ISSN: 2347-467X, Vol. 12, No. (2) 2024, Pg. 672-685

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

The Effect of Prawns Shell Carotenoprotein Insertion on the Quality and Oxidative Stability of Tuna Eye Oil

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Abstract

The oxidation of polyunsaturated fatty acids (PUFAs) reduces the nutritional value of fish oil supplements and poses health risks due to the formation of free radicals and oxidative compounds. Consequently, preventing or minimizing oxidation in these supplements is a critical concern in the production industry. Tuna eye (TE) oil, similar to other fish oils, is highly prone to oxidation, leading to the need for antioxidant enhancement. Carotenoprotein, rich in astaxanthin from shrimp shells, is a powerful natural antioxidant that can potentially stabilize PUFAs in TE oil, but the application to TE oil has not been previously explored. Therefore, this study aimed to evaluate the effect of shrimp shell-derived carotenoprotein on the quality and oxidative stability of TE oil during storage. The proximate composition of tuna eye and shrimp by-products was analyzed during the investigation process. Initial TE oil and TE oil with added carotenoprotein were assessed for free fatty acid content, acid value, peroxide value, p-anisidine value, total oxidation value, and heavy metal content. Carotenoprotein was evaluated for its color and antioxidant activity. The TE oil was combined with 0.4%, 0.6%, and 0.8% (v/v) concentrations of carotenoprotein and examined for stability using the Schaal oven test method at 40°C. The variations in these concentrations were systematically selected to determine potential concentration-dependent effects on TE oil oxidative stability. The results showed that on day 60 of TE oil storage at room temperature, oxidative degradation was significantly influenced by carotenoprotein concentration. Free fatty acids increased to 1.39%, while the values of acid, peroxide, anisidine, and total oxidation identified at the 0.4, 0.6, and 0.8% concentrations were 1.61, 1.50, and 1.24

Article History
Received: 28 April 2024
Accepted: 08 August 2024

Keywords
Astaxanthin;
By-Products;
Biowaste;
Natural Antioxidant;
Omega-3 Fatty Acids;
Oxidative Stability;
Valorization.

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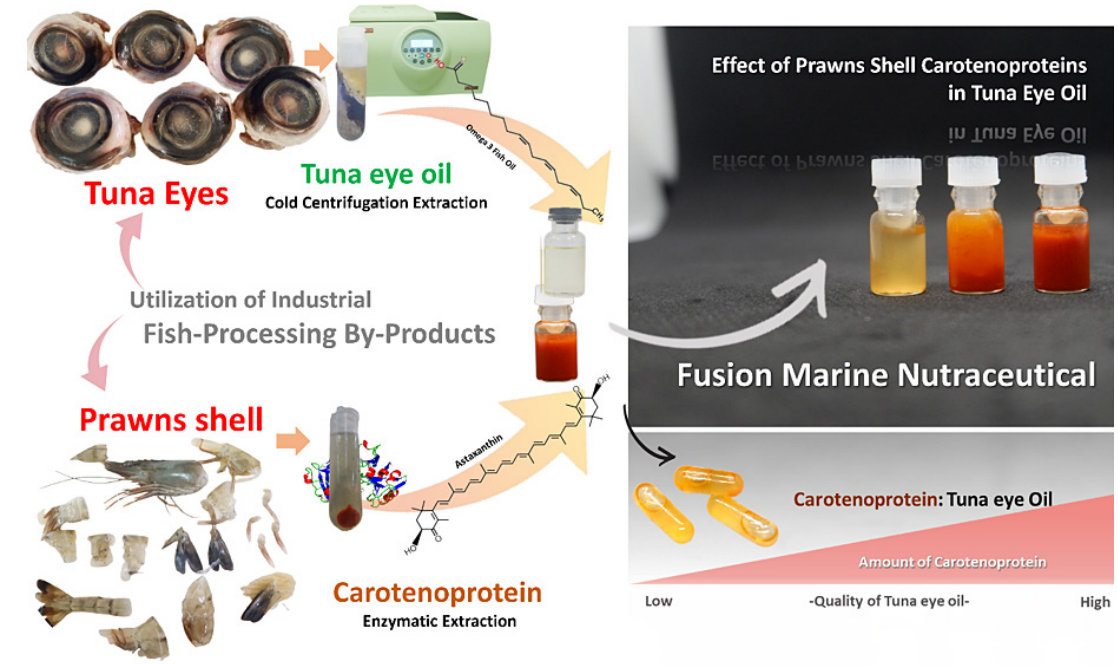
Doi: https://dx.doi.org/10.12944/CRNFSJ.12.2.15

mg KOH/kg, 18.87, 15.98, and 13.29 meq/kg, 10.80, 11.40, and 9.70 meq/kg, as well as 48.54, 43.36, and 36.28 meq/kg, respectively. Moreover, 0.8% carotenoprotein addition was found to effectively prevent TE oil deterioration compared to the control group.

Graphical Abstract



Graphical Abstract

Introduction

The incorporation of omega-3 fatty acids into the diet is recently gaining significant traction due to the essential role played in human nutrition. Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are known to be beneficial in enhancing brain development, the cognitive function of children, and anti-inflammatory properties.[1,2] These fatty acids were reported to reduce cardiovascular disease risk by improving arterial health, lipid levels, heart rate, and blood pressure.[3] However, due to the inability of the body to produce internally, omega-3 fatty acids need to be derived from an external source. Fish oil is a highly demanded option because it serves as the exclusive source of omega-3 fatty acids, including eicosapentaenoic acid (EPA c20:5) and docosahexaenoic acid (DHA c22:6).

Fish oil can be acquired from various sources, of which the most common is fatty fish such as salmon, cod, sardines, and tuna. This is generally obtained from different parts including the flesh, head, bones, fin, tail, skin, and guts. Practically in industry, approximately 50% of the fish body weight generates by-products from the processing method.[4] There is a growing trend in using these by-products as raw materials for oil production, signifying an opportunity for the usage and extraction of value from the by-products. Consequently, current studies have identified the processing of fish by-products as a novel source of omega-3.[5,6] Previously, the potential of tuna eye was reported as a viable source of omega-3, specifically with high DHA content reaching 33.44-34.96%.[7–9] Another investigation showed that the DHA content in tuna eye was 36.72%, which was higher than the 8.67% found

in sardine muscle.[10] However, DHA-rich oil is highly unsaturated and more susceptible to oxidation, leading to quality deterioration.[11]

DHA-rich oil needs to be protected from oxidation to improve product shelf life and stability through an effective approach such as the incorporation of antioxidants. Currently, synthetic antioxidants dominate the market for fish oil products, but there is growing interest in natural antioxidants due to concerns about the adverse effects of synthetic options.[12] Legislation and safety standards globally limit synthetic antioxidant use, prompting the industry to explore natural alternatives. Several studies reported the effectiveness of natural antioxidants in maintaining fish oil quality. For instance, seaweed-derived antioxidants significantly improved the stability of dogfish liver oil during accelerated oxidation studies.[13] Similarly, green tea extract (GTE) enhanced the oxidative stability of DHA-rich oil, reducing peroxide value by 85.8% compared to the control group.[14] Despite these promising results, the potential of many natural antioxidants, particularly from by-products, remains underexplored.

Trilaksani and coworkers found the potency of mangrove fruit extract as an antioxidant that can maintain the stability of the quality of TE virgin fish oil.[15] The potential of these products is often not considered, specifically when solely used for antioxidant purposes despite possessing the capability to serve as valuable single raw materials. This signifies the necessity to source alternative raw materials to replace the antioxidant role, particularly from waste in the fisheries industry. The use of by-products from the industry is becoming a significant trend among scientists and is increasingly being incorporated into the policies of many countries. However, there is a demand to use another source that does not have a conflict of interest with the demand for raw materials in the food industry and the basic human needs, including land. The exploration of a more sustainable source is still considered necessary, and a technical solution to this is using potential antioxidant sources from by-products. Recent studies have found that carotenoprotein can be derived from the by-products of fishery animals such as shrimp.

The by-products of shrimp can serve as an alternative source for carotenoprotein production due to the significant amount which can reach up to 50% of the total shrimp weight.[16] Moreover, the export value of shrimp, excluding heads and shells, is 142,000 tons, leading to a substantial quantity of unused by-products estimated at 60,000 tons.[17] Carotenoprotein, a rich source of astaxanthin, is reported to possess antioxidant properties 10 times stronger than other carotenoids, including lutein, zeaxanthin, canthaxanthin, and β-carotene as well as 100 times better than vitamins C and E.[18–20] Structural features comprising the presence of a lengthy carbon chain with conjugated double bonds and keto (C=O) groups on each ionone ring facilitate antioxidant activity by enabling effective interaction with free radicals by attracting unpaired electrons and donating electrons.[21,22] Due to these reasons, there is great potential for valorization and conversion of processed fish by-products into more valuable products. Currently, no studies have discussed the antioxidant potential of carotenoprotein derived from shrimp by-products in tuna eye (TE) oil. This represents a gap in the literature regarding the use of natural antioxidants from fishery by-products to enhance the stability of omega-3-rich oil. Therefore, this study aimed to investigate the effect of carotenoprotein derived from shrimp shell by-products on the quality and oxidative stability of TE oil during storage. The investigation performed is expected to expand the understanding of natural antioxidant sources and the efficacy in preserving oil quality, particularly in the context of using by-products from the seafood processing industry to enhance the stability and shelf life of TE oil. Additionally, the results should be promising in enhancing the sustainability of the future fish industry. The synergistic use of two valuable by-products in the production of a premium-grade dietary supplement with high health benefits for human consumption provides a novel and ample opportunity to address sustainable development goals and initiatives in the developing world.

Material and Method

Material

The material used in this study included tuna (*Thunnus albacares*) eyes obtained from PT Nutrindo Fresfood International in Bitung, North Sulawesi, Indonesia, and shrimp (*Penaeus monodon*) by-products sourced from PT Wirontono Baru, North Jakarta. The tuna eyes were transported through KM Oriental Galaxy V03 cargo ship equipped with

a freezer from Bitung City to Muara Baru port, North Jakarta. Meanwhile, shrimp by-products were transported to the laboratory in an open pick-up truck with a cool box filled with ice. The by-product materials were obtained with industry consent, ensuring ethical and relevant sourcing for conducting further studies. All samples were stored frozen (<-20 °C) until further analysis, while the entire chemicals applied for the extraction and testing of samples were of analytical grade as well as acquired from Merck (KGaA, Darmstadt, German).

Method

Sample Characteristics

The characteristics of the tuna eyes used were consistent with the investigation conducted by Trilaksani *et al*. (2023).[9] Moreover, proximate analysis was performed to determine the nutritional composition, including moisture, ash, protein, carbohydrate, and lipid content. This study specifically focused on the potential oil content in tuna eyes, which was rich in omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The potential of tuna eyes and shrimp shells as a source of raw materials was evaluated. Similarly, the characteristics of shrimp shells were examined through proximate analysis to determine the nutritional and chemical composition. Proximate analysis was crucial in estimating the carotenoid content in shrimp shells, which was particularly significant due to carotenoprotein presence. All materials, including both tuna eyes and shrimp shells, were obtained with consent from the industry for use as raw materials.

Tuna Eye (TE) Oil Extraction

TE oil extraction was conducted by following the method described by La Dia,[7] where the preparation process included separating tuna eye meat from the sclera and lenses. The eye meat was crushed into a paste which was centrifuged at 4°C and 10,000 rpm for 30 minutes to extract the oil content. TE oil was bleached using 5% magnesol XI at 50 °C for 20 minutes, then centrifuged at 10,000 rpm at 4°C for 10 minutes. Furthermore, it was analyzed for free fatty acid, acid, peroxide, and p-anisidine value, as well as total oxidation, and heavy metal, with Figure 1 presenting the extraction process.

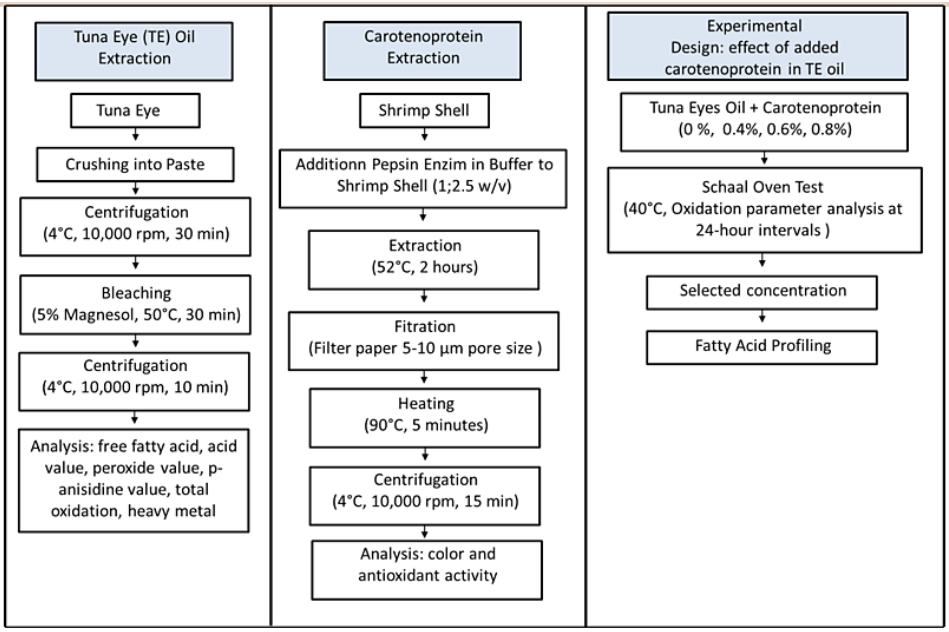


Fig.1: Study flowchart and stages

Carotenoprotein Extraction

Carotenoprotein was extracted from shrimp shells using pepsin following the method described by Babu.[23] Pepsin enzyme 2,500-3,500 units/mg was prepared in a buffer solution pH 2-3 at 52°C for maximum activity, after which shrimp shell samples

(1:2.5 w/v) were added and extracted for 2 hours. The extraction results were separated using filter paper with a pore size of 5-10 µm, then the filtrate was heated at 90°C for 5 minutes to stop the enzyme reaction. The filtrate was centrifuged at 10,000 rpm for 15 minutes at 4°C, and the obtained carotenoprotein was analyzed for color and antioxidant activity, with Figure 1 showing this extraction process.

Experimental Design

The carotenoprotein was added to TE oil with three concentrations of 0.4%, 0.6%, and 0.8% (v/v), which were established from the enhancement and modification of a previous study.[24] The control group consisted of TE oil without carotenoprotein (0%), while the mixed samples were evaluated for stability using the Schaal oven test following a method described by Suseno.[25] Samples were stored at 40°C and analyzed for oxidation parameters including free fatty acid, acid, peroxide, and p-anisidine value, as well as total oxidation in 24-hour intervals, then the best treatment was examined for fatty acid profile, with Figure 1 presenting the experimental design.

Analysis

Proximate analysis

The proximate analysis examined moisture, ash, fat, and protein content according to the guidelines of the Association of Official Analytical Chemists (AOAC), while the carbohydrate content was calculated using the difference method.

Fish Oil Oxidation Analysis

Fish oil oxidation paramater were analysed according to American Oil Chemists Society (AOCS) procedures including free fatty acid (method Ca 5a 40), acid value, peroxide value (method Ca 8-53), p-anisidine value and total oxidation (Method Ca 18-90).

Antioxidant Activity Analysis

The antioxidant activity was analyzed using 2,2'-azinobis 3-ethylbenzothiazoline6sulfonic acid (ABTS) radical according to the procedure described by Sumczynski[26] with a modification in the sample solution at concentrations of 100, 200, 400, 600, 800, and 1000 ppm and The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical according to the procedure described by Wu.[27]

Color Analysis

The color of carotenoprotein was determined using a colorimeter (Lutron RGB-1002, Taiwan) by measuring the intensity of RGB colors with values ranging from 0 to 255, then converting to L*(brightness), a*(redness/greenness), and b*(yellowness/blueness) system (CIE Lab).

Data Analysis

The results of proximate analysis, heavy metal, color measurement, fatty acid profiling, and antioxidant activity were examined descriptively, while the stability value of TE oil was subjected to Analysis of Variance (ANOVA). Post-hoc analysis through the Duncan Multiple Range Test (DMRT) was conducted for significant ANOVA outputs comprising p-value <0.05, and the data were evaluated using IBM SPSS Statistics® software version 25.

Result and Discussion

Sample Characteristics

Tuna Eyes

Proximate composition analysis was conducted in this study to determine the characteristics of tuna eyes, with a specific focus on the fat content. The lipid content in various fish species can be influenced by intrinsic factors, such as size and age, as well as extrinsic factors, including seasonality, water temperature, and geographical location.[28] The accumulation of lipids in tuna eyes is also influenced by several biological and environmental factors. Tuna fish are top predators that consume prey rich in fats and oils, leading to the storage of these lipids in various body tissues, including the eyes.[9] The lipid content observed from the obtained results had the highest percentage after the moisture content. Similar studies have reported the fat content in tuna eyes to be the most substantial component following moisture, with percentages of 18.44±0.39%[29] and 18.15±0.07%,[7] respectively. Trilaksani[9] further reported variations in lipid content in tuna eyes of different sizes, presenting a positive correlation between eye size and lipid content. The results showed that lipid content of 13.11 ± 0.14% in tuna eyes was close to the amount reported by Djamaludin[30] at 13.05 ± 0.01%. These values categorize the characteristics of small-sized tuna eyes based on the classification scheme proposed by Trilaksani.[9] However, the lipid content of the tuna eyes used in this study had the highest percentage

compared to yellowfin tuna (12.04%)31 and Thunnus sp (6.11%)32, with Table 1 presenting the proximate composition of tuna eyes.

Shrimp Shell

Proximate analysis was conducted to investigate the composition of the shell and cephalothorax as the shrimp shell, with a specific focus on lipid and protein content. Lipid and protein content are considered primary parameters in determining fatty acid and carotenoprotein content. Specifically, lipid content is related to fatty acids, while protein content is associated with carotenoids in the form of carotenoprotein.[33,34] The protein content was found to be the second-largest component after moisture, with a percentage of 39.34±0.31 (dry basis). In other studies, lower protein percentages were observed in shell parts from Pacific white shrimp (*Litopenaeus vannamei*), measuring 29.37 ± 0.81%35. Both lipid and protein content showed higher percentages when compared to *L. vannamei*. Similarly, Pattanaik[18] reported that *P. monodon* had higher protein content compared to *M. affinis, P. stylifera,* and *N. tenuipes.* Based on the shell proximate composition results presented in Table 1, shrimp (*Penaeus monodon*) by-products could be recommended as a good source for protein extraction.

Table 1. Proximate composition of shrimp shell and tuna eyes

Parameters	Shrimp shell		Tuna eye	
	%(wet basis)	%(dry basis)	%(wet basis)	%(dry basis)
Moisture	66.67±0.62	-	75.21±0.06	-
Ash	0.89±0.01	2.66±0.07	6.19±0.02	24.95±0.02
Lipid	13.11±0.14	39.34±0.31	2.95±0.05	11.88±0.05
Protein	15.52±0.38	46.57±0.28	12.03±0.09	48.51±0.09
Carbohydrate	3.82±0.11	11.45±0.11	3.64±0.04	14.66±0.04

Note: Data were expressed as mean ± standard deviation

Table 2. Oxidation characteristics of TE oil

Parameter	TE Oil	CODEX
Free Fatty Acid (%)	0.54±0.07	1.50
Acid Value (mg KOH/kg)	0.59±0.07	3
Peroxide Value (meq/kg)	3.63±1.01	<5
P-Anisidine Value (meq/kg)	4.81±2.15	<20
Total Oxidation (meq/kg)	12.04±2.39	<26

Note: Data were expressed as mean ± standard deviation. TE oil = tuna eye oil

Characteristics of TE Oil

Quality of TE Oil

The determination of the quality of TE oil aimed to ascertain the value of oxidation parameters in the oil. The process of oxidation in fish oil, particularly those rich in EPA and DHA, is crucial to understand due to the impact on oil quality and stability. Lipid oxidation is an autoxidation process featuring free radical reactions with unsaturated fatty acids. This process starts with the formation of free radicals from unsaturated fatty acids through initiation, followed by propagation and termination, producing various oxidation products such as hydroperoxides, aldehydes, ketones, and alcohols. The formation of primary oxidation products, including hydroperoxides, is the initial stage in lipid oxidation. These products subsequently decompose into secondary oxidation products, including aldehydes and ketones, which

are known to contribute to the characteristic fishy odor of fish oil.[11,36–38] Specifically, the obtained results showed that TE oil complied with the acceptable limits of oxidation parameters as defined by the CODEX. This was significant considering previous investigation stating that the PV levels in Skipjack Tuna Eyeball oil exceeded the established standard (>5 meq/kg),[39] signifying a higher oxidation level compared to TE oil. The quality of TE oil analysis on free fatty acid, acid value, peroxide value, p-anisidine value, and total oxidation are presented in Table 2.

Heavy Metal

The accumulation of heavy metals in marine environments and the subsequent uptake by marine organisms is a critical aspect of environmental toxicology. Heavy metals such as arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) are known to be some of the most severe pollutants due to the persistence, bioaccumulation, and toxicity. These tend to be stored in various organs, hence the accumulation in fish oil is of utmost concern from a safety perspective. As, Pb, Cd, and Hg are categorized to be highly harmful when consumed above the toxicity levels. Moreover, the presence of heavy metal content in fish oil has been found with the potential to accelerate oxidation.[36]

TE oil used in the experiment conducted in this study was pre-purified based on the optimal method from a previous report.[7] The result showed that As, Pb, Cd, and Hg were not detected in TE oil. This complied with the standards set by CODEX, which state the maximum allowable limits of heavy metal contamination in fish oil to be no more than 5 mg/kg for arsenic, 0.3 mg/kg for cadmium, 10 mg/kg for lead, and 0.5 mg/kg for mercury. However, the previous report showed the presence of mercury and lead in the crude tuna fish oil, signifying the importance of the purification process in reducing heavy metal content. Since the eye is among the organs with the tendency to accumulate heavy metals, a purification step is necessary to ensure the safety of the final product for human consumption. Lee[40] conducted a safety assessment of several commercial fish oil supplements and reported no detectable heavy metals. The purification process used in obtaining TE oil should adhere to the established standards for sourcing fish oil supplements, and the heavy metals are presented in Table 3.

Table 3: Heavy metals of TE oil

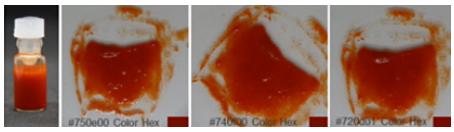
Parameter	Result	Standard
Arsenic (As)	not detected	≤5 mg/kg
Cadmium (Cd)	not detected	≤ 0.3 mg/kg
Lead (Pb)	not detected	10 mg/kg
Mercury (Hg)	not detected	≤ 0.5 mg/kg

Characteristics of Carotenoprotein

Color of Carotenoprotein

Crustaceans such as shrimp and lobsters, comprise astaxanthin specifically bound to certain proteins called crustacyanin (CRCN), forming carotenoprotein. The interaction between astaxanthin and crustacyanin leads to a bathochromic shift, changing the wavelength of light absorbed, thereby altering the visible color of the exoskeleton.[41] Crustacyanin exists in two primary forms including α-crustacyanin and β-crustacyanin, each responsible for different colors observed in crustaceans. The binding of astaxanthin to this protein causes conformational changes in the structure, leading to various color expressions such as purple, blue, blue-black, and yellow.[42] The measurement of color in carotenoprotein, as represented by the Lab* color space, provides quantitative data on the lightness and the red-green and blue-yellow color components. The L* value measures the lightness from 0 (black) to 100 (white), where a* represents the red (positive) to green (negative) axis, and b* represents the yellow (positive) to blue (negative) axis.[43] The L* value signified the lightness of carotenoprotein, with positive a* values suggesting astaxanthin color to be dominated by redness, and positive b* values denoting domination of yellowness. The color name of carotenoprotein with hex code #810000 was deep maroon with RGB (129, 0, 0) and HSV values (hue: 0°, saturation: 100%, brightness: 51%) observed in this study reflecting the high concentration and specific binding interactions of astaxanthin in the shrimp carapace. In the context of fish oil formulations, evaluating the color of carotenoprotein can provide insights into the stability and concentration of astaxanthin, which is not only a significant antioxidant but also a valued pigment for consumer appeal,[44] with Table 4 presenting the carotenoprotein color.

Table 4. Color of shrimp shell carotenoprotein

Parameter	Result	Color Visual
Lightness (L*)	24.67 ± 0.58	
Redness (a*)	42.67 ± 0.58	
Yellowness (b*)	37.00 ± 0.00	

Note: data were expressed as mean ± standard deviation

Antioxidant Activity of Carotenoprotein

The antioxidant activity of carotenoprotein, particularly astaxanthin, is a well-investigated topic due to the remarkable capability to scavenge free radicals. Astaxanthin is a xanthophyll carotenoid with a unique chemical structure that includes hydroxyl (OH) and keto (C=O) groups, as well as a significant number of conjugated double bonds.[45] This chemical structure allows astaxanthin to effectively neutralize free radicals and function as a hydrogen donor, which is crucial for the antioxidant activity.[46] Furthermore, the antioxidant activity is commonly measured by parameters such as IC_{50}(Inhibition Concentration 50), which represents the concentration of a compound required to inhibit 50% of free radical activity, with a lower IC_{50} value <50 signifying a very powerful antioxidant capacity.[47] Figure 1A presents the IC_{50} value of carotenoprotein at 7.90±2.83 µg/mL, signifying that carotenoprotein shows higher antioxidant activity compared to butylated hydroxytoluene (BHT) and ascorbic acid. When compared to BHT and ascorbic acid, carotenoprotein antioxidant activity was 2.5 times stronger and 1.9 times higher, respectively. This result was consistent with Chintong[48] who reported an IC_{50} value of 7.7±0.6 µg/mL for astaxanthin extracted from shrimp by-products. Similarly, the previous report showed that astaxanthin extracted from shrimp waste (Penaeus indicus) had a strong antioxidant capacity, comparable to the well-known antioxidant α-tocopherol49. In this study, the concentration of the sample ranged from 100 to 1000 mg/mL, as presented in Figure 1B. The inhibition of the sample had a linear increase with rising concentration, as shown in Figure 2 presenting the antioxidant activity of shrimp shell carotenoprotein.

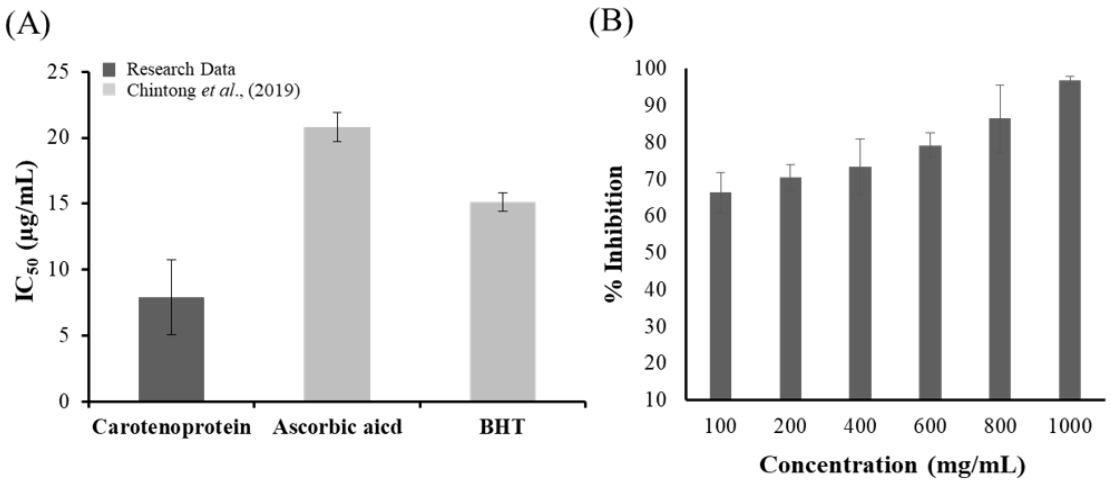


Fig. 2: Antioxidant activity of carotenoprotein. IC_{50} value (A), percent inhibition (B)

Oxidative Stability of TE Oil During Schaal Oven Test

The stability of fish oil refers to the ability to maintain the physical properties and characteristics during storage. To evaluate the temporal-chemical changes occurring during 60 days, the oxidation parameters of TE oil were measured. These

parameters included free fatty acid, acid, peroxide, and p-anisidine value, as well as total oxidation. The results showed significant changes in all oxidation parameters for TE oil without carotenoprotein addition. In Figures 2a and 2b, after 60 days, the free fatty acids and acid values in TE oil still conform to CODEX standards. However, TE oil with added carotenoprotein had significantly slower development of oxidation compared to the control. After 45 days of observation, the control samples exceeded the recommended limit for peroxide value (5 meq/kg) and total oxidation (26 meq/kg), and a similar trend was observed in the fish oil with 0.2% added carotenoprotein. However, the samples with 0.4% and 0.8% added carotenoprotein showed a more gradual increase in oxidation parameters all through the entire experiment.

High concentrations of carotenoprotein were found to significantly delay oxidation parameters, suggesting that carotenoprotein might prevent the production of oxidation products in TE oil. This natural antioxidant has comparable and competitive performance with synthetic antioxidants including TBHQ, BHA, and BHT as reported by Mishra,[50] signifying that the incorporation of antioxidants is a highly efficient method to mitigate lipid oxidation in fish oil. The effectiveness of carotenoprotein as an antioxidant can be attributed to the chemical composition, particularly the presence of conjugated double bonds and functional groups including hydroxyl and keto groups. These components enable carotenoprotein to scavenge free radicals and inhibit the formation of reactive oxygen species (ROS), thereby preserving the integrity of TE oil.

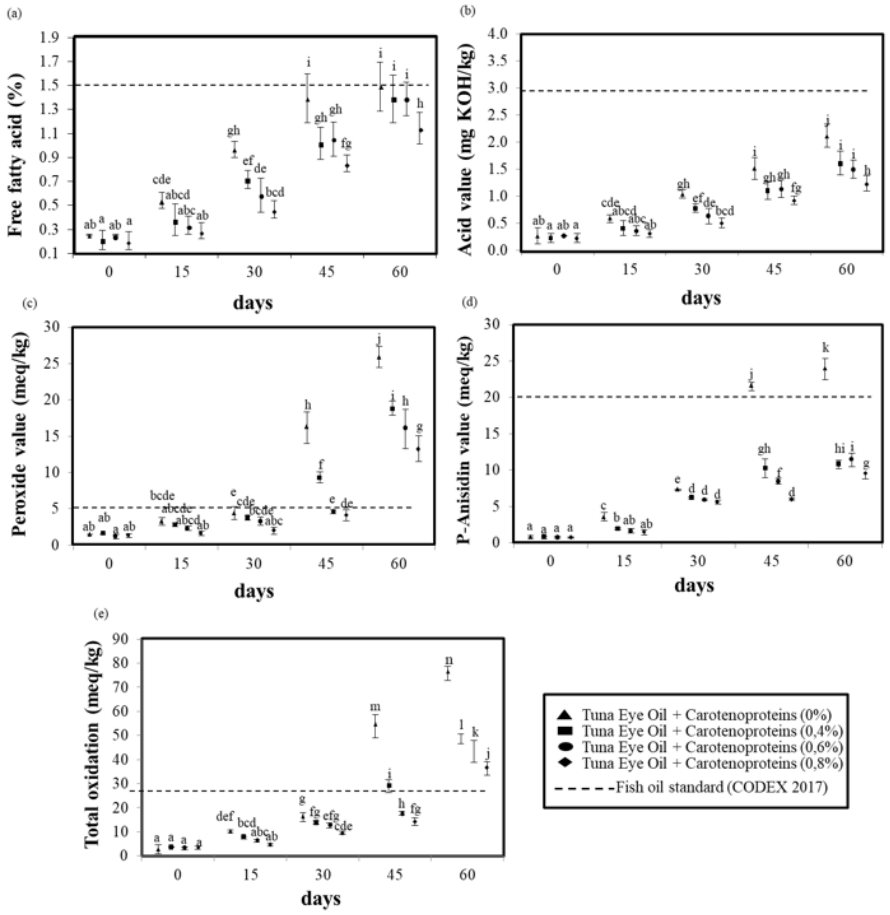


Fig. 3: The effect of carotenoprotein on the quality of TE oil during storage in the Schaal oven test: free fatty acid (a), acid value (b), peroxide value (c), p-anisidine value (d), total oxidation (e). Different lowercase letters indicate statistically significant differences (p< 0.05)

The highest concentration of carotenoprotein (0.8%) included in TE oil was considered suitable for further development studies in supplementation. Successfully demonstrating the proof of concept shows the efficacy of a natural pigment derived from a sustainable source in effectively preserving TE oil quality. The concentration of 0.8% carotenoprotein had optimal efficacy in preserving the quality of TE oil, achieving approximately 50% higher oxidative stability compared to unsupplemented TE oil. This significant improvement was achieved by only increasing the antioxidant concentration in multiples of 0.2%. However, it should be noted that the fixed concentration of carotenoprotein might require adjustments based on the final formulation and regulatory considerations for fish oil supplements in different countries. An inclusion of a higher concentration of carotenoprotein could preserve TE oil quality and contribute other positive effects, including potential benefits for daily oral consumption as an additional antioxidant. Further investigation is needed to explore the potential advantages and optimize the concentration levels accordingly. The antioxidative effects of carotenoproteins on TE oil from tuna eyes are presented in Figure 3.

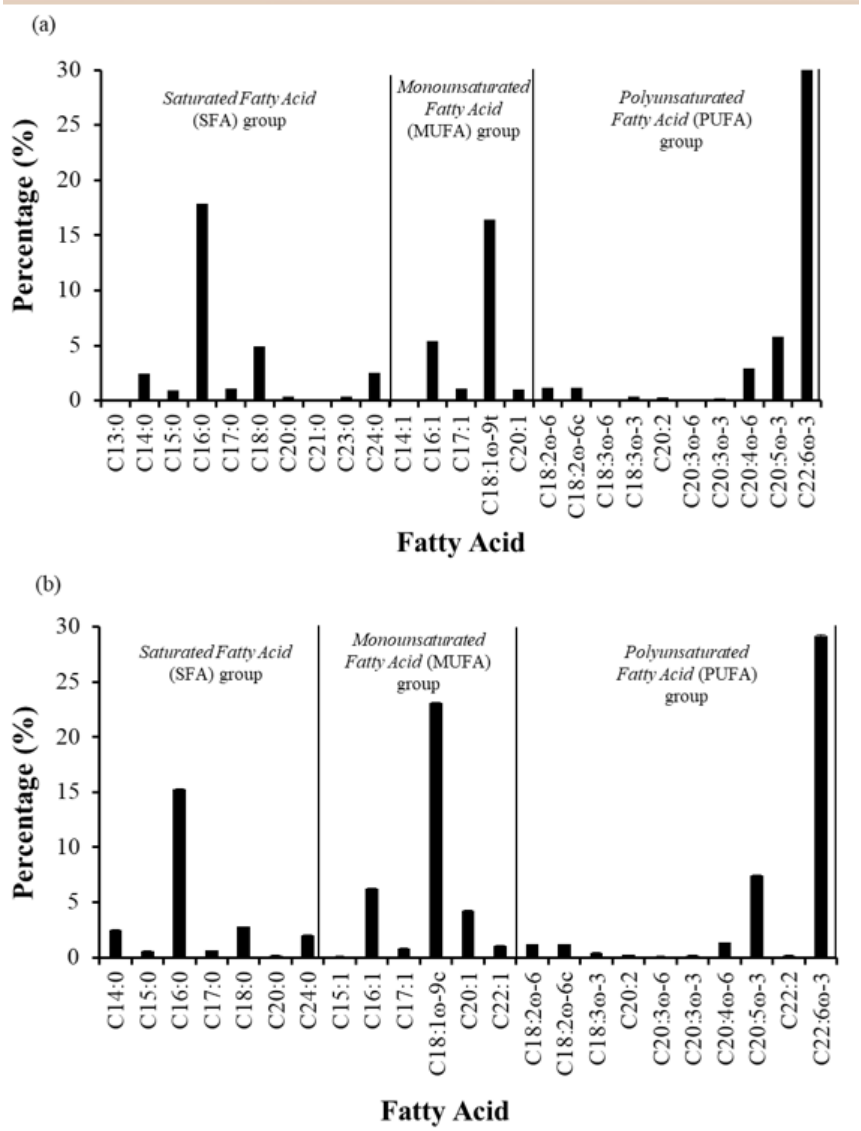


Fig. 4: Fatty acid composition: TE oil (a)7; TE oil with caronetoprotein (b)

Fatty Acid Composition

The fatty acid composition of fish oils, particularly the proportions of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), plays a critical role in determining the nutritional value and health benefits. In this study, TE oil enriched with 0.8% carotenoprotein was analyzed for the fatty acid profile, which showed distinctive characteristics specific to marine oil. The composition of TE oil supplemented with carotenoprotein consisted of 23.99±0.03% SFA, 35.57±0.02% MUFA, and 41.58±0.05% PUFA, with a DHA/EPA ratio of 3.91 and an ω-3/ω-6 ratio of 13.58. These results were consistent with previous reports on TE oil, which showed similar ratios of DHA/EPA (5.55 to 5.65) and ω-3/ω-6 (9.05 to 10.19).[7,51] Additionally, Renuka[31] reported a DHA/EPA ratio of 5.19 and a ω-3/ω-6 ratio of 9.17 in fish oil from yellowfin tuna eye. The ratios of DHA/EPA and ω-3/ω-6 are crucial indicators of the nutritional quality of fish oil, influencing the bioavailability and effectiveness in human health. The addition of carotenoprotein not only helped to maintain the oxidative parameters in TE oil but also showed high standards and had no adverse effects on the PUFA content, as the most crucial aspect of fish oil. However, there was a decrease in the proportion of SFAs and an increase in the percentage of MUFAs, with Figure 4 presenting the fatty acid composition.

Conclusion

In conclusion, this study identified significant progress in the valorization of fisheries by-products and quality maintenance. Additionally, high lipid content in tuna eyes, combined with the absence of heavy metals including As, Pb, Cd, and Hg, detected in TE oil, showed safety and nutritional value. The significant presence of omega-3 DHA in TE oil (35.53%) and the combination with carotenoprotein (29.19%) showed the potential in enhancing nutritional benefits. The high protein and lipid content in shrimp shells, along with the distinctive color characteristics of carotenoprotein, represented the rich antioxidant properties (Lightness (L*) 24.67 ± 0.58; Redness (a*) 42.67 ± 0.58; Yellowness (b*) 37.00 ± 0.00) and antioxidant activity (7.90±2.83 µg/mL). The supplementation of carotenoprotein in TE oil proved effective in preserving the quality during the Schaal oven test, particularly at a concentration of 0.8%, showing the best results till day 4 of observation at room temperature. The identified efficacy of carotenoprotein in enhancing the oxidative stability of TE oil offered a promising avenue for improving the shelf life and nutritional value of fish oil supplements. By using by-products from shrimp shells, this method would contribute to waste reduction and promote sustainable practices in the seafood industry. This study introduced novel possibilities for using natural antioxidants in food preservation, thereby promoting further investigation and potential commercialization of carotenoprotein-enriched fish oil products. Without considering the study limitations the results should not be generalized due to potential variation with other species, since the tuna eyes used were obtained from Thunnus albacares and shrimp shells were sourced from Penaeus monodon. Replicating this study using higher concentrations of carotenoprotein or pure astaxanthin extract is necessary for further advancement of understanding. The recommended method would enable a comprehensive assessment of the dual functionality of compounds in the context of fish oil supplementation. Therefore, the potential allergenicity of shrimp carotenoprotein extract should be explored, specifically concerning a group of individuals with specific seafood dietary restrictions. Understanding the allergenic properties of this extract could aid in developing safer and more inclusive seafood products that cater to diverse dietary needs and preferences.

Acknowledgment

The authors are grateful to the Ministry of Education, Culture, Research, and Technology of Indonesia for the funding assistance provided through the National Competitive Research Grant.

Funding Sources

This study was fully funded through the National Competitive Research Program with the Master's Thesis Research (PTM) scheme – DIKTI Indonesia in 2022-2023 under contract number 3870/IT3.L1/PT.01.03/P/B/2022.

Conflict of Interest

The author(s) declares no conflict of interest.

Authors' Contributions

Authors are required to provide a statement detailing the specific contributions of each author to the manuscript. Each author mentioned has significantly

and directly contributed intellectually to the project and has approved the publication.

Data Availability Statement

The data that support the results of this study are available from the corresponding author upon reasonable request.

Ethics Statement

No ethics statement is required for this study.

Informed Consent Statement

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Clinical Trial Registration

This study was not registered as a clinical trial.

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