil-enriched samples, particularly F2.5 and F3.5, exhibited a slower pH decline (6.55 for F3.5), suggesting that the antioxidant and antimicrobial properties of the astaxanthin mitigated protein degradation and microbial growth, thereby preserving water retention and gel network stability (Nikzade et al., 2019; Seo et al., 2021; Zhu et al., 2022; Draghici, 2025).

WHC closely reflected the structural effects of protein stabilization. Initially the WHC was high (approximately 74–78%) across all samples but decreased during storage due to protein denaturation and moisture loss, with the control experiencing the greatest reduction (approximately 68%). The F2.5 formulation effectively maintained WHC (approximately 74%), likely resulting from interactions between astaxanthin-enriched oil and myofibrillar proteins that reinforced the gel network.

Table 5 pH and functional properties of fish balls during 12-day storage at 4°C (Mean $\pm$ SD, $n = 3$). Fish balls were formulated with astaxanthin-enriched shrimp oil at 0% (F0, control), 0.5% (F0.5), 1.5% (F1.5), 2.5% (F2.5), and 3.5% (F3.5). FBHT = formulation containing synthetic antioxidant treatment; WI = whiteness index; WHC = water-holding capacity.

Parameters	Day	FBHT	F0	F0.5	F1.5	F2.5	F3.5	<i>p</i> Value
pH	0	6.60 $\pm$ 0.02 ^{a, A}	6.59 $\pm$ 0.01 ^{a, A}	6.59 $\pm$ 0.01 ^{a, A}	6.60 $\pm$ 0.03 ^{a, A}	6.61 $\pm$ 0.02 ^{a, A}	6.61 $\pm$ 0.02 ^{a, A}	0.864
	4	6.60 $\pm$ 0.02 ^{a, A}	6.36 $\pm$ 0.02 ^{b, AB}	6.55 $\pm$ 0.02 ^{ab, A}	6.56 $\pm$ 0.02 ^{ab, A}	6.60 $\pm$ 0.02 ^{a, A}	6.60 $\pm$ 0.02 ^{a, A}	0.033
	8	6.58 $\pm$ 0.02 ^{a, A}	6.06 $\pm$ 0.03 ^{b, B}	6.47 $\pm$ 0.03 ^{ab, AB}	6.50 $\pm$ 0.03 ^{ab, AB}	6.56 $\pm$ 0.02 ^{a, AB}	6.58 $\pm$ 0.02 ^{a, A}	0.029
	12	6.56 $\pm$ 0.03 ^{a, A}	5.63 $\pm$ 0.03 ^{c, C}	6.21 $\pm$ 0.03 ^{b, B}	6.35 $\pm$ 0.03 ^{ab, B}	6.51 $\pm$ 0.03 ^{a, AB}	6.55 $\pm$ 0.03 ^{a, AB}	0.021
	<i>p</i> Value	0.051	0.042	0.028	0.035	0.032	0.037	
WHC (%)	0	77.73 $\pm$ 0.28 ^{a, A}	77.87 $\pm$ 0.33 ^{a, A}	77.78 $\pm$ 0.27 ^{a, A}	77.77 $\pm$ 0.31 ^{a, A}	76.15 $\pm$ 0.40 ^{a, A}	74.51 $\pm$ 0.35 ^{a, A}	0.071
	4	76.52 $\pm$ 0.30 ^{a, A}	73.42 $\pm$ 0.33 ^{b, B}	74.84 $\pm$ 0.29 ^{ab, B}	76.34 $\pm$ 0.32 ^{a, A}	76.15 $\pm$ 0.32 ^{a, A}	72.03 $\pm$ 0.37 ^{b, B}	0.048
	8	72.16 $\pm$ 0.31 ^{b, AB}	70.91 $\pm$ 0.34 ^{c, C}	72.34 $\pm$ 0.30 ^{b, C}	75.81 $\pm$ 0.33 ^{a, AB}	72.13 $\pm$ 0.33 ^{b, B}	68.73 $\pm$ 0.36 ^{c, C}	0.022
	12	69.66 $\pm$ 0.33 ^{b, B}	68.34 $\pm$ 0.36 ^{b, D}	68.74 $\pm$ 0.32 ^{b, D}	74.24 $\pm$ 0.34 ^{a, B}	69.8 $\pm$ 0.35 ^{b, C}	68.12 $\pm$ 0.40 ^{b, C}	0.029
	<i>p</i> Value	0.032	0.021	0.035	0.044	0.033	0.028	
WI (%)	0	73.11 $\pm$ 0.24 ^{b, A}	73.97 $\pm$ 0.20 ^{b, A}	74.06 $\pm$ 0.22 ^{ab, A}	74.42 $\pm$ 0.23 ^{ab, A}	75.80 $\pm$ 0.21 ^{a, A}	76.08 $\pm$ 0.24 ^{a, A}	0.046
	4	73.02 $\pm$ 0.25 ^{b, A}	71.28 $\pm$ 0.22 ^{c, B}	73.09 $\pm$ 0.24 ^{b, A}	74.70 $\pm$ 0.24 ^{ab, A}	75.42 $\pm$ 0.23 ^{a, A}	76.00 $\pm$ 0.25 ^{a, A}	0.037
	8	72.97 $\pm$ 0.26 ^{b, A}	70.01 $\pm$ 0.24 ^{b, B}	71.84 $\pm$ 0.25 ^{b, B}	72.57 $\pm$ 0.25 ^{b, B}	74.39 $\pm$ 0.24 ^{a, A}	75.11 $\pm$ 0.25 ^{a, A}	0.021
	12	72.61 $\pm$ 0.27 ^{ab, A}	67.6 $\pm$ 0.26 ^{c, C}	70.64 $\pm$ 0.26 ^{b, B}	71.35 $\pm$ 0.26 ^{b, B}	73.19 $\pm$ 0.26 ^{a, AB}	74.26 $\pm$ 0.27 ^{a, AB}	0.025
	<i>p</i> Value	0.045	0.029	0.035	0.027	0.044	0.047	
Gel strength (g/cm)	0	3650 $\pm$ 45 ^{a, A}	3680 $\pm$ 40 ^{a, A}	3525 $\pm$ 46 ^{a, A}	3350 $\pm$ 44 ^{b, A}	3220 $\pm$ 48 ^{b, A}	3205 $\pm$ 42 ^{b, A}	0.041
	4	3420 $\pm$ 47 ^{a, A}	3406 $\pm$ 42 ^{a, A}	3390 $\pm$ 47 ^{a, B}	3220 $\pm$ 43 ^{ab, A}	3195 $\pm$ 46 ^{b, AB}	3175 $\pm$ 46 ^{b, A}	0.039
	8	3180 $\pm$ 49 ^{a, B}	3180 $\pm$ 43 ^{a, B}	3140 $\pm$ 48 ^{a, C}	3075 $\pm$ 45 ^{b, B}	3055 $\pm$ 45 ^{b, B}	3020 $\pm$ 48 ^{b, B}	0.047
	12	3020 $\pm$ 50 ^{a, B}	2805 $\pm$ 45 ^{b, C}	2980 $\pm$ 49 ^{a, C}	2970 $\pm$ 47 ^{a, B}	3010 $\pm$ 47 ^{a, B}	2950 $\pm$ 50 ^{a, B}	0.031
	<i>p</i> Value	0.034	0.036	0.041	0.024	0.024	0.034	0.026

Fish balls formulated with astaxanthin-enriched shrimp oil at 0% (F0, control), 0.5% (F0.5), 1.5% (F1.5), 2.5% (F2.5) and 3.5% (F3.5); FBHT = synthetic antioxidant treatment; WI = whiteness index; WHC = water holding capacity.

For each parameter, values followed by different lowercase superscript letters within the same row are significantly different among treatments ($p < 0.05$), whereas values followed by different uppercase superscript letters within the same column are significantly different among storage days ($p < 0.05$).

Notably, the highest oil level (F3.5) slightly reduced the WHC, possibly due to the over-aggregation of proteins and oil interfering with uniform gel formation (Niu et al., 2020; Zhu et al., 2022). This retention of water was directly associated with gel strength: while all samples showed gradual weakening of the gel matrix over time, F2.5 retained superior gel strength (approximately 2,970 g/cm² at day 12), illustrating the synergistic relationship between water retention and textural resilience.

Changes in visual quality, measured as whiteness (WI), paralleled these physicochemical and structural trends. Initial WI values were in the range 73–76%; however, protein oxidation and lipid degradation during storage led to notable color loss, particularly in the control (approximately 67.6%). Shrimp oil-supplemented samples, especially F2.5 and F3.5, better preserved whiteness (approximately 74%), reflecting the combined effects of pH stabilization, reduced protein oxidation and maintained gel network integrity (Niu et al., 2020; Zhu et al., 2022; Wang et al., 2024). This interplay underscored the interconnected nature of physicochemical and sensory attributes in determining overall product quality.

Overall, supplementation with the shrimp oil enhanced fish ball storage stability by concurrently slowing pH decline, preserving WHC and gel strength and maintaining visual appeal. Among the tested levels, 2.5% shrimp oil provided the optimal balance, delivering sufficient astaxanthin to protect both structural and sensory attributes without compromising gel formation, representing the most promising formulation for extending shelf life and consumer acceptability.

Evaluation of lipid oxidation, protein degradation and microbial quality of fish balls during cold storage

Lipid oxidation, protein degradation and microbial proliferation are interdependent processes that collectively determine the sensory quality, structural stability and storage life of seafood products. In the current study, the fish balls were formulated with astaxanthin-enriched shrimp oil at 0.5%, 1.5%, 2.5% and 3.5% and compared with a control (F0) and a synthetic antioxidant treatment (FBHT). During 12 d of refrigerated storage, changes in the values for PV, TBARS, TSC, TVB-N and TVC were analyzed to evaluate oxidative, proteolytic and microbial deterioration.

As presented in Table 6, lipid oxidation was suppressed substantially in samples containing higher shrimp oil levels (2.5% and 3.5%). By day 12, these samples had PV values of 0.9–1.3 meq O₂/kg and TBARS levels of 0.27–0.33 mg MDA/kg, which increased at a significantly slower rate compared to the control (2.21 meq O₂/kg and 0.55 mg MDA/kg). The results were comparable to those of the BHT-treated group (0.82 meq O₂/kg and 0.25 mg MDA/kg), confirming that astaxanthin-enriched shrimp oil effectively inhibited both primary and secondary lipid oxidation. This strong antioxidative efficacy could be attributed to the free radical-scavenging and singlet oxygen-quenching abilities of astaxanthin, as well as the synergistic effects of its polyunsaturated lipid matrix (Zhu et al., 2022; Draghici, 2025).

Table 6 Lipid oxidation, protein oxidation, and microbial quality of fish balls during 12 d of storage at 4 °C (mean ± SD, *n* = 3). Fish balls were formulated with astaxanthin-enriched shrimp oil at 0% (F0, control), 0.5% (F0.5), 1.5% (F1.5), 2.5% (F2.5), and 3.5% (F3.5).

Parameter	Day	FBHT	F0	F0.5	F1.5	F2.5	F3.5	<i>p</i> value
PV (meq O ₂ /kg)	0	0.42±0.02 ^{a, A}	0.42±0.01 ^{a, A}	0.41±0.01 ^{a, A}	0.41±0.02 ^{a, A}	0.41±0.02 ^{a, A}	0.42±0.02 ^{a, A}	0.073
	4	0.49±0.02 ^{a, A}	0.81±0.02 ^{c, B}	0.78±0.02 ^{bc, B}	0.71±0.03 ^{b, B}	0.59±0.03 ^{a, B}	0.55±0.03 ^{a, B}	0.045
	8	0.61±0.03 ^{a, B}	1.86±0.03 ^{c, C}	1.12±0.03 ^{b, C}	1.02±0.03 ^{b, C}	0.89±0.03 ^{c, C}	0.70±0.03 ^{b, C}	0.036
	12	0.82±0.03 ^{a, C}	2.21±0.03 ^{a, D}	1.98±0.03 ^{ab, D}	1.78±0.04 ^{b, D}	1.28±0.04 ^{b, D}	0.90±0.04 ^{a, D}	0.031
	<i>p</i> value	0.045	0.023	0.023	0.039	0.031	0.021	
TBARS (mg MDA/ kg)	0	0.20±0.004 ^{a, A}	0.20±0.003 ^{a, A}	0.21±0.004 ^{a, A}	0.21±0.004 ^{a, A}	0.20±0.005 ^{a, A}	0.20±0.005 ^{a, A}	0.092
	4	0.21±0.004 ^{a, A}	0.39±0.004 ^{c, B}	0.34±0.004 ^{c, B}	0.28±0.005 ^{b, A}	0.25±0.005 ^{a, A}	0.23±0.005 ^{a, A}	0.023
	8	0.23±0.01 ^{a, A}	0.78±0.01 ^{d, C}	0.49±0.01 ^{c, C}	0.35±0.02 ^{b, B}	0.30±0.01 ^{b, B}	0.25±0.01 ^{a, B}	0.022
	12	0.25±0.01 ^{a, AB}	0.93±0.02 ^{c, D}	0.53±0.01 ^{d, D}	0.37±0.02 ^{c, B}	0.33±0.01 ^{b, B}	0.27±0.01 ^{a, C}	0.034
	<i>p</i> value	0.046	0.023	0.035	0.036	0.047	0.032	
TVB-N (mg/100g)	0	2.80±0.05 ^{a, A}	2.75±0.04 ^{a, A}	2.81±0.04 ^{a, A}	2.80±0.05 ^{a, A}	2.82±0.05 ^{a, A}	2.82±0.06 ^{a, A}	0.067
	4	2.82±0.05 ^{a, A}	4.88±0.05 ^{c, B}	3.88±0.05 ^{c, B}	2.92±0.06 ^{b, B}	3.19±0.06 ^{b, B}	2.98±0.07 ^{a, A}	0.045
	8	3.74±0.06 ^{a, B}	5.75±0.06 ^{d, C}	4.94±0.06 ^{c, C}	4.18±0.07 ^{b, C}	4.04±0.07 ^{b, C}	3.88±0.08 ^{a, B}	0.034
	12	4.16±0.07 ^{a, C}	10.22±0.07 ^{c, D}	7.22±0.07 ^{d, D}	5.28±0.08 ^{c, D}	4.36±0.08 ^{b, C}	4.41±0.09 ^{b, C}	0.029
	<i>p</i> value	0.035	0.034	0.034	0.041	0.036	0.045	
TSC (μmol/ g protein)	0	42.28±1.05 ^{a, A}	42.15±1.12 ^{a, A}	42.25±0.99 ^{a, A}	42.02±1.07 ^{a, A}	42.84±1.20 ^{a, A}	42.52±1.12 ^{a, A}	0.078
	4	42.16±0.96 ^{a, A}	40.04±1.10 ^{b, B}	40.65±0.9 ^{b, B}	41.44±1.16 ^{ab, AB}	41.12±0.93 ^{ab, A}	41.93±0.96 ^{a, A}	0.032
	8	41.84±0.88 ^{a, A}	37.42±1.25 ^{b, C}	39.29±1.28 ^{ab, B}	40.73±1.08 ^{a, B}	41.42±1.08 ^{a, A}	41.23±1.19 ^{a, A}	0.029
	12	40.58±1.19 ^{a, AB}	35.65±1.19 ^{c, D}	38.13±1.24 ^{b, B}	40.91±0.99 ^{a, B}	40.61±1.09 ^{a, AB}	40.41±1.0 ^{a, AB}	0.035
	<i>p</i> value	0.045	0.032	0.041	0.034	0.047	0.038	

Table 6 Continued

Parameter	Day	FBHT	F0	F0.5	F1.5	F2.5	F3.5	<i>p</i> value
TVC								
(log CFU/g)	0	2.14±0.03 ^{a, A}	2.14±0.03 ^{a, A}	2.15±0.03 ^{a, A}	2.14±0.03 ^{a, A}	2.14±0.03 ^{a, A}	2.14±0.03 ^{a, A}	0.081
	4	2.38±0.04 ^{a, A}	2.89±0.04 ^{d, B}	2.52±0.04 ^{c, B}	2.50±0.04 ^{c, B}	2.48±0.04 ^{b, B}	2.45±0.04 ^{b, B}	0.030
	8	2.87±0.05 ^{a, B}	3.74±0.05 ^{d, C}	3.38±0.05 ^{c, C}	3.05±0.05 ^{b, C}	3.00±0.05 ^{b, C}	2.95±0.05 ^{a, C}	0.025
	12	3.35±0.06 ^{a, C}	4.70±0.06 ^{d, D}	3.90±0.06 ^{c, D}	3.55±0.06 ^{b, D}	3.48±0.06 ^{ab, D}	3.42±0.06 ^{a, D}	0.024
	<i>p</i> value	0.034	0.025	0.028	0.036	0.041	0.018	

FBHT = formulation containing synthetic antioxidant treatment; PV = peroxide value; TBARS = thiobarbituric acid reactive substances; TVB-N = total volatile basic nitrogen; TSC = total sulfhydryl content; TVC = total viable count. For each parameter, mean ± SD followed by different lowercase superscript within the same row are significantly different ($p < 0.05$), whereas values followed by different uppercase superscript letters within the same column are significantly different among storage days ($p < 0.05$).

Protein oxidation followed a consistent trend, as indicated by changes in the TSC. The TSC had declined by 15.5% by day 12, whereas samples containing shrimp oil retained 40.6–40.4 $\mu\text{mol/g}$ protein, comparable to the BHT treatment. The preservation of thiol groups suggested that shrimp oil limited thiol oxidation and protein-lipid crosslinking, thereby maintaining myofibrillar structure and functionality (Nikzade et al., 2019; Seo et al., 2021; Zhu et al., 2022; Draghici, 2025). These findings emphasized the mechanistic relationship between lipid and protein oxidation, where suppression of lipid peroxidation reduces reactive oxygen species formation, consequently protecting protein integrity.

Protein degradation, assessed based on the TVB-N content, also reflected improved stability. After 12 days, TVB-N values reached 10.22 mg N/100 g in the control, while treatments with 2.5% and 3.5% shrimp oil recorded only 4.96 mg N/100 g and 4.41 mg N/100 g, respectively, comparable to BHT (4.16 mg N/100 g) and well below the spoilage limit of 30 mg N/100 g (Nguyen et al., 2024; Bekhit et al., 2021). This correlation between reduced oxidative stress and lower TVB-N levels indicated that shrimp oil slowed proteolytic activity and nitrogenous compound formation effectively, thus preserving product freshness and textural quality (Zhu et al., 2022; Nguyen et al., 2024). Similarly, microbial proliferation was significantly retarded by shrimp oil supplementation. By day 12, the control reached 4.70 log CFU/g, while the shrimp oil-treated samples maintained 3.42–3.48 log CFU/g, closely matching BHT (3.35 log CFU/g). Although all samples remained below the microbiological limit of 6 log CFU/g for chilled seafood (Liu et al., 2011; Nguyen et al., 2024), the lower bacterial counts in the shrimp oil groups indicated potential antimicrobial effects, likely due to the bioactive carotenoids and polyunsaturated fatty acids inherent in shrimp oil.

Overall, incorporation of 2.5–3.5% astaxanthin-enriched shrimp oil provided comprehensive preservation effects by concurrently inhibiting lipid oxidation, maintaining protein stability and suppressing microbial growth. This integrated

protection resulted in pH stability and enhanced structural integrity, yielding preservation performance comparable to synthetic antioxidant BHT. These findings highlight the potential of shrimp oil as a natural, multifunctional preservative for seafood products. Further studies should examine its long-term stability and potential synergistic applications with other preservation technologies, such as freezing or modified-atmosphere packaging, to optimize quality and shelf life.

Conclusion

Shrimp oil extracted from shrimp shells and enriched in astaxanthin demonstrated potent antioxidant and antimicrobial activities, with efficacy comparable to the synthetic antioxidant BHT. When incorporated into *N. chitala* fish balls, the shrimp oil effectively improved color and sensory quality, while mitigating lipid and protein oxidation, protein degradation and microbial proliferation during refrigerated storage. The optimal supplementation level of 2.0–2.5% preserved gel integrity and WHC, simultaneously enhancing visual and sensory attributes. These findings underscored the potential of shrimp oil as a natural, clean-label preservative, offering a sustainable approach to valorizing shrimp byproducts. Furthermore, its application is compatible with industrial-scale seafood production, providing a cost-effective alternative to synthetic preservatives and supporting the development of high-quality, shelf-stable seafood products with improved consumer acceptance.

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Conflicts of interest

The authors declare no conflict of interest.

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