EFFECT OF TREATMENT DURATION WITH ERGOTHIONEINE-LOADED CHITOSAN NANOSCALE PARTICLES ON DISCOLORATION AND LIPID OXIDATION IN REFRIGERATED YELLOWFIN TUNA CUBES

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ABSTRACT

This study aimed to evaluate the effect of treatment duration with ergothioneine-loaded chitosan nanoscale particles (ECNP) on discoloration and lipid oxidation in yellowfin tuna cubes during refrigerated storage at 3 ± 1 °C for 5 days. Yellowfin tuna cubes were treated with ECNP at a dose of 250 mg/kg and kept for different treatment durations: 30 min, 60 min, 90 min, and a control (no treatment). The results showed that treatment duration significantly influenced the ability of ECNP to retard discoloration and lipid oxidation in refrigerated yellowfin tuna cubes. A treatment duration of 60 min effectively preserved the quality of A-grade tuna cubes for up to 3 days of refrigerated storage. Extending the treatment duration to 90 min did not result in a significant improvement compared to 60 min. These findings suggest that a 60-min treatment duration is appropriate for preventing lipid oxidation and discoloration in refrigerated yellowfin tuna cubes.

Keywords: Antioxidant activity, biopolymer, sashimi, quality control, Scombridae.

1. INTRODUCTION

Tuna is an essential global food source and a high-value commodity that supports major fisheries and seafood industries [1]. The yellowfin tuna (*Thunnus albacares*) is best known for its texture and flavor that makes wonderful sashimi as a top choice for high-end products [2]. The bright red color of fresh tuna, which is caused by the oxygen-binding protein myoglobin (Mb), is a key indicator of quality [3]. However, myoglobin gets oxidized to metmyoglobin (metMb) during refrigerated storage, resulting in an unappealing brown color that diminishes consumer attractiveness [4].

The decline of quality in tuna meat is mainly due to the continuous damaging cycle of lipid and myoglobin oxidation. Lipid oxidation causes the generation of reactive oxygen species (ROS) that accelerate myoglobin oxidation; while metMb, in turn, promotes further lipid peroxidation, creating a negative feedback cycle for degrading color and taste [5]. The oxidative process acts as a significant hurdle in refrigerated storage at low temperatures where both enzymatic and non-enzymatic reactions gradually shorten the shelf life [6].

Synthetic antioxidants, such as butylated hydroxytoluene (BHT), have been widely applied in seafood to retard the oxidation processes [7]. However, growing health concerns coupled with consumer demands for clean-label products have driven research toward natural alternatives [8]. Ergothioneine (EGT) is a naturally occurring antioxidant from mushrooms and has provided remarkable inhibition against lipid and Mb oxidation, causing excellent efficacy [9]. Studies indicate that 50 mg/kg EGT can hold tuna meat in its color for around seven days at 4 °C [10]. Despite its effectiveness, the high cost of EGT limits its industrial-scale application.

Chitosan, a biodegradable polysaccharide obtaining from the shell of crustaceans, exhibits inherent antimicrobial and antioxidant properties [11]. Its bioactivity depends on the molecular weight and degree of deacetylation, with nano-formulated chitosan (CNP) giving enhanced functionality due to increased surface area and improved delivery of bioactive compounds [12]. Nano-encapsulation of EGT in chitosan would stabilize, improve bioavailability, and assure controlled release while requiring lower doses of EGT [13]. Bao *et al.* recently reported that ergothioneine-loaded chitosan nanoscale particles (ECNP) can successfully prevent lipid oxidation and discoloration of yellowfin tuna cubes stored in the refrigerator [14]. This is believed to occur via the bioactivity of CNP in simultaneous controlled release of EGT, thereby more effectively interfering with the oxidative pathway.

In light of the previously shown promise of EGT and CNP in oxidative stabilization, this study now explored the effect of treatment duration with ECNP on discoloration and lipid oxidation of refrigerated yellowfin tuna cubes. The results are intended to provide a natural preservation strategy, cost-effective and to enhance the shelf life without compromising sensory quality.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

Yellowfin tuna (*Thunnus albacares*) employed in this investigation was collected from a seafood enterprise located in Khanh Hoa Province, Vietnam. The tuna loins were pre-frozen at the processing site before being delivered to the laboratory at Nha Trang University. Upon arrival, the samples were preserved at -20 ± 2 °C using a Sanaky VH-5699HY4K freezer (Viet Nhat Electronic - Refrigeration Co., LTD, Vietnam) until experimental use.

Low molecular weight chitosan (viscosity not exceeding 110 cPs and deacetylation degree of at least 90.3%) was supplied by Vietnam Food Joint Stock Company (VNF), Vietnam. Ergothioneine, with a purity of no less than 98%, was sourced from Shaanxi Dideu Medichem Co. Ltd. (China). All other chemicals utilized were of analytical grade, procured from Sigma-Aldrich (USA) and Merck (Germany).

2.2. Preparation of ECNP

The synthesis of ECNP followed the method established by Thinh [15], subject to minor procedural refinements. In summary, a 1 mg/mL chitosan solution was prepared by dissolving chitosan in 1 % (w/v) ascorbic acid. Separately, EGT was dissolved and then introduced into the chitosan solution to achieve a final EGT concentration of 0.2 mg/mL. The mixture was continuously stirred at 750 rpm to ensure homogeneity. Subsequently, a 1 mg/mL sodium tripolyphosphate (STPP) solution was introduced dropwise while maintaining stirring at 750 rpm, resulting in a chitosan/STPP mass ratio of 4/1. The dispersion was then left to stabilize for 2 hours before analysis.

The size and distribution of the resulting nanoparticles were characterized via dynamic light scattering (DLS) using a Horiba SZ-100V2 spectrometer. The Z-average particle diameter was 139.6 nm, and the polydispersity index (PDI) was 0.319, indicating moderate monodispersity. The loading capacity of EGT within ECNP was quantified as 159.5 ± 3.4 µg/mg.

2.3. Treatment of tuna cubes

Tuna loins were cut into uniform cubes measuring 20 × 20 × 20 mm and randomly assigned to four treatment groups: Control (no treatment), T30, T60, and T90. The treated groups (T30, T60, T90) were sprayed with ECNP at a dose of 250 mg/kg and kept for 30, 60, and 90 minutes, respectively, to complete the treatment durations. Following treatment, the tuna cubes were placed on foam trays (200 g per tray), wrapped with polyethylene (PE) cling film, and stored under refrigerated conditions at 3 ± 1 °C. Samples from each group were collected at scheduled intervals for analysis of color (L*a*b*), metMb, HPO, and TBARS. Each treatment was conducted in triplicate to ensure reproducibility.

2.4. Methods of analysis

Color parameters (L*, a*, b*) were measured using a Konica Minolta CR-400/CR-40 colorimeter (Japan). The the a*/b* ratio was used to calculate a redness index [16].

The proportion of metMb was assessed based on the protocol developed by Bito [4], with minor modifications. Briefly, 3 g of grinded tuna muscle was homogenized with 10 mL of chilled phosphate buffer (0.04 M, pH 6.8) using a magnetic stirrer equipped with a Teflon-coated bar. The homogenate was centrifuged at 5,000 rpm for 5 minutes, and the supernatant was filtered through Whatman No. 1 paper. Absorbance readings at 540 nm and 503 nm were recorded using a Biochrom Libra S50 UV/VIS spectrophotometer (UK), and metMb percentage was calculated based on the absorbance ratio as per Bito's method for tuna meat.

The HPO content was analyzed following the procedure adapted from Shantha and Decker [17]. Lipids were extracted from 5 g of grinded tuna using the method of Bligh and Dyer [18], and the final extract was adjusted to 10 mL using chloroform/methanol (2:1, v/v). To this, 50 µL each of 30 % ammonium thiocyanate and 2 % ferrous chloride were added, vortexed, and incubated for 5 minutes at ambient temperature. Absorbance at 500 nm was recorded, and HPO levels were expressed as nmol cumene hydroperoxide per gram of sample, based on a standard calibration curve.

TBARS were analyzed following the procedure adapted of Uchiyama and Mihara [19]. A 0.5 g sample of grinded tuna was homogenized in 4.5 mL of 1.15 % KCl solution. A 0.5 mL aliquot of this homogenate was mixed with 0.3 mL of 1 % phosphoric acid and 1.0 mL of 0.6 % thiobarbituric acid. The mixture was heated at 95 °C for 45 minutes and then cooled. After adding 4.0 mL of n-butanol, the mixture was vortexed and centrifuged at 3000 rpm for 10 minutes. Absorbance was recorded at 535 and 520 nm, and the TBARS values were determined from the absorbance difference, expressed in malondialdehyde (MDA) equivalents using a 1,1,3,3'-tetraethoxypropane standard curve.

2.5. Statistical analysis

Data processing, including mean and standard deviation calculations and graph plotting, was done using Microsoft Excel 2013. Statistical analyses were conducted using R software (version 4.5.0). A two-way analysis of variance (ANOVA) was performed to assess the significance of differences among treatment groups and storage times. Where significant effects were detected, Tukey's multiple comparisons test was applied to identify specific differences between groups. A significance level of $p < 0.05$ was used for all statistical tests.

3. RESULTS AND DISCUSSION

3.1. Effect of holding time after treatment with ECNP on lipid oxidation in tuna cubes during refrigerated storage

HPO is commonly used as a reliable indicator of the initial oxidation of fish lipids because they are the primary products generated in the early stages of lipid oxidation. The fluctuation in HPO content during the initial stages of storage serves as an important indicator of lipid oxidation progression in fish muscle [20]. Figure 1 illustrates the impact of treatment duration with ECNP on the HPO levels in yellowfin tuna cubes stored under refrigeration.

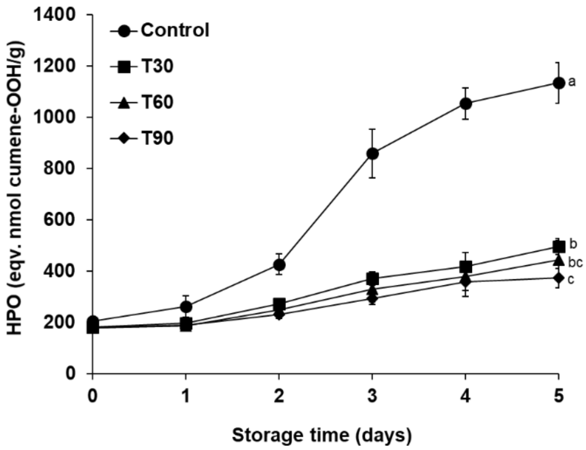

Figure 1. Variation in total lipid hydroperoxide (HPO) content in yellowfin tuna cubes subjected to different treatment durations with ergothioneine-loaded chitosan nanoparticles: 30 minutes (T30), 60 minutes (T60), and 90 minutes (T90), in comparison with untreated controls, during refrigerated storage. Data are presented as mean ± SD (n = 3). Different letters indicate statistically significant differences among treatments at $p < 0.05$.

As shown in Figure 1, there were no statistically significant differences ($p > 0.05$) in HPO levels of yellowfin tuna cubes among the various treatment groups prior to refrigerated storage. Specifically, the HPO values were 205.74 ± 10.04 nmol/g in the control (untreated) samples, 180.06 ± 7.79 nmol/g in the group treated for 30 minutes with ECNP, 179.49 ± 6.29 nmol/g in the 60-minute treatment group, and 179.55 ± 6.35 nmol/g in the 90-minute treatment group. According to Bao and Ohshima, freshly harvested fish muscle generally exhibits HPO levels below 50 nmol/g when stored at temperatures under 4 °C within the first 24 hours post-harvest [21]. Given that all groups in this study exhibited initial HPO values substantially higher than this threshold, it can be inferred that a considerable degree of lipid oxidation had already occurred in the tuna cubes before the onset of refrigerated storage. The upward trend in HPO contents over time in each group (Figure 1) reflects sustained oxidative activity during refrigerated storage ($p < 0.05$). However, treatment with ECNP significantly reduced the formation of HPO compared to the untreated control, and this reduction effect was influenced by the treatment duration. Extending the ECNP treatment time tended to result in lower HPO levels during storage. In particular, a 90-minute treatment led to significantly lower HPO accumulation than a 30-minute treatment ($p < 0.05$). However, no significant difference ($p > 0.05$) was observed between the 60-minute and 90-minute treatments. Therefore, the 60-minute treatment time appeared to be the most effective and consistent duration for minimizing lipid oxidation throughout the storage period.

As HPO formed in fish breaks down during refrigerated storage, they contribute to the formation of secondary oxidation products, such as TBARS, which reflect the extent of lipid oxidation in fish muscle and are commonly used as an indicator of lipid oxidation in fish in particular and food in general [21]. The effect of treatment duration with ECNP on TBARS values in tuna cubes stored at 3 ± 1 °C is illustrated in Figure 2.

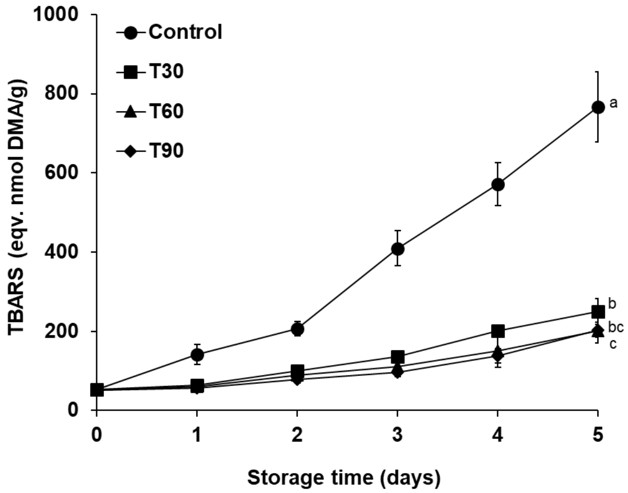

Figure 2. Variation in thiobarbituric acid reactive substances (TBARS) in yellowfin tuna cubes subjected to different treatment durations with ergothioneine-loaded chitosan nanoparticles: 30 minutes (T30), 60 minutes (T60), and 90 minutes (T90), in comparison with untreated controls, during refrigerated storage. Data are presented as mean ± SD (n = 3). Different letters indicate statistically significant differences among treatments at $p < 0.05$.

As illustrated in Figure 2, the initial TBARS values in yellowfin tuna cubes were relatively low across all groups: control group (53.50 ± 5.48 nmol MDA/g), 30-minute treatment group (53.98 ± 4.49 nmol MDA/g), 60-minute treatment group (51.85 ± 3.08 nmol MDA/g), and 90-minute treatment group (51.90 ± 4.26 nmol MDA/g). Statistical analysis indicated that the initial TBARS values did not differ significantly among the groups ($p > 0.05$).

Throughout refrigerated storage, a significant increase in TBARS values was observed in all tuna cube samples over time ($p < 0.05$). This observation backs up 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 more slowly ($p < 0.05$) than that of the untreated control, and treatment duration had a significant effect on TBARS formation, with longer treatment times generally resulting in a slower rate of TBARS increase.

According to Ke *et al.*, fish with TBARS values exceeding 200 nmol MDA/g develop rancid odors [22]. The results in Figure 2 show that among the ECNP-treated groups, the 60-minute treatment was the most effective in delaying the increase in TBARS values, maintaining them below 200 nmol MDA/g for up to 5 days. In contrast, the 30-minute group exceeded this threshold after 4 days, while the control group surpassed it after only 2 days. Although the 90-minute group showed slightly lower TBARS values than the 60-minute group, the difference was not statistically significant ($p > 0.05$). These results suggest that a 60-minute treatment duration is the most appropriate for effectively inhibiting lipid oxidation in refrigerated yellowfin tuna cubes.

3.2. Effect of holding time after treatment with ECNP on RI and metMb concentration of refrigerated tuna cubes

Throughout low-temperature storage, tuna meat undergoes a gradual color transformation from bright red to dark brown, which is caused by the oxidation of deoxyMb and oxyMb molecules, leading to the formation of metMb [4]. Nurilmala *et al*. reported that metMb concentration of yellowfin tuna meat is categorized as follows: grade A (excellent) less than 26 %; grade B (good) less than 46 %; grade C (acceptable) up to 52 %; and grade D (unacceptable) exceeding 52 % [16].

The effect of ECNP treatment duration on metMb accumulation in refrigerated tuna cubes stored at 3 ± 1 °C is shown in Figure 3.

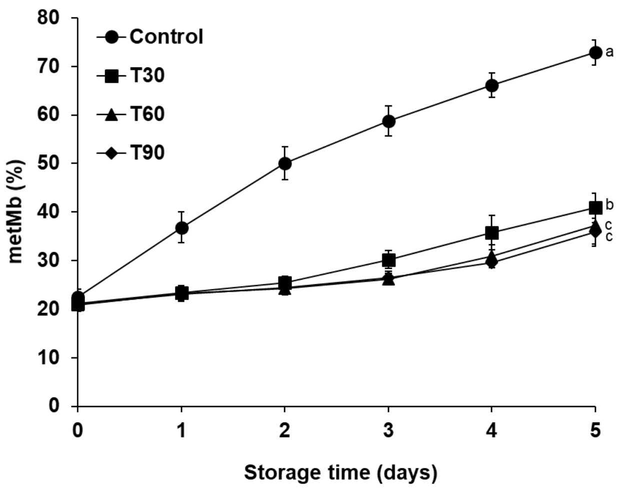

Figure 3. Variation in metmyoglobin (metMb) concentration in yellowfin tuna cubes subjected to different treatment durations with ergothioneine-loaded chitosan nanoparticles: 30 minutes (T30), 60 minutes (T60), and 90 minutes (T90), in comparison with untreated controls, during refrigerated storage. Data are presented as mean ± SD (n = 3). Different letters indicate statistically significant differences among treatments at $p < 0.05$.

The data presented in Figure 3 show that prior to storage, the metMb levels in all tuna cube samples were not significantly different ($p > 0.05$) and remained under 26 %, which corresponds to grade A quality. The metMb concentration of all samples increased progressively ($p < 0.05$) over the storage period. However, the increase in metMb concentration of the samples treated with ECNP was significantly slower ($p < 0.05$) than that of the untreated control, and treatment duration had a significant effect on metMb formation. Longer ECNP treatment times tended to result in lower metMb concentrations during storage. In fact, tuna cube samples treated with ECNP for 60 minutes and 90 minutes maintained their metMb concentration at an acceptable level of grade A for up to 3 days, while the 30-minute treatment samples only maintained their metMb concentration at an acceptable level of grade A for 2 days, in accordance with the classification by Nurilmala *et al*. [16]. The concentration of metMb exhibited a negative correlation with the RI (a*/b*) was further observed in yellowfin tuna meat by the same authors. The RI values for yellowfin tuna are categorized as follows: grade A (excellent) above 1.0; grade B (good) above 0.8; grade C (acceptable) at or above 0.6; and grade D (unacceptable) below 0.6.

The effect of treatment duration with ECNP on the variation in the RI of refrigerated tuna cubes stored at 3 ± 1 °C is presented in Figure 4.

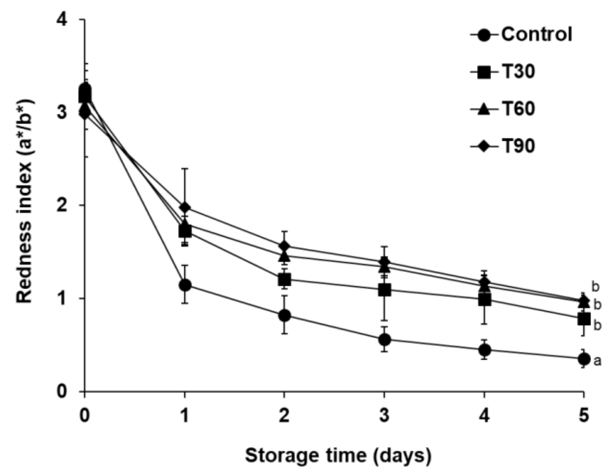

Figure 4. Variation in redness index (RI, a*/b*) in yellowfin tuna cubes subjected to different treatment durations with ergothioneine-loaded chitosan nanoparticles: 30 minutes (T30), 60 minutes (T60), and 90 minutes (T90), in comparison with untreated controls, during refrigerated storage. Data are presented as mean ± SD (n = 3). Different letters indicate statistically significant differences among treatments at $p < 0.05$.

The data presented in Figure 4 reveal 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. Nevertheless, the RI of the treated tuna cubes with ECNP decreased significantly slower ($p < 0.05$) than that of the untreated control under similar storage conditions; with prolonged ECNP treatment, the tendency was for a slower rate of RI decrease during storage. This is consistent with previous studies that reported a negative correlation between RI of tuna meat and metMb concentration [4, 20]. Findings show that a 60-minute treatment duration is the most appropriate for the effective preservation of the color of yellowfin tuna cubes during storage under refrigeration by using ECNP in this particular case study.

4. CONCLUSION

This study demonstrated that treatment with ECNP effectively inhibited lipid oxidation and preserved color stability in yellowfin tuna cubes during refrigerated storage. ECNP treatment significantly reduced the formation of both HPO and TBARS, with the 60-minute treatment duration showing the most consistent and effective performance. Moreover, ECNP also retarded the formation of metMb and maintained a higher RI, which are critical indicators of tuna meat color quality. The 60-minute treatment was sufficient to maintain metMb concentrations and RI values at grade A levels for up to 3 days, while longer treatments offered no significant advantage. Overall, a 60-minute treatment with ECNP is recommended as the sufficient condition for preventing lipid oxidation and discoloration in yellowfin tuna cubes under refrigerated storage.

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TÓM TẮT

ẢNH HƯỞNG CỦA THỜI GIAN XỬ LÝ BẰNG NANO CHITOSAN TẢI ERGOTHIONEINE ĐẾN BIẾN MÀU VÀ OXY HÓA LIPID TRONG CƠ THỊT CÁ NGỪ VÂY VÀNG BẢO QUẢN LẠNH

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Nghiên cứu này nhằm đánh giá ảnh hưởng của thời gian xử lý bằng nano chitosan tải ergothioneine (ECNP) đến quá trình biến màu và oxy hóa lipid trong cơ thịt cá ngừ vây vàng bảo quản lạnh ở 3 ± 1 °C trong vòng 5 ngày. Các mẫu cơ thịt cá ngừ được xử lý với ECNP ở liều lượng 250 mg/kg trong các khoảng thời gian khác nhau: 30 phút, 60 phút, 90 phút và nhóm đối chứng (không xử lý). Kết quả cho thấy thời gian xử lý ảnh hưởng đáng kể đến hiệu quả ức chế biến màu và oxy hóa lipid của ECNP trong cơ thịt cá ngừ bảo quản lạnh. Thời gian xử lý 60 phút giúp duy trì chất lượng cơ thịt cá ngừ loại A trong tối đa 3 ngày bảo quản lạnh. Kéo dài thời gian xử lý đến 90 phút không mang lại cải thiện đáng kể so với 60 phút. Những kết quả này cho thấy thời gian xử lý 60 phút là phù hợp để hạn chế quá trình oxy hóa lipid và biến màu trong cơ thịt cá ngừ vây vàng bảo quản lạnh.

Từ khóa: Hoạt tính chống oxy hóa, polymer sinh học, sashimi, kiểm soát chất lượng, *Scombridae.*