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**ORIGINAL RESEARCH PAPER**

# ANN MODELING OF DOSE-DEPENDENT EFFICACY OF ERGOTHIONEINE-LOADED CHITOSAN NANOPARTICLES AGAINST DISCOLORATION AND LIPID OXIDATION IN REFRIGERATED YELLOWFIN TUNA CUBES

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**Abstract**

The present study evaluates the efficacy of ergothioneine-loaded chitosan nanoparticles (ECNP) in enhancing color and lipid oxidative stability in refrigerated yellowfin tuna cubes based on key indicators, including redness index (RI), metmyoglobin (metMb), total lipid hydroperoxides (HPO), and thiobarbituric acid reactive substances (TBARS). The findings revealed that ECNP effectively delayed discoloration and lipid oxidation, with its efficacy directly linked to the applied dose. Two-way ANOVA confirmed that both ECNP dose and storage time had a significant impact on discoloration and lipid oxidation ($p < 0.001$), with their interaction indicated that ECNP efficacy depends on both factors. Furthermore, artificial neural network (ANN) models were developed to predict ECNP's effectiveness. The predictions confirmed that ECNP's efficacy varies with dosage and storage duration. The ANNs determined that an ECNP dose of 367 mg/kg could maintain the quality of tuna cubes classified as grade A for up to 2.55 days, while a dose of 253 mg/kg could maintain the quality of tuna cubes classified as grade B for 5 days. These results underscore the potential of ECNP as a bio-based preservative, offering a sustainable and viable alternative to synthetic additives for seafood preservation. Additionally, ANN proved to be a valuable tool for rapidly determining the optimal ECNP dose for specific preservation goals. This approach improves cost-effectiveness and enhances practical applicability.

**Keywords**: antioxidant, ANN modeling, biopolymer, sashimi, seafood preservation

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## Introduction

Tuna is a globally important food source and contributes significantly to the economies of many countries. There are more than 48 species of tuna found in the Atlantic, Indian, and Pacific Oceans, as well as the Mediterranean Sea (Herpandi *et al.*, 2011). Tuna meat is characterized by the bright red color, which is primarily attributed to the high myoglobin (Mb) content. Consequently, color is one of the primary criteria used to evaluate the freshness of tuna meat, especially when it is used as sashimi. As tuna meat is stored under refrigerated conditions, the bright red color changes to a dark brown hue, due to the oxidation of deoxymyoglobin (deoxyMb) and oxymyoglobin (oxyMb) to metmyoglobin (metMb) (Palanisamy *et al.*, 2024; Wang *et al.*, 2024). This discoloration is closely linked to lipid oxidation, in which free radicals generated during lipid peroxidation accelerate the oxidative conversion of Mb, thereby promoting the accumulation of metMb. In turn, metMb promotes lipid oxidation by facilitating lipid hydroperoxide decomposition, creating cross-propagation of lipid and myoglobin oxidation that speeds up oxidative deterioration (Lee *et al.*, 2003; Bao *et al.*, 2009; Reeder and Wilson, 2001; Baron and Andersen, 2002). Over time, the accumulation of oxidation products from lipids and Mb results in discoloration and a decline in tuna meat quality.

Antioxidants have been demonstrated to successfully reduce these oxidative processes and help maintain the color and quality of tuna meat (Sohn and Ohshima, 2010; Palanisamy *et al.*, 2024; Palanisamy *et al.*, 2025). However, concerns over the safety and potential health risks of synthetic antioxidants have spurred interest in natural alternatives. One such alternative is ergothioneine (EGT), a naturally occurring antioxidant found in edible mushrooms such as *Flammulina velutipes*. Previous studies by the authors have shown that EGT is a promising agent for preventing discoloration in tuna meat (Bao *et al.*, 2010). The mechanism of discoloration inhibition in tuna meat was demonstrated to be due to EGT inhibiting the oxidation of Mb and lipids, which helps maintain the bright red color of the fish meat (Bao *et al.*, 2009). Minced tuna meat added with EGT at a dose of 150 mg/kg retained its natural red color after 7 days of iced storage (Bao *et al.*, 2008). However, the major limitation for its application is the cost of EGT when used at effective doses. Making industrial applications of EGT more favorable will require the development of techniques that increase its efficacy. One promising approach is to encapsulate EGT into biopolymer-based nanoparticles to protect its bioactivity and control its release during fish storage.

Chitosan is a biopolymer with natural antimicrobial and antioxidative properties, so it has been widely studied for its potential in seafood preservation (Kulawik *et al.*, 2020). The preservative effectiveness of chitosan is dependent on its degree of deacetylation and molecular weight. Generally, its preservative efficacy is enhanced with a higher degree of deacetylation and a lower molecular weight. Recent studies have revealed that the bioactivity of native chitosan is less than that of chitosan nanoparticles (CNP). Ghorabi and Khodanazary (2020) found that CNP better preserved *Cynoglossus arel* compared to native chitosan. Similarly, Binh *et al.* (2021) reported that CNP more effectively enhanced lipid oxidative stability in

seasoned-dried pangasius fillets when compared with native chitosan of equivalent degree of deacetylation and molecular weight.

CNP can be synthesized by the ionic gelation method employing sodium tripolyphosphate (TPP) as a crosslinking agent. This is a common practice in the encapsulation of bioactive compounds to control their release as well as enhance their stability, bioavailability, and effectiveness ( Sarp *et al.*, 2018; Othman *et al.*, 2018; Picchi *et al.*, 2021; Zaboon *et al.*, 2021).

The correlation between independent variables (antioxidant dosage/storage time) and dependent variables (redness index (RI), metMb, thiobarbituric acid reactive substances (TBARS) and total lipid hydroperoxides (HPO)) determined from the experimental data is typically used to determine the extent to which antioxidants can prevent the discoloration and lipid oxidation in refrigerated tuna meat (Bao *et al.*, 2009; Wang *et al.*, 2024). Fish quality degradation during refrigerated storage can occur due to the complex interactions of many processes, such as enzyme activity, bacterial growth, and chemical reactions, and therefore these correlations are typically nonlinear (Khoshnoudi-Nia and Moosavi-Nasab, 2019). Nie *et al.* (2022) reported that these processes normally occur simultaneously and affect each other. Although traditional studies primarily rely on linear statistical methods, they fail to adequately capture the complex, nonlinear oxidative interactions due to the combined effects of enzyme activity and chemical deterioration during the storage period. Moreover, their reliance on linear assumptions when applied to real-world, nonlinear data is likely to create huge errors (Dalatu *et al.*, 2017; O'Brien and Silcox, 2024). Furthermore, there remains a significant gap in integrating nanotechnology with predictive machine learning techniques to develop cost-effective treatment protocols consistent with commercial quality grading specifications for sashimi-grade tuna.

Artificial neural networks (ANNs) have the capability to learn from experimental data, adapt to new inputs, and provide complex, nonlinear modeling of the relationships between variables. ANNs are beneficial for analyzing changing datasets since the systems refine their predictions and grow more accurate over time (Barthwal *et al.*, 2024). This capability allows ANNs to minimize the effects of random and extraneous variations while enabling a more accurate assessment of the complex interactions between a multitude of variables (Zhang *et al.*, 2020), such as evaluating the impact of ECNP on refrigerated tuna cubes.

To address these challenges, the present study represents a novel integration of ANNs to assess the effectiveness of ECNP against color degradation and lipid oxidative deterioration of yellowfin tuna cubes in the course of refrigeration. ANNs enable modeling of intricate nonlinear relationships, providing a robust tool to minimize random variations and accurately assess complex variables and predict major quality parameters based on experimental data. The use of ANNs in the present study enables the optimal estimation of the most economical ECNP dose, which can sufficiently enhance preservation measures while reducing treatment costs.

## Materials and methods

### *Materials and chemicals*

Yellowfin tuna (*Thunnus albacares*) sourced from a seafood company in Khanh Hoa Province, Vietnam, was immediately frozen on-site and transferred to the laboratory at Nha Trang University. The tuna was stored in a Sanaky VH-5699HY4K freezer (Viet Nhat Electronic - Refrigeration Co., Ltd, Vietnam) at -20 ± 2 °C until required for this project.

Low-molecular-weight chitosan (viscosity ≤ 110 cP, degree of deacetylation ≥ 90.3%) was obtained from Vietnam Food Joint Stock Company (VNF), Vietnam. Ergothioneine (purity ≥ 98%) was supplied by Shaanxi Dideu Medichem Co. Ltd. (Dideu Group, Shaanxi Province, China). All other chemicals used in this project were of analytical grade and were supplied by Sigma-Aldrich (USA) and Merck (Germany).

### *Preparation of ECNP*

ECNP was prepared using a procedure previously reported by Thinh (2023), with minor modifications. In brief, chitosan was solubilized in a 1% ascorbic acid medium to obtain a 0.1% (w/v) chitosan solution. Subsequently, EGT was incorporated into the chitosan solution to achieve a final concentration of 0.2 mg/mL. The resulting mixture was agitated at 750 rpm to produce a homogeneous mixture. Next, a TPP solution (1 mg/mL) was introduced dropwise under constant agitation at 750 rpm to maintain a 4:1 chitosan-to-TPP ratio (w/w) for the spontaneous formation of ECNP. After 2 h, the particle size and polydispersity index (PDI) were measured by dynamic light scattering using a Horiba SZ-100V2 spectrometer (Horiba Ltd., Kyoto, Japan). The Z-average diameter and PDI were 139.6 nm and 0.319, respectively. The amount of EGT loaded into the nanoparticles was 159.5 ± 3.4 µg/mg.

### *Treatment of tuna cubes*

Tuna cubes were prepared with dimensions of 20 × 20 × 20 mm using tuna loins and were randomly assigned to four groups: Control (untreated), 100 mg/kg, 200 mg/kg, and 400 mg/kg (ECNP spray at the respective concentrations). The tuna cubes were then arranged on foam trays (200 g/tray), wrapped with PE cling film, and stored under refrigerated conditions at 3 ± 1°C. Samples were periodically taken to measure color parameters (L*a*b*), metMb, HPO, and TBARS. Each treatment was replicated three times.

### *Measurement of color*

The color (L*a*b*) of tuna cubes was measured using a Konica Minolta CR-400/CR-40 Chroma Meter (Konica Minolta Inc., Tokyo, Japan). The RI was calculated as the ratio of a*/b* (Nurilmala *et al.*, 2013).

### *Determination of metMb*

The metMb concentration was measured using a modified version of the method described by Bito (1965). A 3 g portion of minced tuna meat was blended with 10 mL of chilled phosphate buffer (0.04 M, pH 6.8). The blended sample was stirred with a Teflon-coated magnetic stir bar for 10 min, after which the mixture was

centrifuged at 5000 rpm for 5 min. The supernatant was then filtered through Whatman No.1 filter paper before the measurement of absorbance at 540 and 503 nm using a Biochrom Libra S50 UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, UK). The absorbance ratio at these two wavelengths was used to calculate metMb concentration based on the equation proposed by Bito for tuna Mb.

***Determination of HPO***

HPO concentrations were determined using a modified version of the method described by Shantha and Decker (1994). Five grams of ground tuna meat were used for lipid extraction according to the method of Bligh and Dyer (1959). The final extraction volume was adjusted to 10 mL with a chloroform/methanol mixture (2:1, v/v). Aliquots were mixed with 50 µL of 30% ammonium thiocyanate and 50 µL of 2% ferrous chloride solution, vortexed, and incubated at room temperature for 5 min. The absorbance of the mixture was determined at 500 nm using the spectrophotometer. HPO concentrations were expressed as nmol of cumene hydroperoxide equivalents per gram of tuna meat based on a standard curve.

***Determination of TBARS***

TBARS values were determined using a modified version of the method described by Uchiyama and Mihara (1978). Half a gram of ground tuna meat was homogenized with 4.5 mL of a 1.15% potassium chloride solution. Subsequently, a reaction mixture consisting of 0.5 mL of the homogenate, 0.3 mL of a 1% phosphoric acid solution, and 1.0 mL of a 0.6% thiobarbituric acid solution was incubated at 95 °C for 45 min. After the reaction mixture was cooled to ambient temperature, 4.0 mL of n-butanol was added and the mixture was vortexed and centrifuged at 3000 rpm for 10 min. The absorbance of the resulting solution was determined at 535 nm and 520 nm using the spectrophotometer. TBARS values were calculated based on the difference between the absorbance values. Results were reported as malondialdehyde (MDA) equivalents using a standard curve with 1,1,3,3'-tetraethoxypropane.

***Statistical analysis***

Microsoft® Excel (Version 2013) was used for calculating both the mean and standard deviation, for in generating graphs. Statistical analyses were conducted using R (version 4.5.2). A two-way ANOVA was performed to identify statistically significant differences, followed by Tukey's multiple comparison test to compare sample means with a significance level of $p < 0.05$.

***Development of ANN models***

ANN models were developed to predict the potential effects of ECNP dose and storage time on the discoloration and lipid oxidation of yellowfin tuna cubes during refrigerated storage. The experimental dataset was normalized to a range of 0-1 for both independent (input) and dependent (output) variables. The dataset was randomly partitioned into two groups: 70% of the data was used to develop and train the models (training set), while the remaining 30% was used to evaluate model performance (testing set).

Overfitting was evaluated based on the gap in the coefficient of determination ($R^2$) between the training and testing sets. A difference in $R^2$ of less than 0.10 between the training and testing sets was considered indicative of good generalization performance. ANN models with different hidden layer architectures were trained using the "neuralnet" package in R. These models employed a feedforward backpropagation architecture to model the relationship between the two input variables (ECNP dose and storage time) and four output variables (RI, metMb, HPO, or TBARS). Various hidden layer configurations, including 3-2, 5-3, 7-5, and 10-7 neurons, were tested to identify the most effective network architecture. Model performance was evaluated using mean squared error (MSE), root mean squared error (RMSE), mean absolute error (MAE), and the coefficient of determination ($R^2$). The optimal ANN model for each quality parameter was selected based on a composite score derived from these metrics.

## Results and discussion

### *Influence of ECNP treatment on the oxidative stability of lipids in refrigerated tuna cubes*

HPO are the primary oxidation products formed during the initial stages of lipid oxidation, and their accumulation serves as an early indicator of lipid oxidation in fish. The variations in HPO levels in tuna cubes treated with ECNP during refrigerated storage are presented in Figure 1.

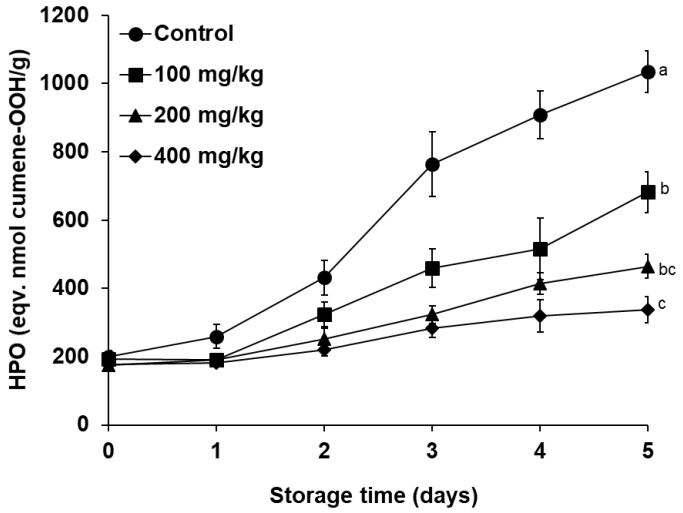



**Figure 1.** Changes in total lipid hydroperoxides (HPO) of yellowfin tuna cubes treated with ECNP at doses of 100 mg/kg fish (■), 200 mg/kg fish (▲), 400 mg/kg fish (◆), and the control without treatment (●) during refrigerated storage. Data are presented as mean ± SD (n = 3). Curves with different letters represent significant differences ($p < 0.05$).

The degree of lipid oxidation is reflected in changes in the HPO levels of fish muscle throughout the initial stage of storage (Sohn and Ohshima, 2010). Typically, fresh fish kept below 4°C within one day of harvesting usually contain fewer than 50 nmol/g of HPO in their muscle (Bao and Ohshima, 2014). However, in the present study, the tuna cubes had significantly higher HPO concentrations before storage: 207.43 ± 14.73 nmol/g (control), 198.96 ± 18.91 nmol/g (100 mg/kg), 175.85 ± 11.16 nmol/g (200 mg/kg), and 177.21 ± 13.41 nmol/g (400 mg/kg). These results showed that substantial lipid oxidation was already evident in the tuna cubes prior to storage. However, there were no significant differences ($p > 0.05$) in the initial HPO concentrations among the treatment groups, which confirms that the samples had comparable baseline oxidation levels. Therefore, changes in the HPO concentration of yellowfin tuna cubes during storage can be due to the different ECNP treatments rather than to differences in initial raw material variability.

Figure 1 shows that the HPO concentration of tuna cubes in all groups increased gradually ($p < 0.05$) over storage time, reflecting the lipid oxidation process that was ongoing during the refrigerated storage period. However, the increase in HPO concentration of the samples treated with ECNP was dose-dependent and significantly slower ($p < 0.05$) compared to the control sample.

As HPO formed in fish muscle breaks down during refrigerated storage, they contribute to the formation of secondary oxidation products, such as TBARS, which are commonly used as indicators of lipid oxidation in refrigerated fish (Suárez-Medina *et al.*, 2024).

The influence of ECNP treatment on TBARS values in tuna cubes stored at 3 ± 1°C is presented in Figure 2.

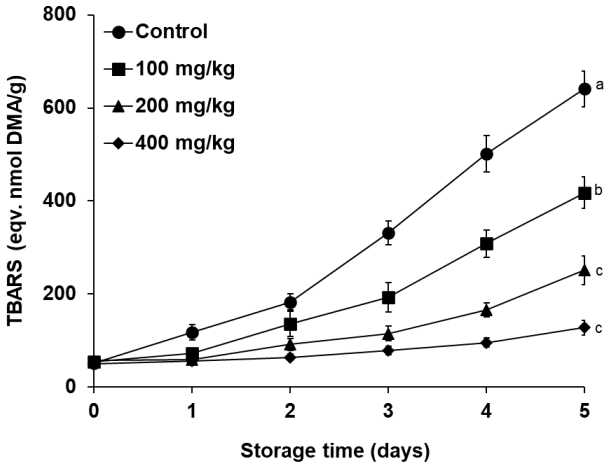



**Figure 2.** Changes in thiobarbituric acid reactive substances (TBARS) of yellowfin tuna cubes treated with ECNP at doses of 100 mg/kg fish (■), 200 mg/kg fish (▲), 400 mg/kg fish (◆), and the control without treatment (●) during refrigerated storage. Data are presented as mean ± SD (n = 3). Curves with different letters represent significant differences ($p < 0.05$).

TBARS are an index of secondary products formed during the oxidation of lipids, and their concentration in fish muscle during storage reflects the progression and intensity of oxidative deterioration (Bao and Ohshima, 2014). Figure 2 shows that the level of TBARS in the muscle of tuna cubes prior to storage was relatively low: the control group (51.63 ± 8.61 nmol MDA/g), the 100 mg/kg group (54.98 ± 6.31 nmol MDA/g), the 200 mg/kg group (57.84 ± 4.02 nmol MDA/g), and the 400 mg/kg group (50.67 ± 5.68 nmol MDA/g). Consistent with the HPO results, the absence of significant differences ($p > 0.05$) in TBARS values among the groups prior to storage confirms that the initial raw material exhibited comparable baseline oxidation levels.

During refrigerated storage, the TBARS levels in each treatment group of tuna cubes increased considerably ($p < 0.05$) over storage time. This observation supports the HPO results (Figure 1) and clearly indicates that lipid oxidation occurred progressively in tuna cubes during the 5-day refrigerated storage period. However, the TBARS values of the samples treated with ECNP increased significantly slower ($p < 0.05$) than those of the control sample. According to Bao *et al.* (2020), fish with TBARS values exceeding 200 nmol MDA/g develop rancid odors. Figure 2 shows that the TBARS value of the control sample exceeded 200 nmol MDA/g after 2 days of refrigerated storage. In contrast, the TBARS values of the samples treated with ECNP at doses of 100 mg/kg and 200 mg/kg exceeded this threshold after 3 and 4 days, respectively. After 5 days of refrigerated storage, the TBARS values of the samples treated with ECNP at a dose of 400 mg/kg still remained below 150 nmol MDA/g. These findings clearly demonstrate the dose-dependent efficacy of ECNP against lipid oxidation in refrigerated tuna cubes.

***Effect of ECNP treatment on RI and metMb concentration of refrigerated tuna cubes***

Throughout chilled storage, metMb in tuna meat is formed by the oxidative conversion of deoxyMb and oxyMb, which consequently causes the color of tuna meat to undergo a progressive transition from bright red to dark brown (Palanisamy *et al.*, 2024; Wang *et al.*, 2024). According to Nurilmala *et al.* (2013), yellowfin tuna meat was graded based on metMb concentration as follows: Grade A (excellent, metMb < 26%), Grade B (good, 26% ≤ metMb < 46%), Grade C (acceptable, 46% ≤ metMb ≤ 52%, and Grade D (unacceptable, metMb > 52%.

The impact of ECNP treatment on changes in metMb concentration of refrigerated tuna cubes stored at 3 ± 1°C is shown in Figure 3.

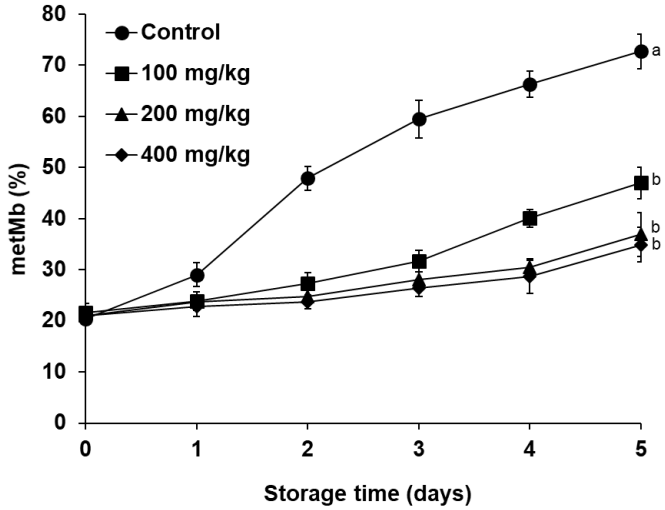



**Figure 3.** Changes in metmyoglobin (metMb) concentration of yellowfin tuna cubes treated with ECNP at doses of 100 mg/kg fish (■), 200 mg/kg fish (▲), 400 mg/kg fish (◆), and the control without treatment (●) during refrigerated storage. Data are presented as mean ± SD (n = 3). Curves with different letters represent significant differences ($p < 0.05$).

As shown in Figure 3, metMb concentrations did not differ significantly ($p > 0.05$) among the tuna cube samples prior to storage, and all values were below 26%, which indicated Grade A quality. MetMb levels increased gradually ($p < 0.05$) throughout the storage period in all treatments. However, ECNP significantly retarded metMb formation ($p < 0.05$) compared with the control group. The antioxidant effect of CNP in inhibiting lipid oxidation, which reduces peroxide and free radical formation, may also play a role in suppressing metMb formation (Binh *et al.*, 2021). The inhibitory effect of EGT on metMb formation in yellowfin tuna and beef has been previously demonstrated by Bao *et al.* (2008). The efficacy of EGT in preventing metMb formation in tuna meat was dose-dependent (Bao *et al.*, 2009). The present study found that ECNP efficacy in preventing metMb formation in refrigerated tuna cubes was dose-dependent under the present experimental conditions. In fact, tuna cube samples treated with ECNP at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg maintained their metMb concentration at an acceptable level of Grade A after 1, 2, and 3 days of refrigerated storage, respectively.

Nurilmala *et al.* (2013) also reported that an increase in metMb levels corresponded to a reduction in RI (a*/b*) values and that yellowfin tuna meat was graded based on RI as follows: Grade A (excellent, RI >1.0); Grade B (good, $0.8 < RI \leq 1.0$); Grade C (acceptable, $0.6 \leq RI \leq 0.8$); Grade D (unacceptable, RI <0.6).

The protective effect of ECNP in preventing RI decline in refrigerated tuna cubes stored at 3 ± 1°C is presented in Figure 4.

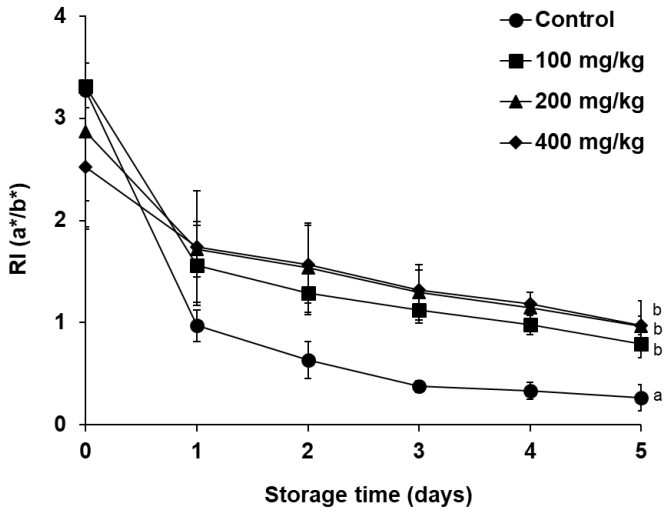



**Figure 4.** Changes in redness index (RI, a*/b*) of yellowfin tuna cubes treated with ECNP at doses of 100 mg/kg fish (■), 200 mg/kg fish (▲), 400 mg/kg fish (◆), and the control without treatment (●) during refrigerated storage. Data are presented as mean ± SD (n = 3). Curves with different letters represent significant differences ($p < 0.05$).

Figure 4 shows that the RI of all tuna cube samples before storage was above 2.5, corresponding to Grade A, with no significant difference ($p > 0.05$). The RI of all samples decreased considerably ($p < 0.05$) over storage time. However, the RI values of tuna cubes subjected to ECNP treatment decreased at a significantly slower rate ($p < 0.05$) than those of the control under similar storage conditions. Previous studies reported that the RI of tuna meat had a negative correlation with metMb concentration (Bao *et al.*, 2009; Nurilmala *et al.*, 2013; Palanisamy *et al.*, 2024). The present study also found a similar trend in which tuna cubes treated with higher ECNP doses had lower metMb concentrations and higher RI at the same storage time under similar storage conditions. This finding supports to confirm the dose-dependent efficacy of ECNP in preventing discoloration of refrigerated tuna cubes.

***Performance of ANN models***

The performance metrics for the ANN models across different architectures, including MSE, RMSE, MAE, and $R^2$ values for each quality parameter (RI, metMb, HPO, and TBARS) and each network architecture, are presented in Table 1.

The results presented in Table 1 reflect that out of the four parameters, the ANN models achieved strong predictive performance with $R^2$ values between 0.91 and 0.97 and were accompanied by low error metrics (MSE, RMSE, MAE), signifying accuracy while estimating the impact of ECNP on the color and lipid oxidative deterioration of refrigerated tuna cubes. This affirms the potential of ANN to capture the complex relationships between ECNP dosage, storage time, and quality parameters. These results corroborate prior observations that pointed out the

effectiveness of ANN models in predicting food quality parameters for complex systems (Huang, 2009; Guiné, 2019; Bhagya Raj and Dash, 2022).

**Table 1.** Performance metrics of ANN models predicting different quality parameters of refrigerated tuna cubes.

| Quality Parameter | ANN Architecture(*) | MSE | RMSE | MAE | $R^2$ |
|---|---|---|---|---|---|
| RI | 3-2 | 0.00467 | 0.06834 | 0.04712 | 0.91210 |
| | 5-3 | 0.00405 | 0.06364 | 0.04311 | 0.92380 |
| | 7-5 | 0.00422 | 0.06496 | 0.04574 | 0.92060 |
| | 10-7 | **0.00388** | **0.06228** | **0.04376** | **0.92700** |
| metMb | 3-2 | 0.00276 | 0.05252 | 0.04148 | 0.93820 |
| | 5-3 | 0.00176 | 0.04192 | 0.03229 | 0.96060 |
| | 7-5 | 0.00175 | 0.04179 | 0.03532 | 0.96080 |
| | 10-7 | **0.00167** | **0.04083** | **0.03480** | **0.96260** |
| HPO | 3-2 | 0.00299 | 0.05473 | 0.04035 | 0.92510 |
| | 5-3 | 0.00325 | 0.05697 | 0.04311 | 0.91880 |
| | 7-5 | 0.00261 | 0.05105 | 0.03833 | 0.93480 |
| | 10-7 | **0.00236** | **0.04862** | **0.03472** | **0.94090** |
| TBARS | 3-2 | **0.00121** | **0.03478** | **0.02504** | **0.97100** |
| | 5-3 | 0.00202 | 0.04495 | 0.03393 | 0.95160 |
| | 7-5 | 0.00140 | 0.03742 | 0.02612 | 0.96640 |
| | 10-7 | 0.00139 | 0.03730 | 0.02587 | 0.96670 |

(*)The numbers (3-2, 5-3, 7-5, 10-7) indicate the number of neurons in the first and second hidden layers, respectively, in the ANN architecture.

Dose-dependent efficacy of ECNP was captured by these models demonstrating a consistent response relationship in which higher dosages consistently yielded better quality parameter outcomes. It was also revealed that the optimal network architecture was parameter-specific: the 10-7 network architecture outperformed all others for RI, metMb, and HPO, while the simpler 3-2 architecture proved optimal for TBARS. This denotes that the sophistication of the ANN for accurate prediction of each parameter may be different, with TBARS being more straightforward to model compared to the other parameters.

The reliability of the selected optimal ANN models was verified through the performance comparison presented in Table 2. All models achieved high $R^2$ values for both the training (0.9529-0.9844) and the testing (0.9270-0.9710) datasets. The resulting Gap $R^2$ values (0.0130-0.0259) remained well below the threshold value of 0.10, which indicates that the models effectively captured the complex interactions between ECNP dose and storage time without overfitting. This high predictive

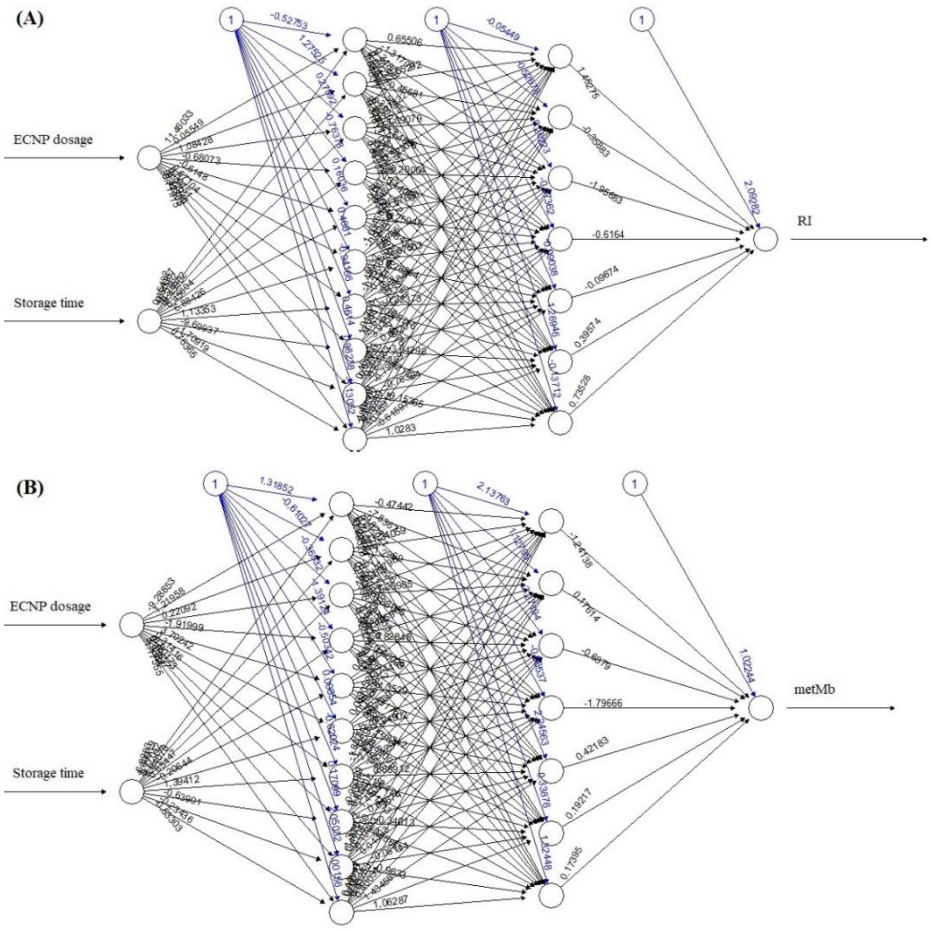
stability provides a robust scientific foundation for the subsequent determination of optimal industrial dosages based on commercial quality standards.

**Table 2.** Evaluation of overfitting for the selected optimal ANN models.

| Quality Parameter | Optimal Architecture | Train $R^2$ | Test $R^2$ | Gap $R^{2(*)}$ | Status(**) |
|---|---|---|---|---|---|
| RI | 10-7 | 0.9529 | 0.9270 | 0.0259 | OK |
| metMb | 10-7 | 0.9756 | 0.9626 | 0.0130 | OK |
| HPO | 10-7 | 0.9622 | 0.9409 | 0.0213 | OK |
| TBARS | 3-2 | 0.9844 | 0.9710 | 0.0134 | OK |

(*)Gap R2 = Train R2 – Test R2; (**)Status "OK" indicates a gap ≤ 0.10.

The architectures of the selected ANN models for each quality parameter are shown in Figure 5.



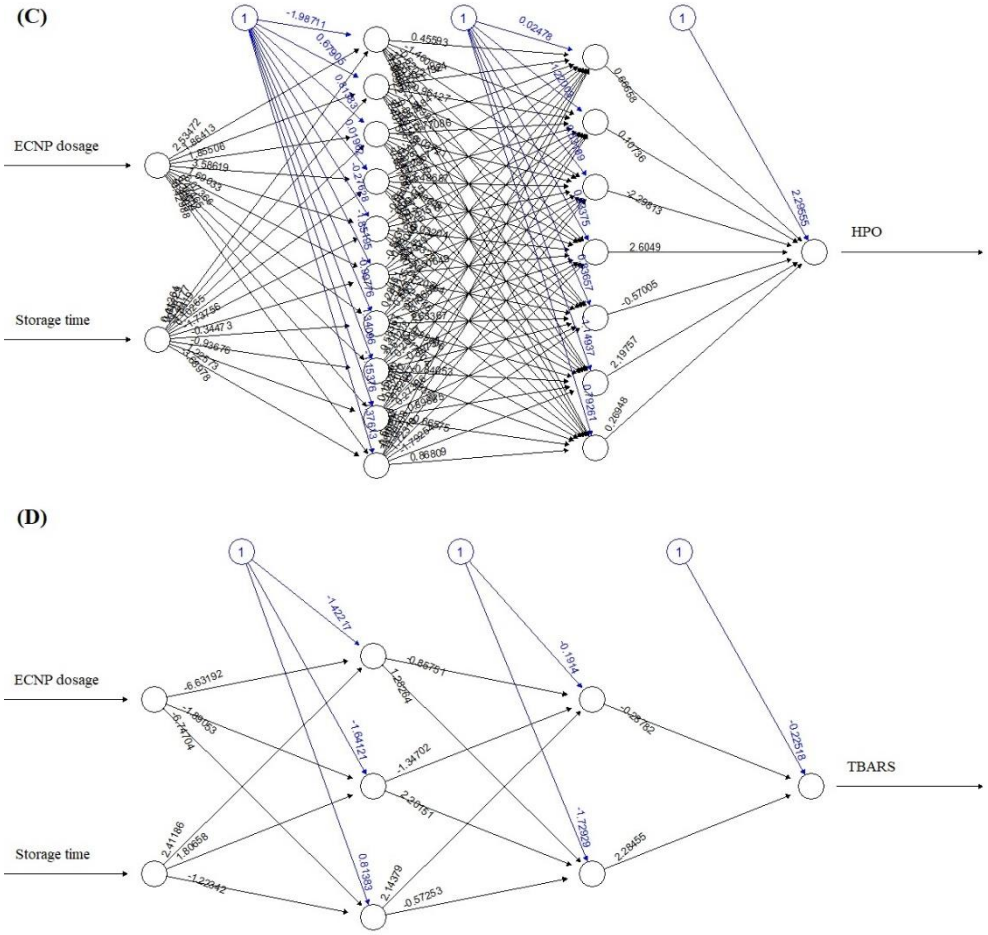



**Figure 5.** Architecture of the selected artificial neural network (ANN) models for the prediction of redness index (RI, A), metmyoglobin (metMb, B), total lipid hydroperoxides (HPO, C), and thiobarbituric acid reactive substances (TBARS, D) of refrigerated tuna cubes based on ergothioneine-loaded chitosan nanoparticles (ECNP) dose and storage time.

Figure 5 highlights the differences in model complexity required to accurately predict each quality parameter. The greater number of neurons in the hidden layers of the 10-7 architecture was required for RI, metMb, and HPO, suggesting that there are more intricate relationships between ECNP dose, storage time, and these quality parameters. In contrast, the simpler 3-2 architecture was sufficient for TBARS, suggesting a less complex relationship.

The predictive performance of the selected ANN models for each quality parameter is visualized in Figure 6, where three-dimensional surface plots illustrate the relationship between ECNP dose, storage time, and the predicted values for RI, metMb, HPO, and TBARS.

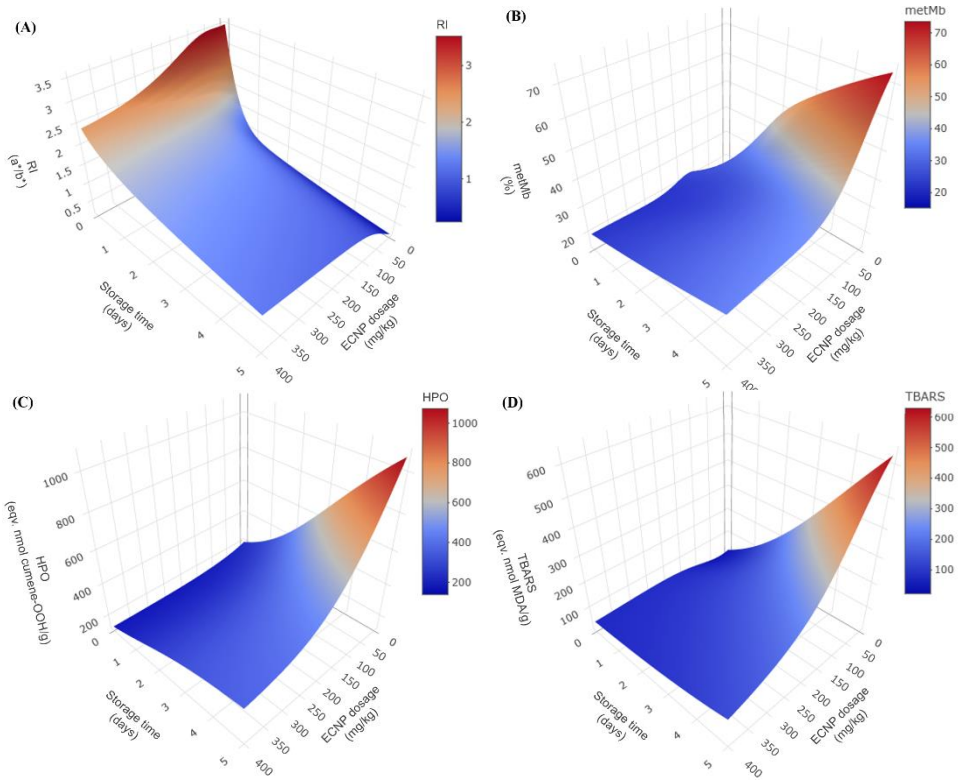



**Figure 6.** Three-dimensional surface plots of predicted values of redness index (RI, A), metmyoglobin (metMb, B), total lipid hydroperoxides (HPO, C), and thiobarbituric acid reactive substances (TBARS, D) based on ergothioneine-loaded chitosan nanoparticles (ECNP) dose and storage time for refrigerated tuna cubes using the selected artificial neural network (ANN) models.

Figure 6 shows the predicted values of RI, metMb, HPO, and TBARS as functions of ECNP dose and storage time for refrigerated tuna cubes. The results indicate that ECNP significantly improved both color and lipid oxidative stability in yellowfin tuna cubes, with higher doses generally providing more effects. However, the dose-response efficacy of ECNP is not strictly linear, which is evidenced by the ANN predictions. For instance, while higher ECNP doses (200-400 mg/kg) showed the best retention of RI and metMb, lower doses (100-200 mg/kg) were sufficient to maintain acceptable levels of TBARS and HPO over storage time.

For RI, the dose-dependent efficacy of ECNP is evident. At lower doses (0-100 mg/kg), RI values decreased rapidly over time, indicating significant color degradation. However, at higher doses (200-400 mg/kg), RI values remained stable, often above 1.0, even after extended storage. This demonstrates that ECNP, in a dose-dependent manner, effectively preserves the color of tuna cubes, which is critical for consumer acceptance. The ability of ECNP to stabilize RI of meats, which aligns with its antioxidant properties as highlighted in previous studies (Bao *et al.*,

2008; Cheng *et al.*, 2021; Safari *et al.*, 2023), demonstrates that higher doses of ECNP result in better color preservation, further emphasizing its dose-dependent efficacy.

In the same vein, there is a noteworthy dose-response relationship in the formation of metMb. At lower ECNP doses, metMb concentration increased progressively, which indicates a significant discoloration due to the oxidation of Mb. However, at more elevated ECNP doses, ranging from 200-400 mg/kg, metMb percentages remained below 30% even after extended periods of storage. This indicates that the dose-dependency of ECNP effectively inhibits metMb formation, thereby reducing discoloration. This is in agreement with the findings of Bao *et al.* (2009), who reported the dose-dependent efficacy of antioxidants against discoloration in meat and fishery products.

The predicted results for HPO and TBARS also provide specific evidence regarding the efficacy of ECNP. Lower doses of ECNP were associated with considerable increases in both HPO and TBARS, signifying great lipid oxidation. On the other hand, higher doses ranging from 200-400 mg/kg were found to sustain low levels of HPO and TBARS, demonstrating that ECNP has the ability to slow down or inhibit lipid oxidation in a dose-dependent manner. This has increased significance when high quality and extended shelf life of refrigerated fish products are required, as lipid oxidation leads to spoilage. The result highlights the finding that higher doses of ECNP inhibited lipid oxidation, again, showing its dose-dependent efficacy.

The ANN predictions also enabled the determination of the optimal ECNP dose as well as the required storage duration under refrigeration to achieve the specified quality grades of tuna cubes. The minimal doses required to maintain the quality of tuna cubes at grade A (TBARS value < 200 nmol DMA/g, metMb concentration < 26%, RI > 1) for up to 2.55 days and at grade B (TBARS value < 200 nmol DMA/g, metMb concentration < 46%, RI > 0.8) for up to 5 days are 367 mg/kg and 253 mg/kg, respectively. These results indicate that lower doses of ECNP can be used for less stringent quality requirements, thereby reducing potential costs and minimizing possible side effects associated with higher ECNP doses.

In the present investigation, ANN modeling facilitated the analysis of the complex interactions among ECNP dose, storage time, and quality parameters of tuna cubes (RI, metMb, HPO, and TBARS). The reliability of these models was substantiated by the remarkably high $R^2$ values and the exceptionally low error metrics (MSE, RMSE, and MAE). All of the models demonstrate that ANN approach was effective in predicting the impact of ECNP on the color and lipid oxidative stability of tuna cubes under refrigeration. This methodology is useful for analyzing the broader context of other food preservation studies involving the complex interplay among multiple factors.

## Conclusions

This study demonstrated the dose-dependent efficacy of ECNP in preserving the quality of refrigerated yellowfin tuna cubes. Higher ECNP concentrations (200-400

mg/kg) proved significantly more effective than lower concentrations (0-100 mg/kg) in maintaining color, reducing discoloration, and inhibiting lipid oxidation. By leveraging ANN models, accurate predictions of quality parameters were achieved, supporting the potential of this approach for optimizing ECNP dosage and storage conditions. These findings also highlight the potential of ECNP as a bio-based preservative in the fisheries sector, offering a promising solution for extending shelf life and maintaining the quality of refrigerated seafood products.

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## References

Bao, H.N.D., Ohshima, T. 2014. Strategies to minimize lipid oxidation of aquatic food products post harvest, In: *Antioxidants and Functional Components in Aquatic Foods*. Hordur G.K., John Wiley & Sons, Inc., pp. 95–124.

Bao, H.N.D., Osako, K., Ohshima, T. 2010. Value-added use of mushroom ergothioneine as a colour stabilizer in processed fish meats. *Journal of the Science of Food and Agriculture*, **90**(10), 1634–1641.

Bao, H.N.D., Ushio, H., Ohshima, T. 2008. Antioxidative activity and antidiscoloration efficacy of ergothioneine in mushroom (*Flammulina velutipes*) extract added to beef and fish meats. *Journal of Agricultural and Food Chemistry*, **56**(21), 10032–10040.

Bao, H.N.D., Ushio, H., Ohshima, T. 2009. Antioxidative activities of mushroom (*Flammulina velutipes*) extract added to bigeye tuna meat: Dose-dependent efficacy and comparison with other biological antioxidants. *Journal of Food Science*, **74**(2), C162–C169.

Baron, C.P., Andersen, H.J. 2002. Myoglobin-induced lipid oxidation. A review. *Journal of Agricultural and Food Chemistry*, **50**(14), 3887–3897.

Barthwal, R. Kathuria, D., Joshi, S., Kaler, R.S.S., Singh, N. 2024. New trends in the development and application of artificial intelligence in food processing. *Innovative Food Science and Emerging Technologies*, **92**, 103600.

Bhagya Raj, G.V.S., Dash, K.K. 2022. Comprehensive study on applications of artificial neural network in food process modeling. *Critical Reviews in Food Science and Nutrition*, **62**(10), 2756–2783.

Binh, N.T.T., Bao, H.N.D., Prinyawiwatkul, W., Trung, T.S. 2021. Antioxidative and antimicrobial effects of low molecular weight shrimp chitosan and its derivatives on seasoned-dried Pangasius fillets. *International Journal of Food Science and Technology*, **56**(10), 119–5129.

Bito, M. 1965. Studies on the retention of meat color of frozen tuna-II. Effect of storage temperature on preventing discoloration of tuna meat during freezing storage. *Nippon Suisan Gakkaishi*, **31**(7), 534–539.

Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**(8), 911–917.

Cheng, Y., Hu, J. and Wu, S. 2021. Chitosan based coatings extend the shelf-life of beef slices during refrigerated storage. *LWT*, **138**, 110694.

Dalatu, P.I., Fitrianto, A., Mustapha, A. 2017. A comparative study of linear and nonlinear regression models for outlier detection. *Advances in Intelligent Systems and Computing*, **549**, 316–326.

Bao, H.N.D., Hien, P.T., Quyen, V.L. 2020. Antioxidant and antimicrobial activities of hydrophilic extracts from shallot and garlic bulbs, and their effects on round scad during iced storage. *Annals of the University Dunarea de Jos of Galati, Fascicle VI: Food Technology*, **44**(1), 26–44.

Ghorabi, R.S., Khodanazary, A. 2020. Effects of chitosan and nano-chitosan as coating materials on the quality properties of large scale tongue sole *Cynoglossus arel* during super-chilling storage. *Iranian Journal of Fisheries Sciences*, **19**(5), 2242–2257.

Guiné, R.P.F. 2019. The use of artificial neural networks (ANN) in food process engineering. *ETP International Journal of Food Engineering*, **5**(1), 15-21.

Herpandi, N.H, Rosma, A., Nadiah, W.A.W. 2011. The tuna fishing industry: a new outlook on fish protein hydrolysates. *Comprehensive Reviews in Food Science and Food Safety*, **10**(4), 195–207.

Huang, Y. 2009. Advances in artificial neural networks - Methodological development and application. *Algorithms*, **2**(3), 973-1007.

Khoshnoudi-Nia, S., Moosavi-Nasab, M. 2019. Prediction of various freshness indicators in fish fillets by one multispectral imaging system. *Scientific Reports*, **9**, 14704.

Kulawik, P., Jamróz, E., Özogul, F. 2020. Chitosan role for shelf-life extension of seafood. *Environmental Chemistry Letters*, **18**(1), 61–74.

Lee, S., Joo, S.T., Alderton, A.L., Hill, D.W., Faustman, C. 2003. Oxymyoglobin and lipid oxidation in yellowfin tuna (*Thunnus albacares*) loins. *Journal of Food Science*, **68**(5), 1664–1668.

Nie, X., Zhang, R., Cheng, L., Zhu, W., Li, S., Chen, X. 2022. Mechanisms underlying the deterioration of fish quality after harvest and methods of preservation. *Food Control*, **135**, 108805.

Nurilmala, M., Ushio, H., Kaneko, G., Ochiai, Y. 2013. Assessment of commercial quality evaluation of yellowfin tuna *Thunnus albacares* meat based on myoglobin properties. *Food Science and Technology Research*, **19** (2), 237-243.

O'Brien, T.E., Silcox, J.W. 2024. Nonlinear Regression Modelling: A Primer with Applications and Caveats. *Bulletin of Mathematical Biology*, **86**, 40.

Othman, N., Masarudin, M.J., Kuen, C.Y., Dasuan, N.A., Abdullah, L.C., Jamil, S.N.A.M. 2018. Synthesis and optimization of chitosan nanoparticles loaded with L-ascorbic acid and thymoquinone. *Nanomaterials*, **8**(11), 920.

Palanisamy, S., Singh, A., Zhang, B., Zhao, Q., Benjakul, S. 2024. Effects of Different phenolic compounds on the redox state of myoglobin and prevention of discoloration, lipid and protein oxidation of refrigerated longtail tuna (*Thunnus tonggol*) slices. *Foods*, **13**(8), 1238.

Palanisamy, S., Singh, A., Zhang, B., Kim, J.T., Benjakul, S. 2025. Effects of polyphenols in combination with L-cysteine/L-ascorbic acid: Myoglobin redox state, color and quality of refrigerated longtail tuna (*Thunnus tonggol*) slices. *Food Chemistry*, **465**, Part 1, 141983.

Picchi, V., Gobbi, S., Fattizzo, M., Zefelippo, M., Faoro, F. 2021. Chitosan nanoparticles loaded with N-acetyl cysteine to mitigate ozone and other possible oxidative stresses in durum wheat. *Plants*, **10**(4), 691.

Reeder, B.J., Wilson, M.T. 2001. The effects of pH on the mechanism of hydrogen peroxide and lipid hydroperoxide consumption by myoglobin: A role for the protonated ferryl species. *Free Radical Biology and Medicine*, **30**(11),1311-1318.

Safari, R., Yaghoubi, M., Marcinkowska-Lesiak, M., Paya, H., Sun, X., Rastgoo, A., Rafiee, M., Alirezalu, K. 2023. The effects of double gelatin containing chitosan nanoparticles-calcium alginate coatings on the stability of chicken breast meat. *Food Science and Nutrition*, **11**(12), 7673–7685.

Sarp, Ö., Yurdakoç, K., Altinisik Tagac, A. 2018. Controlled release of vitamin-C from chitosan nanoparticles. *Hacettepe Journal of Biology and Chemistry*, **1**(46), 69–77.

Shantha, N.C., Decker, E.A. 1994. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC International*, **77**(2), 421–424.

Sohn, J.H., Ohshima, T. 2010. Control of lipid oxidation and meat color deterioration in skipjack tuna muscle during ice storage. *Fisheries Science*, **76**(4), 703–710.

Suárez-Medina, M.D., Sáez-Casado, M.I., Martínez-Moya, T., Rincón-Cervera, M.Á. 2024. The effect of low temperature storage on the lipid quality of fish, either alone or combined with alternative preservation technologies. *Foods*, **13**(7), 1097.

Thinh, N.T. 2023. Development of a technological process for producing antioxidant-active ergothioneine-loaded chitosan nanoscale particles at laboratory scale. Master's thesis, Nha Trang University, Vietnam. (in Vietnamese)

Uchiyama, M., Mihara, M. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, **86**(1), 271–278.

Wang, B., Wang, S., Xu, Y., Jiang, Q., Xia, W. 2024. Colour stability improving of yellowfin tuna slices by compound antioxidant against oxidation of myoglobin, *International Journal of Food Science & Technology*. **59**(8), 5616–5629.

Zaboon, M.H., Saleh, A.A., Al-Lami, H.S. 2021. Synthesis, characterization and cytotoxicity investigation of chitosan-amino acid derivatives nanoparticles in human breast cancer cell lines. *Journal of the Mexican Chemical Society*, **65**(2), 178–188.

Zhang, S., Yang, X., Wang, Y., Zhao, Z., Liu, J., Liu, Y., Sun, C., Zhou, C. 2020. Automatic fish population counting by machine vision and a hybrid deep neural network model. *Animals*, **10**(2), 364.