Turk. J. Fish.& Aquat. Sci. 23(11), TRJFAS23374
https://doi.org/10.4194/TRJFAS23374

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Influence of Epigallocatechin Gallate on Quality of Cooked Harpiosquillid Mantis Shrimp (*Harpiosquilla raphidea*) Subjected to Multiple Freeze-Thaw Cycles

Wattana Temdee[1], Avtar Singh[1], Hui Hong[2], Bin Zhang[3], Ahmet Faruk Yesilsu[4], Soottawat Benjakul[1,*]

[1]Prince of Songkla University, Faculty of Agro-Industry, International Center of Excellence in Seafood Science and Innovation (ICE-SSI), Hat Yai, Songkhla 90110, Thailand
[2]China Agricultural University, College of Food Science and Nutritional Engineering, Beijing Laboratory for Food Quality and Safety, Beijing, China
[3]Zhejiang Ocean University, College of Food Science and Pharmacy, Key Laboratory of Health Risk Factors for Seafood of Zhejiang Province, Zhoushan 316022, China
[4]Central Fisheries Research Institute, Trabzon, Türkiye.

How to Cite

Temdee, W., Singh, A., Hong, H., Zhang, B., Yesilsu, A.F., Benjakul, S. (2023). Influence of Epigallocatechin Gallate on Quality of Cooked Harpiosquillid Mantis Shrimp (*Harpiosquilla raphidea*) Subjected to Multiple Freeze-Thaw Cycles. *Turkish Journal of Fisheries and Aquatic Sciences*, *23(11)*, *TRJFAS23374*. https://doi.org/10.4194/TRJFAS23374

Article History
Received 07 January 2023
Accepted 12 June 2023
First Online 23 June 2023

Corresponding Author
Tel.: +6674286334
E-mail: soottawat.b@psu.ac.th

Keywords
Cooked harpiosquillid mantis shrimp
Quality
Freezing
Thawing
EGCG
Oxidation

Abstract

Effects of epigallocatechin gallate (EGCG) at 200 or 400 ppm on quality changes of cooked Harpiosquillid mantis shrimp (*Harpiosquilla raphidea*) (CHMS) meat subjected to varying freeze-thaw cycles (F-T-C; 0, 1, 3, and 5) were studied. With augmenting F-T-C, the CHMS meat underwent deterioration to higher extent as indicated by upsurges in exudate loss, tougher texture along with increased microbial counts. The addition of EGCG could retard those changes in a dose-dependent manner. Furthermore, EGCG prevented microbial growth as ascertained by lower counts and total volatile basic nitrogen (TVB) content. Lipid oxidation was also impeded in the presence of EGCG, especially at a high concentration as evidenced by lowered malondialdehyde (MDA), while maintaining polyunsaturated fatty acids, particularly EPA and DHA when F-T-C raised. Total disulfide bond content and surface hydrophobicity of CHMS added with EGCG were lower than those of the control. Those alterations were more retarded as EGCG concentration increased. Therefore EGCG at 400 ppm effectively prevented quality loss of CHMS meat during repeated F-T process.

Introduction

Harpiosquillid mantis shrimp (*Harpiosquilla raphidea*) (HMS) is a preferable crustacean in either local or global markets such as Singapore, Vietnam, Korea, China, and Japan (Bo et al., 2020; Lee et al., 2022). It has become an economically important species and is regarded as an excellent source of nutrients such as polyunsaturated fatty acids, vital amino acids, and vitamins. Its consumption has been rising noticeably each year. The volume of mantis shrimp caught from the sea of Thailand was approximately 700–800 tons per year with a value of 10.4 million USD in year 2022 (Yarnpakdee et al., 2022). Raw HMS are prone to degradation associated with softened meat within a few days of iced storage, thus shortening shelf life and consumer acceptability (Lee et al., 2022; Temdee et al., 2022a). HMS could be stored on ice for only 2 days, but cooked HMS (CHMS) having a core temperature of 85°C showed a longer shelf life (9 days) when stored at 4°C (Temdee et al., 2022b). Overall, both raw HMS and CHMS still have a short shelf life and are susceptible to spoilage during storage or distribution. Hence, frozen storage is a promising preservation method for keeping the quality of HMS (Shi et al., 2022).

While frozen storage could limit bacterial activity and enzymatic reactions, it often causes undesirable physical, chemical, and structural changes in fish/shellfish associated with color, texture, and flavor alteration (Lan et al., 2020; Shi et al., 2022; Du et al.,

2021; Zhang et al., 2021), In general, extrinsic factors such as freezing rate, storage temperature, freeze-thawing condition, and distribution methods, directly determine and display the quality change of frozen seafood (Lorentzen et al., 2020; Lan et al., 2020, Yasemi, 2017; Kingwascharapong and Benjakul, 2016). Furthermore, increased size of ice and recrystallization during repeated freeze-thawing enhance injury of muscle cells. The loss of cellular components is associated with muscle dehydration and toughened texture (Du et al., 2021; Du et al., 2023).

Green tea polyphenols, as natural and non-toxic compounds, exhibited antioxidant and antibacterial properties in seafood (Tian et al., 2022; Nirmal & Benjakul, 2009). Catechins are the most important and abundant biomolecule in tea. Epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and EGCG are four major catechin derivatives in green tea (Li et al., 2022; Khan & Mukhtar, 2019). EGCG (400 ppm) in combination with squid pen chitooligosaccharides (400 ppm) could extend shelf life of yellowfin tuna fillets stored at 4°C for 12 days (Singh et al., 2021). Apart from serving as antioxidant or antibacterial agent, EGCG exhibited cross-linking effect toward myofibrillar proteins and EGCG could improve myofibrillar protein gel properties (Li et al., 2021). Furthermore, EGCG aids in the preservation of food freshness (Sae-leaw & Benjakul, 2019; Singh et al., 2021; Nirmal & Benjakul, 2009; Mittal et al., 2021).

EGCG from green tea could act as a natural antioxidant and antimicrobial agent, replacing synthetic antioxidants such as BHA and BHT as well as other antimicrobial agents. It could be applied to CHMS meat after peeling, followed by freezing or frozen storage. Also, it might retard the deterioration of CHMS during multiple freeze-thawing processes. However, no detailed information on the use of EGCG in CHMS meat for quality maintenance during frozen storage exists. The goal of this study was to elucidate the impact of EGCG at varying levels on the quality alteration of CHMS meat subjected to multiple freeze-thaw cycles (F-T-C).

Materials and Methods

Chemicals

Ammonium molybdate, 5-5´-dithio-bis (2-nitrobenzoic acid) (DTNB), 1-anilinonaphthalene-8-sulphonic acid (ANS), bovine serum albumin, malondialdehyde (MDA) and sodium acid pyrophosphate and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Tetrasodium pyrophosphate was purchased from Loba Chemie Pvt. Ltd (Colaba, Mumbai, India). (-)-epigallocatechin gallate (EGCG) was purchased from Chengdu Biopurify Phytochemicals Ltd., (Sichuan, China). Chloroform, methanol and hydrochloric acid were purchased from RCI Labscan (Bangkok, Thailand). All chemicals were of analytical grade.

Preparation of CHMS Meat

HMS weighing 58.8-66.7 g were acquired from the Tamalung fishing port in Satun province, Thailand. Two-hundred live HMS were embedded in polystyrene boxes with iced water by the vendor and brought to the laboratory within 3 h. After being cleaned with fresh water upon arrival, HMS were steamed for 5 min using a steaming pot, achieving a core temperature of 85°C. CHMS were promptly submerged for 2 min in cold water (4±2°C) after steaming. Shells were manually removed after beheading. CHMS meat was soaked in a solution (1:2; w/v) comprising 2.5% NaCl, 2.625% tetrasodium pyrophosphate, and 0.875% sodium acid pyrophosphate for 2 h at 4°C and stirred every 30 min (Rattanasatheirn et al., 2008). Treated HMS meat was spread on a plastic screen for 5 min (4 °C) to allow the solution to drain off. The sample was labeled as 'CHMS'.

Effect of EGCG at Various Levels on Quality Changes of CHMS Subjected to Different F-T-C

Prepared CHMS was treated without (control) and with EGCG at several two levels (200 and 400 ppm). A certain amount of EGCG was mixed with a little volume of distilled water and the solution was mixed with CHMS to obtain the desired concentration. Thereafter each sample was placed in a polyethylene tray (193 × 130 × 12 mm) and inserted in LLDPE/polyamide bag (35 µm each) (180 × 280 mm2). The bag had a thickness of 0.070 mm and certain gas permeability (O_2 transmission rate at 38°C = 47.62 cm^3 /m^2 /day). The samples were vacuum-packed using an Audionvac VM203 vacuum sealer (Audionvac, Weesp, the Netherlands). Subsequently, the packed samples were frozen at -20°C with the aid of an air blast freezer for 24 h and subsequently thawed at 4°C for 12 h, in which core temperature was finally 0-2°C. The F-T process was carried out for 0, 1, 3, and 5 cycles and the obtained thawed samples were analyzed.

pH

The samples were left at room temperature for 30 min. The meat sample (2 g) was mixed with deionized water (18 mL) and homogenized (11,000 rpm, 1 min) using a homogenizer (IKA Labortechnik, Selangor, Malaysia). The pHs of the homogenates were determined with a pH meter (Eutech Instrument-pH 700, Eutech Instrument, Singapore) according to Benjakul et al. (1997).

Moisture Content and Exudate Loss

The approved oven drying method was implemented to determine the moisture content at 102-105°C (AOAC, 1999). Exudate loss was determined, computed and reported as the percentage of water loss, relative to that of initial weight (without freeze-thawing) (Pastoriza et al., 1996).

Shear Force

The texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, England), fitted with a Warner-Bratzler shear apparatus, was used to examine shear force of the samples (Temdee et al., 2022b). The operational condition consisted of a cross-head speed of 2 mm/s and a 50 kg load cell. The fourth segment of abdomen was used to determine the shear force and the required force (N) for shearing was recorded.

Color

The color of samples was evaluated using a colorimeter (Hunter Lab, Colorflex, Reston, Virginia, USA) (Temdee et al., 2022b). Measurements were made at the dorsal portion of samples (4 to 8 segments). Lightness (L*), greenness/redness (a*), and yellowness/blueness (b*) were determined.

Total Volatile Base-nitrogen (TVB-N) Content, Trimethylamine-nitrogen (TMA-N) Contents, Peroxide Value, and Thiobarbituric Acid Reactive Substances

TVB-N and TMA-N contents were determined by the Conway method (Conway & Byrne 1933) as tailored by Benjakul et al. (2002). For lipid oxidation, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) values were measured (Singh & Benjakul, 2020).

Total Disulfide Bond, Surface Hydrophobicity, and Fluorescence Intensity

Before analysis, myofibrillar proteins were extracted from the samples (Du et al., 2021) Two grams of chopped CHMS were combined with 10 mL of cold distilled water and homogenized for 1 min. Homogenate was subsequently centrifuged at 14,000 *g* for 10 min. To get rid of other water-soluble proteins, the pellets were washed with 15 mL of cold 0.3% NaCl for 30 min at 4°C, followed by centrifugation as previously described. The precipitate was mixed with 30 ml of 0.6 M NaCl in 20 mM Tris-maleate (pH 7.0) buffer and homogenized for 10 sec. The homogenate was centrifuged at 14,000 *g* and 4°C for 10 min after keeping at 4°C for 1 h to extract myofibrillar proteins. Four volumes of cold water were added into the supernatant. The precipitated myofibrillar protein was collected by centrifugation, further dissolved in cold 0.6 M NaCl, and centrifuged at 4°C (14,000 *g* for 5 min). The supernatant was used as 'myofibrillar protein'. Protein content of myofibrillar protein solution was determined using the Biuret assay and adjusted to 4 mg/ml.

The content of disulfide bond and surface hydrophobicity were determined as tailored by Benjakul et al. (2003). The fluorescence spectrum was measured by RF-1501 Fluorescence Spectrophotometer (Shimadzu, Kyoto, Japan) according to the method of Matmaroh et al., (2006). Myofibrillar protein solution (0.5 mg/ml) was used. Excitation wavelength was 295 nm. The emission slit was 2.5 nm. The emission wavelength was fixed between 310 and 400 nm and the spectral resolution was 1 nm.

Microbiological Analysis

Samples were collected aseptically and enumerated for total viable count (TVC), psychrophilic bacteria count (PBC), *Pseudomonas* count (PC), *Enterobacteriaceae* count (EC), hydrogen sulphide-producing bacteria count (HSBC), and lactic acid bacteria count (LABC) (Olatunde et al., 2019).

Fatty Acid Composition

Fatty acid profile of samples were analyzed after the extraction of lipid from the samples using the Bligh & Dyer method (1959). Gas chromatography equipped with a flame-ionized detector was used and all analysis conditions as tailored by Singh and Benjakul (2020) were adopted. Prior to analysis, fatty acid methyl esters (FAMEs) were prepared. Fatty acid content was reported as g/100g lipid.

Statistical Analysis

The experiments were performed using a completely randomized design (CRD). Three distinct batches of HMS were used for the experiment. Analyses were also run in triplicate. Analysis of variance was used and mean comparison was made using Duncan's multiple range test. T-test was used for paired comparison. With the help of the SPSS software (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA), statistical analyses were carried out.

Results and Discussion

Changes in pH, Moisture Content, Exudate Loss, and Shear Force

The pHs of CHMS samples added without and with EGCG at two different levels after different F-T-C are presented in Figure 1A. The pH value of samples decreased with increasing F-T-C, irrespective of EGCG levels. At 0 cycle, the control had the higher pH, followed by those added with 200 and 400 ppm EGCG, respectively ($p<0.05$). Tian et al. (2022) also documented that pH of surimi gel from *Hypophythalmalmichthys molitrix* added with 0.01-0.02% EGCG was decreased with increasing F-T-C (0-6 cycles). However, when F-T-C increased, the pH decreased at higher rate in the control than that of EGCG treated samples. Hydrolysis of neutral lipids or phospholipids might result in the generation of free fatty acids, thus causing the lower pH of the samples (Maqsood et al., 2014). Furthermore, during F-T process, particularly after thawing, microorganisms could grow

and degrade small organic molecules into acidic compounds (Tian et al., 2022; Pei et al., 2022; Guo et al., 2014). Deamidation of asparagine or glutamine by some microorganisms could trigger the formation of aspartic acid and glutamic acid, respectively (Kempkes et al., 2016). The decomposition of some amino acids mediated by the spoilage bacteria was dramatically reduced in the EGCG-added samples during thawing. According to Tan et al. (2021), pH shift attenuation might lessen the denaturation of myofibrillar proteins induced by freezing process. The pHs of the EGCG-added samples in the present study were more constant than those of the control samples, suggesting that EGCG might be able to lessen myofibrillar protein denaturation as induced by acidic conditions. As a result, quality of CHMS could be more maintained during freeze-thawing. The lower decrease in the pH of CHMS added with 200 ppm or 400 ppm EGCG might retard the change of muscle protein during frozen storage and thawing.

The moisture contents of samples containing EGCG at varying levels as affected by various F-T-C are depicted in Figure 1B. With upsurge in F-T-C, lower moisture content was obtained, regardless of EGCG addition. This was more likely due to water loss of meat during the F-T process. Moreover, aggregation of protein induced by repeated F-T process could reduce water holding capacity of muscle proteins (Kingwascharapong & Benjakul, 2016; Jiang et al., 2019). Mechanical injury of muscle cells associated with the formation of ice crystals could result in the loss of cellular components (Jiang et al., 2019). For EGCG-treated samples, CHMS added with 200 ppm EGCG had the highest moisture content during whole F-T-C ($p<0.05$). When EGCG concentration was increased to 400 ppm, lower moisture content was noticed, especially after 3 and 5 F-T-C ($p<0.05$). EGCG at high level plausibly caused the myofibrillar protein to crosslink excessively, in which dense aggregate could not hold water potentially (Tian et al., 2022; Li et al.,

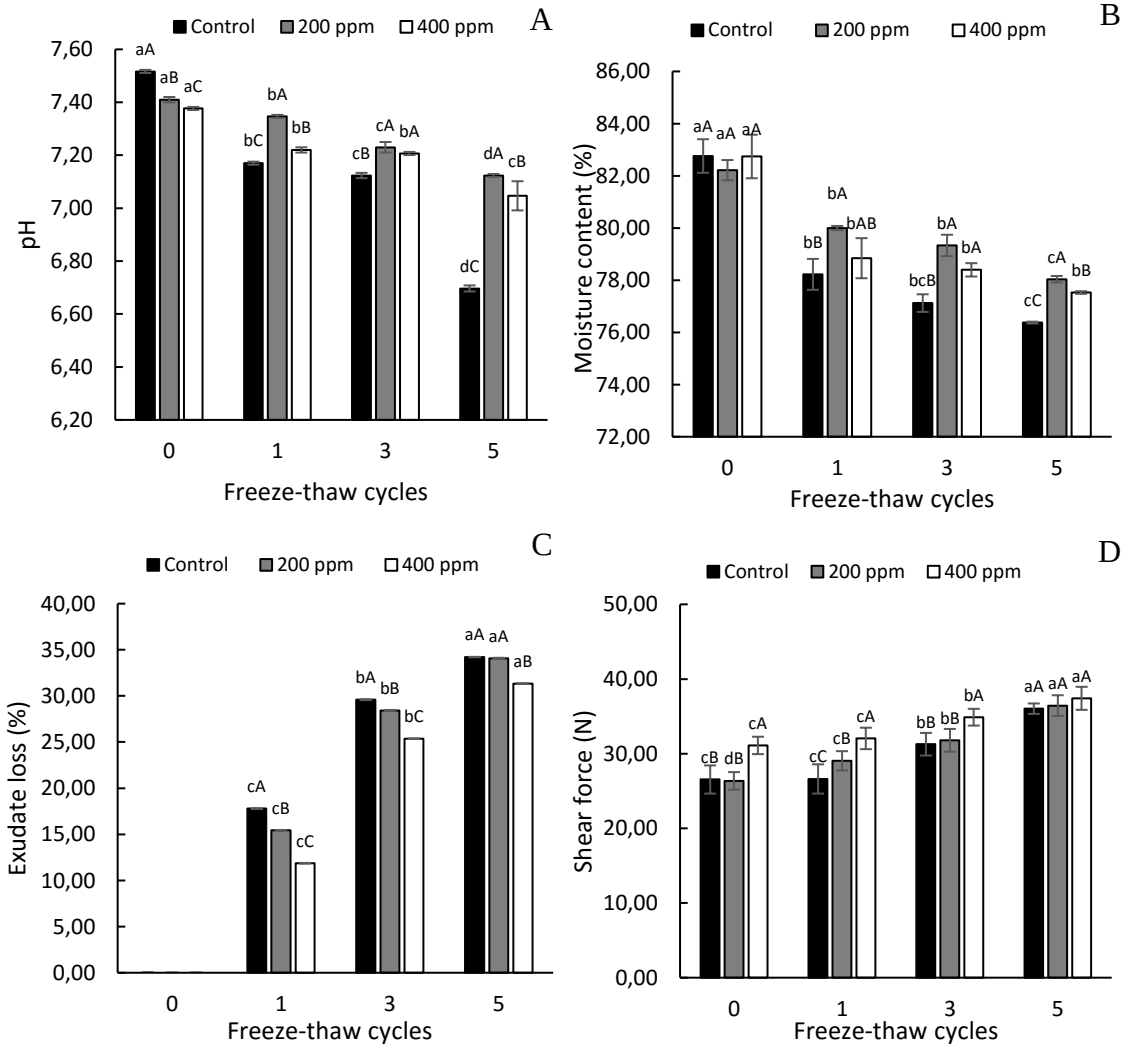

Figure 1. Change in pH (A), moisture content (B), exudate loss (C) and shear force (D) of cooked harpiosquillid mantis shrimp (*Harpiosquilla raphidea*) meat subjected to different freeze-thaw cycles in the absence and presence of EGCG (200 and 400 ppm).

Different lowercase letters on the bars indicate significant differences within the same sample ($p<0.05$). Different uppercase letters on the bars indicate significant differences within the same freeze-thaw cycles ($p<0.05$). Bars represent the standard deviation (n = 3).

2021). EGCG was reported as potential protein cross-linker (Quan et al., 2020). The declining consistency and density of muscle are associated with poorer water-holding capacity of muscle structure (Tian et al., 2022). As a consequence, CHMS was not able to adequately entrap water.

Numerous F-T-C could enhance the alteration of textural and thermophysical characteristics of CHMS, which resulted in water loss or exudate formed (Figure. 1C). At 1 and 3 F-T-C, the highest exudate loss was noticed in the control sample. However, in the presence of 400 ppm EGCG, exudate loss was reduced ($p<0.05$), particularly with 5 F-T-C. Nevertheless, similar exudate loss between the control and the sample incroporated with 200 ppm EGCG ($p>0.05$) after 5 F-T-C was attained. In general, combination of internal forces induced by connective tissue shrinkage and reduction in water holding capacity of proteins owing to denaturation could induce the exudate loss (Kingwascharapong & Benjakul, 2016). Heat was able to induce myofibrillar protein denaturation and collagen shrinkage, resulting in the tightening and stiffness of the muscle (Kingwascharapong & Benjakul, 2016). As a result, there was less water trapped inside the protein structure. With the aid of phosphate pretreatment, more water could be more retained. However, due to the repeated freeze-thawing, some remaining water could be leached out as ascertained by the augmented exudate loss. Increased F-T-C generally reduced the moisture content of CHMS (Figure 1B). Protein aggregation accelerated by EGCG could enhance bound water, especially via OH-group of EGCG. Therefore, transformation of immobilized water to free form was restricted by dense protein networks.

Figure 1D shows the shear force of samples without and with EGCG addition after different F-T-C. Normally shear force determines the firmness or toughness of the shrimp flesh. The control had lower shear force than those added with EGCG at 400 ppm ($p<0.05$) at day 0. This was probably owing to the increased aggregation of proteins in the presence of 400 ppm EGCG. The shear force of all the samples up-surged with augmenting F-T-C ($p<0.05$). With 1 F-T-C, the highest shear force was noticed for the sample containing 400 ppm EGCG followed by that added with 200 ppm EGCG. The lowest shear force was attained for the control ($p<0.05$). However, shear force was similar among all samples incorporated with EGCG at both levels and the control after 5 F-T-C ($p>0.05$). When F-T-C increased, more compact muscle fibers were plausibly developed (Tan, et al.,2021; Han & Gokoglu, 2022; Kingwascharapong & Benjakul, 2016). After 5 F-T-C, the control without EGCG might under oxidation to higher extent. The oxidation products such as aldehyde, etc. might induce the protein cross-linking to high extent. This was generally associated with decreased moisture content (Figure. 1B) and increased exudate loss (Figure. 1C).

Changes in Color

Color is a well-known market pricing influencer and determines consumer acceptance of the product. All samples without F-T process had similar L*, a*, and b*

Table 1. Change in color, TVB, TMA, PV and TBAR of cooked harpiosquillid mantis shrimp meat subjected to different freeze-thaw cycles in the absence and presence of EGCG (200 and 400 ppm).

Parameter		EGCG level (ppm)	Freeze–thaw cycles			
			0	1	3	5
Color	L*	0	71.38 ± 0.55^{aA}	67.16 ± 1.72^{bA}	64.95 ± 0.46^{cA}	64.39 ± 0.30^{cA}
		200	71.43 ± 1.62^{aA}	66.72 ± 0.23^{bB}	62.92 ± 0.66^{cB}	61.29 ± 0.49^{cB}
		400	71.71 ± 0.83^{aA}	65.65 ± 0.12^{bC}	62.28 ± 0.23^{cB}	61.03 ± 0.28^{dB}
	a*	0	8.67 ± 0.06^{aA}	6.42 ± 0.12^{bA}	6.04 ± 0.23^{cA}	5.83 ± 0.16^{dA}
		200	8.70 ± 0.06^{aA}	5.81 ± 0.15^{bB}	5.60 ± 0.03^{cB}	5.20 ± 1.02^{dA}
		400	8.55 ± 0.01^{aA}	6.48 ± 0.24^{bA}	4.99 ± 0.45^{cC}	2.62 ± 0.09^{dB}
	b*	0	6.24 ± 0.15^{aA}	5.19 ± 0.30^{bA}	4.04 ± 0.18^{cA}	3.95 ± 0.06^{cA}
		200	6.36 ± 0.02^{aA}	4.21 ± 0.19^{bB}	4.00 ± 0.12^{cA}	3.21 ± 0.36^{dA}
		400	6.43 ± 0.10^{aA}	2.53 ± 0.32^{bC}	2.37 ± 0.05^{bB}	1.69 ± 0.10^{cB}
TVB (mg/100g)		0	0.79 ± 0.14^{cA}	1.70 ± 0.16^{bA}	2.25 ± 0.16^{aA}	2.51 ± 0.14^{aA}
		200	0.83 ± 0.12^{cA}	1.46 ± 0.27^{bA}	1.86 ± 0.16^{abB}	2.08 ± 0.31^{aB}
		400	0.90 ± 0.16^{bA}	1.30 ± 0.03^{aB}	1.35 ± 0.01^{aC}	1.40 ± 0.01^{aC}
TMA (mg/100g)		0	-	-	0.66 ± 0.08^{aA}	0.69 ± 0.03^{aA}
		200	-	-	0.62 ± 0.01^{aA}	0.68 ± 0.05^{aA}
		400	-	-	0.60 ± 0.05^{aA}	0.64 ± 0.02^{aA}
PV (mg cumene hydroperoxide/ kg)		0	0.23 ± 0.05^{dB}	0.74 ± 0.03^{cA}	1.02 ± 0.13^{bA}	1.72 ± 0.03^{aA}
		200	0.45 ± 0.05^{cA}	0.54 ± 0.07^{cB}	0.95 ± 0.03^{aB}	1.57 ± 0.15^{aB}
		400	0.47 ± 0.01^{cA}	0.50 ± 0.01^{cB}	0.84 ± 0.05^{bC}	1.41 ± 0.10^{aC}
TBARS (mg MDA/kg)		0	0.56 ± 0.23^{cA}	0.60 ± 0.17^{cA}	1.11 ± 0.50^{bA}	1.31 ± 0.55^{aA}
		200	0.46 ± 0.31^{cA}	0.58 ± 0.55^{cB}	1.09 ± 0.11^{bB}	1.23 ± 0.23^{aB}
		400	0.50 ± 0.47^{cA}	0.53 ± 0.51^{cC}	0.74 ± 0.24^{bC}	1.10 ± 0.35^{aC}

Mean ± SD (n = 3). Different lowercase superscripts in the same row indicate significant difference ($P<0.05$). Different uppercase superscripts in the same column within the same parameter tested indicate significant difference ($P<0.05$).

values (p>0.05) (Table 1). These findings showed that EGCG did not affect the color of CHMS (Figure 2). Lightness became lower (p<0.05) as F-T-C increased. The decline in the lightness of samples was attributable to the aggregation of myofibrillar protein caused by repeated F-T process. Moreover, protein aggregation might diminish free water content in meat structure, resulting in the lower light scattering (Li et al., 2019; Ye et al., 2022). When compared to the control, the EGCG-treated samples had lower lightness as F-T-C upsurged (p<0.05). Among all samples, CHMS incorporated with 400 ppm EGCG had the highest reduction in a* value (p<0.05) after 1 F-T-C. The formation of dark colored quinones produced from EGCG oxidation might take place during repeated F-T process (Tian et al., 2022). Similarly, Thanonkaew et al. (2008) found a decrease in L* values for cuttlefish during the first 4 weeks of frozen storage. For a* value, all samples without F-T process exhibited the same value. The a* value decreased with augmenting F-T-C in all samples (p<0.05). Similar result was observed for b* value. In general, color, particularly the redness and yellowness, of seafood products is affected by degree of lipid oxidation and astaxanthin degradation (Tian et al., 2022). Denaturation of carotenoprotein took place in HMS meat during cooking. As a result, free astaxanthin became dominant showing the orange-red color of the samples. EGCG at level higher than 200 ppm caused the discoloration of sample due to their oxidation as induced by multiple F-T processes. Moreover, polyphenols at higher concentration might act as pro-oxidant (Lambert & Elias, 2010). However, no obvious difference in color of all samples after 5 F-T-C was detected visually (Figure 2).

Changes in TVB and TMA Contents

Initial TVB-N values of the control, samples added with 200 ppm EGCG, and 400 ppm EGCG were 0.79, 0.83, and 0.90 mg N/100 g, respectively. Irrespective of EGCG addition, TVB-N content was augmented with increasing F-T-C. The control had the highest TVB-N value (2.51 mg/100 g) (p<0.05), regardless of F-T-C. However, the addition of EGCG resulted in lower TVB-N values, especially at higher concentration of EGCG (p<0.05). After 5 F-T-C, the sampled containing 400 ppm EGCG had the lowest TVB-N (1.40 mg/100 g). In seafood products, volatile basic compounds are decomposed substances mediated by microbial action (Temdee et al., 2022b). In CHMS, EGCG particularly at 400 ppm impeded the growth of spoilage bacteria. Polyphenols were efficient in delaying oxidation and degradation of proteins, particularly myofibrils (Wei et al., 2021). Thus, small peptides, which could be used by microorganisms, were also reduced.

No TMA was detected in CHMS with F-T-C of 0 and 1.TMA was found after 3 F-T-C (Table 1). With theincrease in F-T-C, TMA could be produced by bacteria with TMAO reductase (Benjakul et al., 2004), particularly during or after thawing process. In general, TMA-N content at 5 mg/100g is the permitted upper limit for shrimp (Okpala et al. 2014). EGCG had no impact on TMA content in CHMS, while it showed a profound effect on the retardation of TVB-N. Thus, EGCG might not be able to inhibit bacteria having TMAO reductase.

Changes in PV and TBARS Values

Cellular membranes of several crustaceans have high content of polyunsaturated fatty acids (PUFA) (Okpala et al., 2014). Those PUFA are prone to oxidation. During the freeze-thawing process, tissues or muscles underwent damages, thus releasing PUFA. PUFA could be oxidized during both thawing and frozen storage (Nazemroaya et al., 2009). PV frequently refers to primary lipid oxidation products, especially hydroperoxide (Okpala et al., 2014). Table 1 displays changes in PV of all CHMS samples as influenced by F-T-C. PV of the control increased (p<0.05) from 0.23 to 1.72

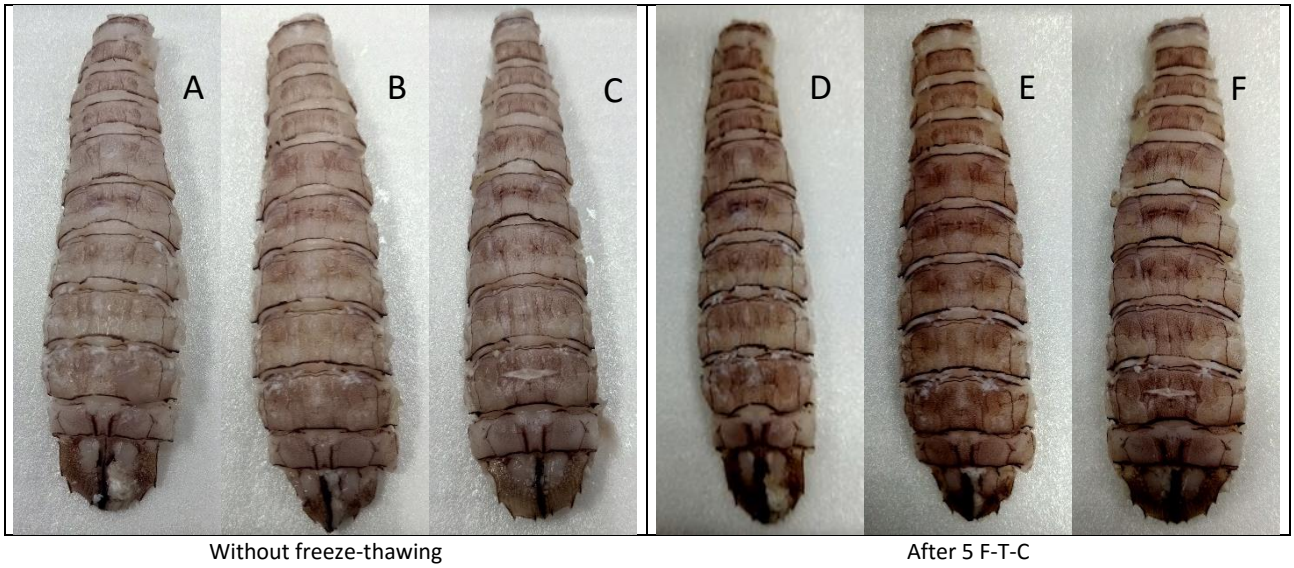

Figure 2. Pictures of cooked harpiosquillid mantis shrimp meat without (A, B, C) and with 5 freeze-thaw cycles (D, E, F) in the absence of EGCG (A, D), presence of 200 ppm EGCG (B, E) and 400 ppm EGCG (C, F).

mg cumene hydroperoxide/ kg when F-T-C increased from 0 to 5 cycles. The lowest increase in PV was attained in the sample incorporated with EGCG at 400 ppm, indicating the efficacy of EGCG in the prevention of lipid oxidation. EGCG was reported to have radical scavenging activities (Singh et al., 2021; Quan et al., 2020; He et al., 2018). In the present study, the samples were packaged under vacuum. Vacuum packaging could remove the air containing oxygen to high degree, thus lowering oxidation of lipids, especially those containing polyunsaturated fatty acids (Erikson et al., 2021). Vacuum packaging effectively prevented lipid oxidation in sturgeon and rainbow trout fillets subjected to multiple freeze-thawing (Du et al., 2023; Vilkova et al., 2022).

Similar results were obtained for TBARS value of all the samples (Table 1). Generally, the TBARS represent secondary lipid oxidation products, associated with rancidity and loss of product quality (Tian et al., 2022; Yasemi, 2017). The TBARS values of samples increased continuously as F-T-C upsurged. This suggested that CHMS was vulnerable to oxidation during freeze-thawing ($p<0.05$). In many frozen marine species, lipid oxidation takes place as induced by various endogenous enzymes such as lipase, peroxidases, lipoxygenases, oxidases, etc. (García-Soto et al., 2015). Some enzymes might be produced by microorganisms grown in CHMS during freeze-thawing process. Additionally, freezing and thawing may result in the release of prooxidants like Cu2+ in the hemocyanin of mantis shrimp. Hemocyanin is the pigment rich in copper found in crustacean blood (Singh & Good, 2022; Taylor & Anstiss, 1999). For the sample added with EGCG, lipid oxidation occurred at a lower extent. This mainly resulted from the excellent antioxidant activity of EGCG, which prevented the production of free radicals and interrupted their propagation (Pei et al., 2022). EGCG includes eight phenolic hydroxyl groups, which may scavenge free radicals and effectively terminate free radical chain reactions by acting as hydrogen donor (Tian et al., 2022; Nikoo et al., 2018; Quan et al., 2020). Overall, malonaldehyde levels CHMS remained lower than 2.0 mg MDA/kg sample, which is the level that panelists would be unable to detect or perceive any off-flavors or aromas.

Changes in Total Disulfide Bond Content

Disulfide bond content in myofibrillar protein increased as F-T-C increased from 0 to 5 cycles ($p<0.05$) (Figure 3A). The content of disulfide bond in myofibrillar protein was lower in the presence of EGCG, regardless of F-T-C. This indicated that EGCG could prevent disulfide bond formation via oxidation of free sulfhydryl groups. During frozen storage, sulfhydryl groups in fish muscle could undergo oxidation with coincidental

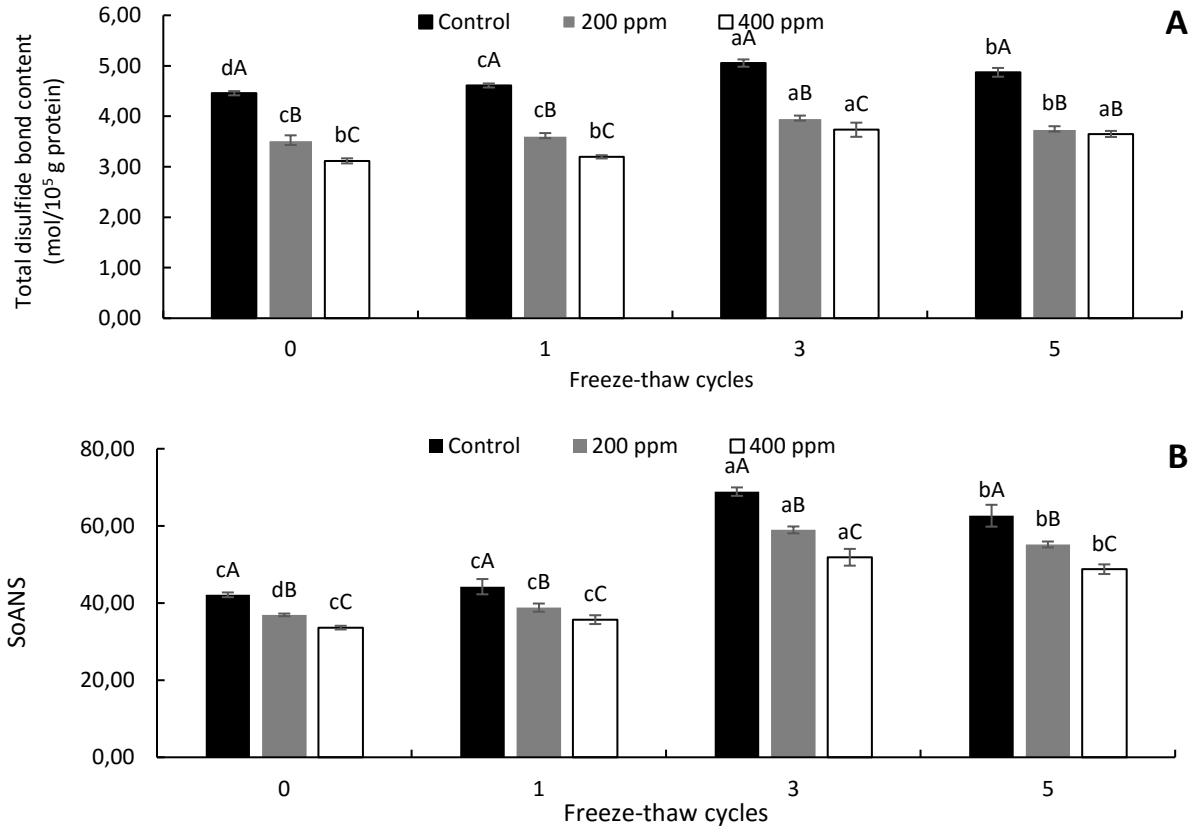

Figure 3. Changes in the total disulfide bone content (A), and surface hydrophobicity (S_0ANS) (B) of myofibrillar proteins of cooked harpiosquillid mantis shrimp (*Harpiosquilla raphidea*) treated without and with EGCG (200 ppm, 400 ppm) and subjected to different freeze-thaw cycles. Different lowercase letters on the bars within the same EGCG level indicate significant differences ($p<0.05$). Different uppercase letters on the bars within the same freeze-thaw cycle indicate significant differences ($p<0.05$). Bars represent standard deviations (n=3).

formation of disulfide bonds (Leelapongwattana et al., 2005). Phenolic compounds have the ability to transform into quinone. Quinone has the potential to interact with sulfhydryl groups to generate covalent C-S bonds. This phenomenon could diminish free sulfhydryl groups for further oxidation (Cao & Xiong, 2015). In addition, hydroxyl groups provided by EGCG might form hydrogen bonds with protein, thus preventing myofibrillar protein to align closely for development of disulfide bonds (Shi et al., 2017).

Changes in Surface Hydrophobicity

Figure 3B shows the changes in the surface hydrophobicity of myofibrillar protein from CHMS during different F-T-C. The surface hydrophobicity increased up to 3 F-T-C, followed by a decrease up to 5 F-T-C. The increased surface hydrophobicity showed that myofibrillar protein in CHMS underwent structural and conformational changes during freeze-thawing, which could have caused the protein to unfold and expose the hydrophobic residues or domains (Shi et al., 2017; Tan et al., 2022). In the presence of EGCG, especially at 400 ppm, the lower surface hydrophobicity was observed. The results suggested that EGCG could decrease the alteration of myofibrillar protein spatial arrangement. EGCG might interact with protein via hydrogen bonds or hydrophobic interaction. As a result, the unfolding of proteins could be retarded as witnessed by a lower increase in surface hydrophobicity. In addition, EGCG might attach to the exposed hydrophobic amino acids. On the other hand, phenolic compounds may cause the exposure of hydrophobic amino acid residues in proteins (Xu et al., 2021). Those exposed hydrophobic amino acids could interact with each other. The aggregation of unfolded protein molecules and the concealment of hydrophobic regions that had previously been exposed could contribute to the reduction in surface hydrophobicity towards the end of storage (Shi et al., 2017).

Intrinsic Fluorescence Intensity (IFI)

Tryptophan (Trp) is a particular aromatic amino acid commonly found in the interior portion of native proteins and it has been used to indicate the degree of protein unfolding. Due to the presence of Trp residues in both rod and head domains of myosin, intrinsic fluorescence measurement is frequently employed to assess the exposure of Trp and conformational alteration in myofibrillar proteins (Guo et al., 2021; Tan et al., 2022). Myofibrillar protein fluorescence spectra of samples without and with EGCG addition after multiple F-T-C are shown in Figure 4. The fluorescence intensity at 315-325 nm of all the samples decreased with augmenting F-T-C. The fluorescence intensity of myofibrillar protein in CHMS decreased to a higher extent for the control. In the presence of EGCG, myofibrillar protein fluorescence intensity was lower than the control, suggesting that EGCG could interact with myofibrillar protein, especially Trp, thus lowering fluorescence intensity (Tan et al., 2022). In addition, EGCG could interact with protein molecules in a way that hydrophobic domain became less exposed. Phenolic compounds can interact with proteins through some non-covalent interactions, e.g. hydrogen bonds or hydrophobic interaction (Tan et al., 2022; Jia et al., 2019). With higher level of EGCG, the stronger interactions between protein molecules might occur. This could contribute to the lower exposure of Trp localized inside the protein molecules.

Microbiological Changes

Table 2 shows TVC, PBC, PC, EC, HSBC, and LABC in the CHMS added without and with EGCG at 200 and 400 ppm as affected by F-T-C. The microbiological shelf life endpoint of the CHMS meat was 5 log CFU/g for TVC (Temdee et al., 2022b). For all the samples, TVC was below the acceptable limit at all F-T-C used. In the control, no difference was found in TVC ($p>0.05$) between 0 and 1 F-T-C. The highest TVC was found for the control than the remaining samples at all F-T-C used. Notably, EGCG-added samples had lower TVC ($p<0.05$) than the control, irrespective of EGCG concentration. The result suggested antibacterial activity of EGCG. CHMS prepared following the conventional procedure (steaming to have core temperature of 85°C) and packed under vacuum had shelf-life of 15 days at 4°C (Temdee et al., 2022b). Microbial structural integrity was disrupted by EGCG, which led to the leakage of intracellular components and functional impairment, thus causing the death of bacteria. EGCG also chelated metal ions required for various metabolic process (Tian et al., 2022; Klancnik, et al.,2012). Catechin (0.2%) inhibited bacteria growth in Pacific white shrimp via complexation with proteins in cell wall and lysing the cell wall (Nirmal & Benjakul, 2009). Phenolics generally have the potential to damage the cell walls of microorganisms (Tian et al., 2022; Klancnik, et al.,2012; Nirmal & Benjakul, 2009).

PBC showed a similar trend with TVC for all samples (Table 2). The samples with 1 F-T-C had the lowest PBC, when compared to samples with higher F-T-C ($p<0.05$). Sample with 5 F-T-C showed the highest PBC. During freeze-thawing, nutrients might be more released and available for the growth of psychrophilic bacteria in samples (Nirmal & Benjakul, 2009). Regardless of F-T-C, EGCG prevented the growth of bacteria during refrigerated storage. Therefore, the use of EGCG, especially at 400 ppm could retard the increase in PBC of CHMS during freeze-thawing process.

PC showed a similar pattern to TVC and PBC. Notably, PC of sample added with 200 ppm and 400 ppm EGCG was less than that of the control, regardless of F-T-C ($p<0.05$). *Pseudomonas* spp. were dominant bacteria causing spoilage of fish. *Pseudomonas* spp. were the specific spoilage bacteria in Pacific white

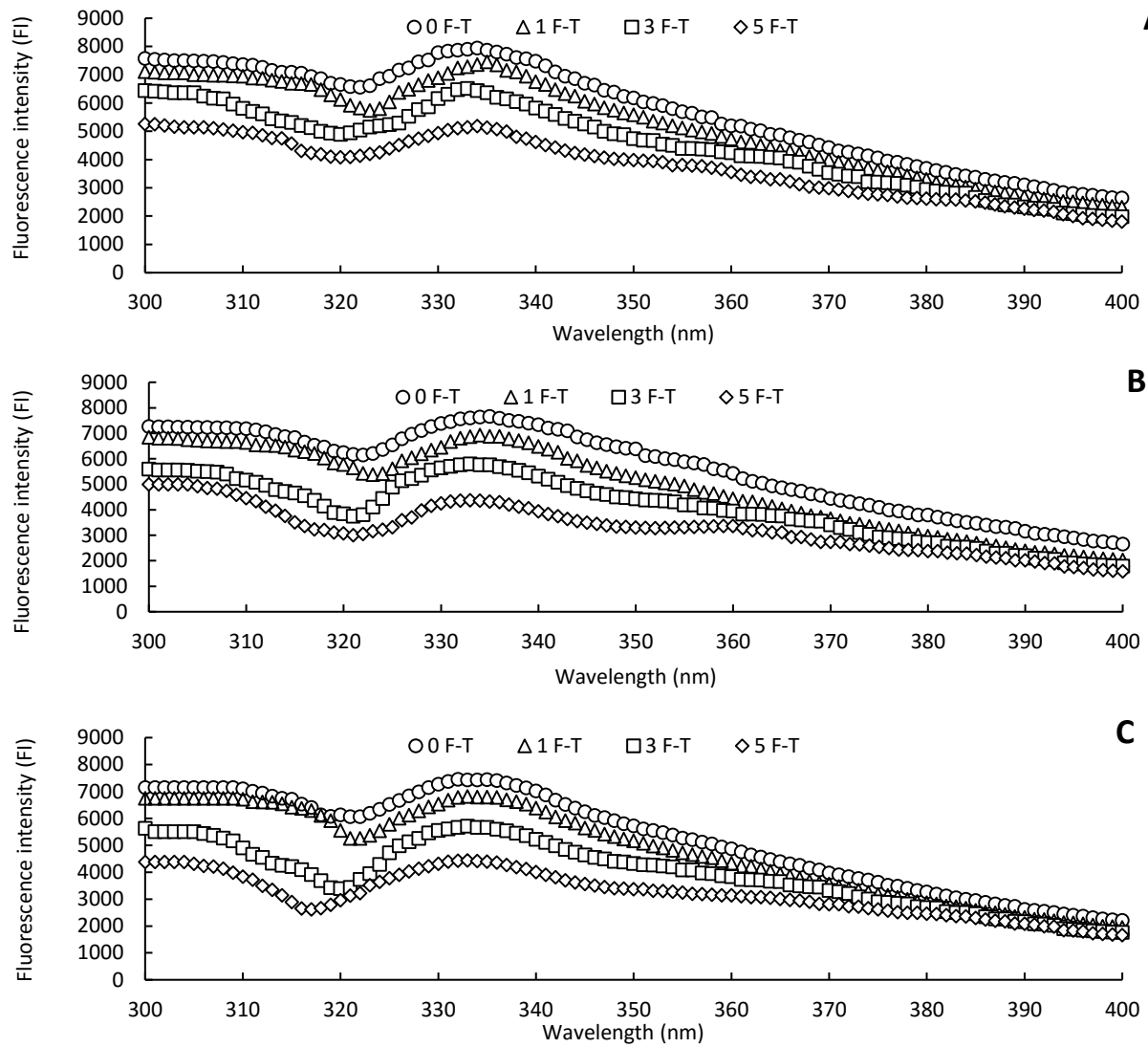

Figure 4. Changes in the fluorescence intensity of myofibrillar proteins from cooked harpiosquillid mantis shrimp (*Harpiosquilla raphidea*) meat subjected to different freeze-thaw cycles after treated without (A) and with EGCG at 200 ppm (B), and 400 ppm (C).

Table 2. Changes in TVC, PBC, *Pseudomonas count*, hydrogen sulphide producing bacteria count, Enterobacteriaceae count and lactic acid bacteria count of cooked harpiosquillid mantis shrimp meat subjected to different freeze-thaw cycles in the absence and presence of EGCG (200 and 400 ppm).

Microbial count (log CFU/g)	EGCG level (ppm)	Freeze–thaw cycles			
		0	1	3	5
TVC	0	2.02 ± 0.25^{bA}	2.08 ± 0.03^{bA}	4.67 ± 0.03^{aA}	4.96 ± 0.58^{aA}
	200	1.87 ± 0.07^{cB}	2.05 ± 0.28^{cA}	4.14 ± 0.05^{bB}	4.54 ± 0.87^{aB}
	400	1.54 ± 0.03^{cC}	2.00 ± 0.16^{cA}	4.08 ± 0.11^{bB}	4.14 ± 0.09^{aC}
PBC	0	1.54 ± 0.58^{cA}	1.87 ± 0.13^{cA}	4.33 ± 1.00^{bA}	4.67 ± 0.15^{aA}
	200	1.54 ± 0.40^{cA}	1.71 ± 0.40^{cB}	3.97 ± 0.58^{bB}	4.35 ± 0.52^{aA}
	400	1.50 ± 1.73^{cA}	1.67 ± 0.47^{cB}	3.67 ± 0.53^{bB}	4.19 ± 0.21^{aB}
Pseudomonas	0	1.75 ± 1.15^{cA}	2.07 ± 0.58^{bA}	4.71 ± 1.67^{aA}	4.94 ± 1.33^{aA}
	200	1.51 ± 1.00^{dB}	1.95 ± 0.43^{cB}	4.04 ± 0.21^{bB}	4.57 ± 0.73^{aB}
	400	1.47 ± 0.53^{dB}	1.53 ± 0.15^{cC}	3.87 ± 0.35^{bC}	4.21 ± 0.58^{aC}
Enterobacteriaceae	0	1.05 ± 0.05^{dA}	1.97 ± 0.73^{cA}	3.10 ± 0.51^{bA}	3.97 ± 0.15^{aA}
	200	1.00 ± 1.00^{dA}	1.62 ± 0.21^{cB}	2.30 ± 0.73^{bB}	3.85 ± 0.08^{aA}
	400	1.05 ± 0.53^{dA}	1.21 ± 0.08^{cC}	2.00 ± 0.05^{bC}	3.63 ± 0.47^{aA}
Hydrogen sulphideproducing bacteria	0	1.25 ± 0.58^{dA}	1.90 ± 0.08^{cA}	3.35 ± 1.00^{bA}	4.37 ± 0.16^{aA}
	200	1.20 ± 0.47^{cA}	1.50 ± 0.58^{bB}	3.18 ± 0.53^{aB}	3.33 ± 0.28^{aB}
	400	1.21 ± 0.03^{dA}	1.50 ± 0.58^{cB}	3.00 ± 0.08^{bC}	3.18 ± 0.09^{aC}
Lactic acid bacteria	0	ND*	ND	1.53 ± 0.21^{bA}	3.47 ± 0.35^{aA}
	200	ND	ND	1.30 ± 0.47^{bB}	3.31 ± 0.71^{aB}
	400	ND	ND	1.31 ± 0.35^{bB}	3.25 ± 0.58^{aB}

Mean ± SD (n=3). Different lowercase superscripts in the same row indicate significant difference ($P<0.05$). Different uppercase superscripts in the same column within the same parameter tested indicate significant difference ($P<0.05$). * ND = Not detected.

shrimp (Sae-leaw & Benjakul, 2019), European anchovy and sardine (Bono et al., 2017), large yellow croaker (*Larimichthys crocea*) (Pei et al., 2022), red king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*) (Lian et al., 2022a). The microbial load of 4 log CFU/g for *Pseudomonas* spp. was considered as the microbial shelf life endpoint of the cooked seafood product (Lian et al., 2022a). Although PC exceeded 104 CFU/g after 3 and 5 F-T-C, TVC was still lower than the limit (105 CFU/g). Thus, it was still acceptable for consumption. The growth of *Pseudomonas* spp. increased when F-T-C increased. Pseudomonadaceae are gram-negative bacteria, in which cell walls are mostly damaged by EGCG (Pei et al., 2022). Superoxide is produced as a result of the OH moiety of EGCG dissociating H+ in solution and reducing oxygen with an electron on the phenol. Superoxide is further reduced by EGCG, resulting in the formation of O22-. In addition, the proton interacts with superoxide to generate hydrogen peroxide. The antioxidant phenoxy radical is produced by EGCG as an intermediary, leading to the generation of the quinone structure of the catechin (Arakawa et al., 2004; Cui et al., 2012). Thawing process should therefore be conducted properly to avoid microbial growth of *Pseudomonas* spp. in CHMS. Also EGCG could help retard the growth of these bacteria, preventing the spoilage of HMS meat.

EC and HSBC of the control were 1.05 and 1.25 log CFU/g, respectively and increased with augmenting F-T-C. Pacific white shrimp treated with cashew leaf extract had lower increase in HSBC after 12 days at 4°C (Sae-leaw & Benjakul, 2019). For both EC and HSBC, there was no difference in LABC when EGCG at both levels was added ($p>0.05$). Enterobacteriaceae are bacteria found in several seafoods, causing spoilage (Sae-leaw & Benjakul, 2019; Bono et al., 2017; Nirmal & Benjakul., 2009). H2S-producing bacteria contribute to formation of offensive odor and cause the rejection by consumers (Sae-leaw & Benjakul, 2019; Nirmal & Benjakul., 2009). For EC, the sample added with EGCG, especially at 400 ppm, had the lower LABC than the control for all F-T-C, except the samples after 5 F-T-C, which had similar counts for all samples. No lactic acid bacteria were detected in all the samples up to 1 F-T-C. However, lactic acid bacteria were found at 3 and 5 F-T-C in all the samples. Similarly, with other bacterial counts, lower LABC was found in samples added with 200 and 400 ppm EGCG than the control. The reduced LABC in CHMS treated with EGCG was plausibly a result of antimicrobial action of EGCG (Shiekh & Benjakul, 2020).

Changes in Fatty Acid Composition

Fatty acid composition in CHMS is shown in Table 3. Nine saturated fatty acids (SFAs), 3 monounsaturated fatty acids (MUFAs) and 6 polyunsaturated fatty acids (PUFAs) were found in CHMS. SFAs were the most abundant and accounted for 44% - 59% of total fatty acids, followed by PUFAs (19% - 28%) and MUFAs (14% - 22%). The major SFAs were palmitic acid (C16:0, 17% - 24%), stearic acid (C18:0, 9% - 15%) and myristic acid (C14:0, 5% - 12%). Palmitoleic acid (C16:1) was the predominant MUFA (6% - 12%), followed by oleic acid (C18:1, 7% - 9%) (Table 3). The prominent PUFAs were eicosapentaenoic acid (EPA, 20:5n-3, 9% - 14%) and docosahexaenoic acid (DHA, 22:6n-3, 7% - 9%) (Table 3). These findings are in line with those obtained by other crustacea (Lian et al., 2022b; Mili et al., 2011; Cherif et

Table 3. Fatty acid composition of cooked harpiosquillid mantis shrimp meat in the absence and presence of EGCG (200 and 400 ppm) before and after freeze-thawing for 5 cycles.

Fatty acids composition		Without freeze-thawing			After 5 freeze-thaw cycles		
		0	200 ppm EGCG	400 ppm EGCG	0	200 ppm EGCG	400 ppm EGCG
C14:0	Myristic	5.87±0.12^{aB}	5.97±0.10^{aB}	5.14±0.40^{aB}	12.12±0.39^{aA}	6.37±0.05^{bA}	6.05±0.16^{bA}
C15:0	Pentadecanoic	1.02±0.04^{aA}	0.97±0.03^{aB}	0.81±0.02^{bB}	0.45±0.07^{bB}	1.35±0.18^{aA}	1.17±0.03^{aA}
C16:0	Palmitic	17.13±0.77^{bB}	18.05±0.40^{aB}	17.02±0.11^{bB}	20.76±0.39^{cA}	22.02±0.46^{bA}	24.41±0.19^{aA}
C16:1	Palmitoleic	12.52±0.65^{aA}	12.15±0.08^{aA}	12.17±0.13^{aA}	8.11±0.10^{aB}	6.55±0.13^{bB}	6.94±0.48^{bB}
C17:0	Heptadecanoic	1.43±0.03^{aA}	1.43±0.03^{aA}	1.38±0.01^{bB}	0.73±0.08^{bB}	1.10±0.03^{aB}	1.12±0.01^{aB}
C18:0	Stearic	9.65±0.36^{aB}	9.57±0.15^{aB}	9.38±0.03^{aB}	15.65±0.84^{aA}	13.00±0.25^{bA}	10.88±0.01^{cA}
C18:1cis	Oleic	9.16±0.05^{aA}	9.08±0.08^{aA}	9.08±0.13^{aA}	7.52±0.02^{aB}	7.57±0.15^{aB}	7.52±0.01^{aB}
C18:2cis	Linoleic	1.63±0.02^{aA}	1.66±0.25^{aA}	1.61±0.05^{aA}	1.26±0.03^{bcB}	1.30±0.03^{bB}	1.45±0.04^{aB}
C20:0	Arachidic	0.82±0.04^{aB}	0.74±0.34^{bB}	0.75±0.04^{bB}	1.22±0.34^{cA}	1.37±0.01^{bA}	1.42±0.04^{aA}
C18:3 is	α-Linolenic	1.86±0.05^{abA}	1.98±0.08^{aA}	1.87±0.02^{abA}	0.30±0.01^{cB}	0.45±0.05^{bB}	0.87±0.03^{aB}
C20:2 is	Eicosadienoic	0.85±0.01^{aA}	0.74±0.04^{bA}	0.80±0.39^{aA}	0.58±0.04^{aB}	0.57±0.11^{aB}	0.59±0.05^{aB}
C20:0	Docosanoic	0.87±0.05^{aB}	0.89±0.01^{aB}	0.86±0.32^{aB}	1.46±0.02^{aA}	1.44±0.10^{aA}	1.45±0.06^{aA}
C23:0	Tricosanoic	8.40±0.08^{aB}	8.42±0.32^{aB}	8.55±0.11^{aB}	11.24±0.12^{aA}	11.15±0.15^{aA}	11.22±0.13^{aA}
C22:2cis	Docosadienoic	0.38±0.05^{aA}	0.35±0.07^{aA}	0.35±0.14^{aA}	0.21±0.01^{aB}	0.19±0.04^{aB}	0.20±0.00^{aB}
C24:0	Lignoceric	0.63±0.04^{aB}	0.54±0.09^{bB}	0.57±0.05^{abB}	1.06±0.02^{bA}	1.17±0.18^{abA}	1.58±0.06^{aA}
C20:5 is EPA	Eicosatrienoic	14.09±0.16^{aA}	14.02±0.33^{aA}	14.64±0.21^{aA}	9.57±0.13^{bB}	12.07±0.23^{aB}	12.25±0.03^{aB}
C24:1cis	Nervonic	0.41±0.05^{aA}	0.46±0.01^{aA}	0.46±0.03^{aA}	0.39±0.01^{aA}	0.36±0.01^{aB}	0.39±0.05^{aB}
C22:6cis DHA	Docosahexaenoic	9.57±0.36^{aA}	9.78±0.03^{aB}	9.73±0.12^{aA}	7.71±0.69^{cB}	8.11±0.03^{bB}	8.58±0.19^{aB}
Saturated Fatty Acid		45.82	46.58	44.46	64.69	58.97	59.3
Monounsaturated Fatty Acid		22.09	21.69	21.71	16.02	14.48	14.85
Polyunsaturated Fatty Acid		28.38	28.53	29	19.63	22.69	23.94

Mean ± SD (n=3). Different lowercase superscripts in the same row within the same freeze-thawing cycle indicate significant difference ($P<0.05$). Different uppercase superscripts in the same row within the same treatment indicate significant difference ($P<0.05$).

al., 2008). After freezing and thawing for 5 cycles, SFAs level increased ($p<0.05$). Conversely, level of MUFAs and PUFAs decreased in samples ($p<0.05$). SFAs in seafood generally rose as storage time increased, but PUFAs and MUFAs decreased, suggesting hydrolysis or oxidation (Temdee et al., 2022b; Sae-leaw & Benjakul 2014). Overall, the addition of 400 ppm EGCG lowered the loss of MUFAs and PUFAs after 5 F-T-C. Conversely, higher decreases in both MUFAs and PUFAs were found in the control after 5 F-T-C. For control, EPA and DHA decreased by 32.08% and 19.43%, respectively, after 5 F-T-C. Nevertheless, EPA and DHA decreased by 16.32% and 11.81%, in the sample added with 400 ppm EGCG after 5 F-T-C. This was in tandem with lower TBARS and PV of the sample added with 400 ppm EGCG after freeze-thawing (Table 1). EGCG has eight phenolic hydroxyl groups, which act as hydrogen donors, free radical scavengers, and potential inhibitors of free radical chain reactions (Tan, et al., 2021).

Conclusions

The addition of EGCG in CHMS meat could prevent the microbial growth and lipid oxidation induced by multiple F-T-C. The use of EGCG could decrease the exudate loss, especially at a higher level (400 ppm). Disulfide bond content, surface hydrophobicity, and intrinsic fluorescence index in CHMS myofibrillar proteins decreased with augmenting concentration of EGCG, regardless of F-T-C. EGCG could lower the reduction of MUFAs and PUFAs of CHMS meat, especially when F-T-C increased. Thus, EGCG could serve as a natural additive to retard the change of myofibrillar protein as influenced by repeated freeze-thawing.

Ethical Statement

Ethics approval was not required for this research.

Funding Information

This research was funded by PSU under Prachayacharn program (AGR6502111N).

Author Contribution

1. Author contributes to the conception and acquisition of data, analysis, and interpretation of data, and drafting of the article.

2. Author contributes to the conception and acquisition of data.

3. Author participates in revising it critically for important content.

4. Author participates in revising it critically for important content.

5. Author participates in edting the article and revising it critically for important content.

6. Author makes substantial contributions to the conception, funding acquisition, and approval of the submitted version.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

Authors would like to express their sincere thanks to Prince of Songkla University (PSU) for the financial support.

References

AOAC. (1999). Official methods of analysis. Vol. 16th ed., Washington, D.C.: Association of Official Analytical Chemists.

Arakawa, H., Maeda, M., Okubo, S., & Shimamura, T. (2004). Role of hydrogen peroxide in bactericidal action of catechin. *Biological and Pharmaceutical Bulletin*, 27(3), 277-281. https://doi.org/10.1248/bpb.27.277

Benjakul, S., Visessanguan, W., Riebroy, S., Ishizaki, S., & Tanaka, M. (2002). Gel-forming properties of surimi produced from bigeye snapper, *Priacanthus tayenus* and *P macracanthus*, stored in ice. *Journal of the Science of Food and Agriculture*, 82, 1442-1451. https://doi.org/10.1002/jsfa.1207

Benjakul, S., Visessanguan, W., & Tanaka, M. (2004). Induced formation of dimethylamine and formaldehyde by lizardfish (*Saurida micropectoralis*) kidney trimethylamine-N-oxide demethylase. *Food Chemistry*, 84, 297-305.
https://doi.org/10.1016/S0308-8146(03)00214-0

Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2003). Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. *Food Research International*, 36, 787-795. https://doi.org/10.1016/S0963-9969(03)00073-5

Benjakul, S., Seymour, T. A., Morrissey, M. T., & An, H. (1997). Physicochemical changes in Pacific whiting muscle proteins during iced storage. *Journal of Food Science*, 62(4), 729-733.
https://doi.org/10.1111/j.1365-2621.1997.tb15445.x

Bligh, E. G., & Dyer, W. J. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917. https://doi.org/10.1139/o59-099

Bo, Q. K., Lu, Y. Z., Mi, H. J., Yu, Y. G., Gu, D. X., You, H. Z., & Hao, S. (2020). Feeding intensity and molecular prey identification of the mantis shrimp, *Oratosquilla oratoria* (De Haan, 1844), in the Tianjin coastal zone of Bohai Bay. *Marine ecology*, 41 (4), e12594.
https://doi.org/10.1111/maec.12594

Bono, G., Okpala, C. O. R., Vitale, S., Ferrantelli, V., Noto, A. D., Costa, A., Bella, C. D., & Monaco, D. L. (2017). Effects of different ozonized slurry-ice treatments and superchilling storage (-1°C) on microbial spoilage of two important pelagic fish species. *Food Science & Nutrition*, 5, 1049-1056. https://doi.org/10.1002/fsn3.486.

Cao, Y., Xiong, Y. L. (2015). Chlorogenic acid-mediated gel formation of oxidatively stressed myofibrillar protein. *Food Chemistry*, 180, 235-243.
https://doi.org/10.1016/j.foodchem.2015.02.036

Cherif, S., Frikha, F., Gargouri, Y., & Miled, N. (2008). Fatty acid composition of green crab (*Carcinus mediterraneus*) from the Tunisian mediterranean coasts. *Food Chemistry*, 111 (4), 930-933.
https://doi.org/10.1016/j.foodchem.2008.05.007

Conway, E. J., & Byrne, A. (1933). Micro-diffusion analysis of TVN. *Biochemical Journal*, 27(2), 419-429.

Cui, Y., Oh, Y.J., Lim, J., Youn, M., Lee, I., Pak, H.K., Park, W., Jo, W., & Parka, S. (2012). AFM study of the differential inhibitory effects of the green tea polyphenol (-)-epigallocatechin3-gallate (EGCG) against gram-positive and gram-negative bacteria. *Food Microbiology*, 29, 80-87.https://doi.org/10.1016/j.fm.2011.08.019

Du, N., Sun, Y., Chen, Z., Huang, X., Li, C., Gao, L., Bai, S., Wang, P., & Hao, Q. (2023). Effects of multiple freeze-thaw cycles on protein and lipid oxidation, microstructure and quality characteristics of Rainbow Trout (*Oncorhynchus mykiss*). *Fishes*, 8(2), 108.
https://doi.org/10.3390/fishes8020108

Du, X., Li, H., Nuerjiang, M., Rui, L., Kong, B., Xia, X., & Shao, M. (2021). Influence of repeated freeze-thaw treatments on the functional and structural properties of myofibrillar protein from mirror carp (*Cyprinus carpio* L.). *Food Biophysics*, 16, 492-501.
https://doi.org/10.1007/s11483-021-09689-5

Erikson, U., Uglem, S., & Greiff, K. (2021). Freeze-chilling of whitefish: effects of capture, on-board processing, freezing, frozen storage, thawing, and subsequent chilled storage-a review. *Foods*, 10(11), 2661. https://doi.org/10.3390/foods10112661

García-Soto, B., Miranda, J. M., Barros-Velázquez, J., & Aubourg, S. P. (2015). Quality changes during the frozen storage of the crustacean lobster krill (*Munida* spp.). *European Journal of Lipid Science and Technology*, 117, 431-439. https://doi.org/10.1002/ejlt.201400309

Guo, A. Q., Jiang, J., True, A. D., & Xiong, Y. L. (2021). Myofibrillar protein cross-linking and gelling behavior modified by structurally relevant phenolic compounds. *Journal of Agricultural and Food Chemistry*, 69, 1308-1317. https://doi.org/10.1021/acs.jafc.0c04365

Guo, Y., Kong, B., Xia, X., Yu, T., & Li, Q. (2014). Changes in physicochemical and protein structural properties of common carp (*Cyprinus carpio*) muscle subjected to different freeze-thaw cycles. *Journal of Aquatic Food Product Technology*, 23, 579-590. http://dx.doi.org/10.1080/10498850.2012.741663

Han, A. T., & Gokoglu, N. (2022). Effects of different freezing and thawing methods on the quality of giant red shrimp (*Aristaeomorpha foliacea*). *Aquatic Sciences*, 9, 46-53.

He, J., Xu, L., Yang, L., & Wang, X. (2018). Epigallocatechin gallate is the most effective catechin against antioxidant stress via hydrogen peroxide and radical scavenging activity. *Medical Science Monitor*, 24, 8198-8206. http://doi.org/10.12659/MSM.911175

Jia, N., Zhang, F. X., Liu, Q., Wang, L. T., Lin, S. W., & Liu, D. Y. (2019). The beneficial effects of rutin on myofibrillar protein gel properties and related changes in protein conformation. *Food Chemistry*, 301, 125206. https://doi.org/10.1016/j.foodchem.2019.125206

Jiang, Q., Nakazawa, N., Hu, Y., Osako, K., & Okazaki, E. (2019). Changes in quality properties and tissue histology of lightly salted tuna meat subjected to multiple freeze-thaw cycles. *Food Chemistry*, 293, 178-186. https://doi.org/10.1016/j.foodchem. 2019.04.091

Kempkes, L. J. M., Martens, J., Grzetic, J., Berden, G., & Oomens, J. (2016). Deamidation reactions of asparagineand glutamine-containing dipeptides investigated by ion spectroscopy. *Journal of the American Society for Mass Spectrometry*, 27, 1855-1869. https://doi.org/10.1007/s13361-016-1462-5

Khan, N., & Mukhtar, H. (2019). Tea polyphenols in promotion of human health. *Nutrients*, 11, 39.
https://doi.org/10.3390/nu11010039

Kingwascharapong, P., & Benjakul, S. (2016). Effect of phosphate and bicarbonate replacers on quality changes of raw and cooked Pacific white shrimp as influenced by the repeated freeze–thawing. *International Journal of Refrigeration*, 67, 345-354. https://doi.org/10. 1016/j.ijrefrig.2016.01. 010

Klancnik, A., Mozina, & S. S., Zhang, Q. (2012). Anti-Campylobacter activities and resistance mechanisms of natural phenolic compounds in Campylobacter. *PLoS ONE*, 7(12), e51800.
https://doi:10.1371/journal.pone.0051800

Lambert, J. D., & Elias, R. J. (2010). The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Archives of Biochemistry and Biophysics*, 501, 65-72.
https://doi.org/10.1016/j.abb.2010.06.013

Lan, W., Hu, X., Sun, X., Zhang, X., & Xie, J. (2020). Effect of the number of freeze-thaw cycles number on the quality of Pacific white shrimp (*Litopenaeus vannamei*): An emphasis on moisture migration and microstructure by LF-NMR and SEM. *Aquaculture and Fisheries*, 5(4), 193–200. https://doi.org/10.1016/j.aaf.2019.05.007

Lee, L. Y., Normaiyudin, N. A., Wong, S. C., Chien, A. C. S., Rahmah, S., & Ram, A. J. (2022). First description of mantis shrimp, *Miyakella nepa* (Latreille, 1828), feeding preference behaviour in captive conditions. *Aquaculture Reports*, 22, 100969.
https://doi.org/ 10.1016/j.aqrep.2021.100969

Leelapongwattana, K., Benjakul, S., Visessanguan, W., & Howell, N. K. (2005). Physicochemical and biochemical changes during frozen storage of minced flesh of lizardfish (*Saurida micropectoralis*). *Food Chemistry*, 90, 141-150.
https://doi.org/10.1016/j.foodchem.2004.03.038

Li, F., Wang, B., Kong, B., Shi, S., & Xia, X. (2019). Decreased gelling properties of protein in mirror carp (*Cyprinus carpio*) are due to protein aggregation and structure deterioration when subjected to freeze-thaw cycles. *Food Hydrocolloids*, 97, 105223.
https://doi.org/10.1016/j.foodhyd.2019.105223

Li, J., Munir, S., Yu, X., Yin, T., You, J., Liu, R., Xiong, S., & Hu, Y. (2021). Double-crosslinked effect of TGase and EGCG on myofibrillar proteins gel based on physicochemical properties and molecular docking. *Food Chemistry*, 345, 128655.
https://doi.org/10.1016/j.foodchem.2020.128655

Li, Y., Lei, Y., Tan, Y., Zhang, J., Hong, H., & Luo, Y. (2022). Efficacy of freeze-chilled storage combined with tea polyphenol for controlling melanosis, quality deterioration, and spoilage bacterial growth of Pacific white shrimp (*Litopenaeus vannamei*). *Food Chemistry*, 370, 130924.
https://doi.org/10.1016/j.foodchem.2021.130924

Lian, F., Jostensen, O., Siikavuopio, S. I., & Lorentzen, G. (2022a). Live holding of red king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*) Effect on microbial growth in processed leg meat during refrigerated storage. *Food Microbiology*, 104, 103973. https://doi.org/10.1016/j.fm.2021.103973

Lian, F., Siikavuopio, S. I., Harrison, S. M., Vang, B., Brunton, N. P., Esaiassen, M., & Lorentzen, G., (2022b). Fatty acid profile of cooked leg meat and raw hepatopancreas of red king crab (*Paralithodes camtschaticus*) during three-month live holding without feeding at 5 and 10°C. *Journal of Food Composition and Analysis*, 105, 104206. https://doi.org/10.1016/j.jfca.2021. 104206

Lorentzen, G., Hustad, A., Lian, F., Grip, A. E., Schrodter, E., Medeiros, T., & Siikavuopio, I. S. (2020). Effect of freezing methods, frozen storage time, and thawing methods on the quality of mildly cooked snow crab (*Chionoecetes opilio*) clusters. *LWT - Food Science and Technology*, 123, 109103.
https://doi.org/10.1016/j.lwt.2020.109103

Maqsood, S., Benjakul, S., Abushelaibi, A., & Alam, A. (2014). Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: A detailed review. *Comprehensive Reviews in Food Science and Food Safety*, 13(6), 1125-1140. https://doi.org/10.1111/1541-4337.12106

Matmaroh, K., Benjakul, S., & Tanaka, M. (2006). Effect of reactant concentrations on the Maillard reaction in a fructose–glycine model system and the inhibition of black tiger shrimp polyphenoloxidase. *Food Chemistry*, 98(1), 1-8.
https://doi.org/10.1016/j.foodchem.2005.05.029

Mili, S., Bouriga, N., Missaoui, H., & Jarboui, O. (2011). Morphometric, reproductive parameters and seasonal variations in fatty acid composition of the mantis shrimp *Squilla mantis* (Crustacea: Stomatopoda) in the gulf of Gabes (Tunisia). *Journal of Life Sciences*, 5, 1058-1071.

Mittal, A., Singh, A., & Benjakul, S. (2021). Use of nanoliposome loaded with chitosan-epigallocatechin gallate conjugate for shelf life extension of refrigerated Asian seabass (*Lates calcarifer*) slices. *International Journal of Food Science*, 56, 3795-3806.
https://doi.org/10.1111/ijfs. 14995

Nazemroaya, S., Sahari, M. A., & Rezaei, M. (2009). Effect of frozen storage on fatty acid composition and changes in lipid content of *Scomberomorus commersoni* and *Carcharhinus dussumieri*. *Journal of Applied Ichthyology*, 25, 91-95.
https://doi.org/10.1111/j.1439-0426.2008.01176.x

Nikoo, M., Regenstein, J. M., & Gavlighi, H. A. (2018). Antioxidant and antimicrobial activities of (-)-Epigallocatechin-3-gallate (EGCG) and its potential to preserve the quality and safety of foods. *Comprehensive Reviews in Food Science and Food Safety*, 17, 732-753. https://doi.org/10.1111/1541-4337.12346

Nirmal, N. P., & Benjakul., S. (2009). Melanosis and quality changes of pacific white shrimp (*Litopenaeus vannamei*) treated with catechin during iced storage. *Journal of Agricultural and Food Chemistry*, 57, 3578-3586. https://doi.org/10.1021/jf900051e

Okpala, C. O. R., Choo, W. S., & Dykes, G. A. (2014). Quality and shelf life assessment of Pacific white shrimp (*Litopenaeus vannamei*) freshly harvested and stored on ice. *LWT - Food Science and Technology*, 55(1), 110-116. https://doi.org/10.1016/j.lwt.2013. 07.020

Olatunde, O. O., Benjakul, S., & Vongkamjan, K. (2019). Combined effects of high voltage cold atmospheric plasma and antioxidants on the qualities and shelf life of Asian sea bass slices. *Innovative Food Science & Emerging Technologies*, 54, 113-122.
https://doi.org/10.1016/j.ifset.2019.03.012

Pastoriza, L., Sampedro, G., Herrera, J. J., & Cabo, M. L. (1996). Effect of modified atmosphere packaging on shelf life of iced fresh hake slices. Journal of the Science of Food and Agriculture, 71, 541-547. https://doi.org/10.1002/(SICI)1097-0010(199608) 71:4<541:: AID-JSFA615>3.0.CO;2-H

Pei, J., Mei, J., Yu, H., Qiu, W., & Xie, J. (2022). Effect of gum tragacanth-sodium alginate active coatings incorporated with epigallocatechin gallate and lysozyme on the quality of large yellow croaker at superchilling condition. *Frontiers in Nutrition*, 8, 812741. https://doi.org/10. 3389/fnut.2021.812741

Quan, T. H., Benjakul, S., & Hozzein, W. N. (2020). Quality and storage stability of fish tofu as affected by duck albumen hydrolysate-epigalocatechin gallate conjugate. *LWT - Food Science and Technology*, 120, 108927. https://doi.org/10.1016/j.lwt.2019.108927

Rattanasatheirn, N., Benjakul, S., Visessanguan, W., & Kijroongrojana, K. (2008). Properties, translucence, and microstructure of pacific white shrimp treated with mixed phosphates as affected by freshness and deveining. *Journal of Food Science*, 73(1), 31-40. https://doi.org/10.1111/j.1750-3841.2007.00603.x.

Sae-leaw, T., & Benjakul, S. (2019). Prevention of quality loss and melanosis of Pacific white shrimp by cashew leaf extracts. *Food Control*, 95, 257-266.
https://doi.org/10.1016/j.foodcont.2018.08.014

Sae-leaw, T., & Benjakul, S. (2014). Fatty acid composition, lipid oxidation, and fishy odor development in seabass (*Lates calcarifer*) skin during iced storage. *European Journal of Lipid Science and Technology*, 116, 885-894. https:// doi.org/10.1002/ejlt.201300381

Shi, J., Zhang, L., Lu, H., Shen, H., Yu, X., & Luo, Y. (2017). Protein and lipid changes of mud shrimp (*Solenocera melantho*) during frozen storage: chemical properties and their prediction, *International Journal of Food Properties*, 20(2), 2043-2056.
https://doi.org/10.1080/10942912.2017.1361973

Shi, Y., Wang, H., Zheng, Y., Qiu, Z., & Wang, X. (2022). Effects of ultrasound-assisted vacuum impregnation antifreeze protein on the water-holding capacity and texture properties of the yesso scallop adductor muscle during freeze–thaw cycles. *Foods*, 11, 320. https://doi.org/10.3390/foods11030320

Shiekh, K. A., & Benjakul, S. (2020). Effect of high voltage cold atmospheric plasma processing on the quality and shelf life of Pacific white shrimp treated with Chamuang leaf extract. *Innovative Food Science & Emerging Technologies*, 64, 102435.
https://doi.org/10.1016/j.ifset.2020.102435

Singh, A., & Benjakul, S. (2020). The combined effect of squid pen chitooligosaccharides and high voltage cold atmospheric plasma on the shelf life extension of Asian sea bass slices stored at 4°C. *Innovative Food Science & Emerging Technologies*, 64, 1-10.
https://doi.org/10.1016/ j.ifset.2020.102339

Singh, A., Benjakul, S., Zhou, P., Zhang, B., & Deng, S. (2021). Effect of squid pen chitooligosaccharide and epigallocatechin gallate on discoloration and shelf life of

yellowfin tuna slices during refrigerated storage. *Food Chemistry*, 351, 129296.
https://doi.org/10.1016/j.foodchem.2021.129296

Singh, H., & Good, J.P. (2022). Bioaccumulation of copper in *Cambarus georgiea*. *BIOS*, 92(3), 68-75. https://doi.org/10.1893/BIOS-D-20-00005

Tan, M., Ye, J., & Xie, J. (2021). Freezing-induced myofibrillar protein denaturation: Role of pH change and freezing rate. *LWT - Food Science and Technology*, 152, 112381. https://doi.org/10.1016/ j.lwt.2021.112381

Tan, C., Xu, Q. D., Chen, N., He, Q., Sun, Q., Zeng, W. C. (2022). Cross-linking effects of EGCG on myofibrillar protein from common carp (*Cyprinus carpio*) and the action mechanism. *Journal of Food Biochemistry*, 46(12), e14416. https://doi.org/10.1111/jfbc.14416

Taylor, H. H., & Anstiss, J. M. (1999). Copper and haemocyanin dynamics in aquatic invertebrates. *Marine and Freshwater Research*, 50(8), 907-931.
https://doi.org/10.1071/ MF99117

Temdee, W., Singh, A., & Benjakul, S. (2022a). Rapid quality deterioration of harpiosquillid mantis shrimp (*Harpiosquilla raphidea*) during iced storage. *Journal of Food Science and Technology*, 59, 1812-1822. https://doi.org/10.1007/s13197-021-05192-4

Temdee, W., Singh, A., Zhang, B., & Benjakul, S. (2022b). Effect of vacuum packaging on shelf life extension of cooked and peeled harpiosquillid mantis shrimp (*Harpiosquilla raphidea*) during refrigerated storage. *International Journal of Food Science & Technology*, 57(7), 4451-4462. https://doi.org/10.1111/ijfs.15778

Thanonkaew, A., Benjakul, S., Visessanguan, W., & Decker, E.A. (2008). The effect of antioxidants on the quality changes of cuttlefish (*Sepia pharaonis*) muscle during frozen storage. *LWT - Food Science and Technology*, 41(1), 161-169. https://doi.org/10.1016 /j.lwt.2007.02.008

Tian, Z., Jiang, X., Xiao, N., Zhang, Qiang., Shi, W., & Guo, Q. (2022). Assessing the gel quality and storage properties of *Hypophythalmalmichthys* molitrix surimi gel prepared with epigallocatechin gallate subject to multiple freeze-thaw cycles. *Foods*. 11, 1612.
https://doi.org/10.3390/foods11111612

Vilkova, D., Kondratenko, E., Chèné, C., & Karoui R. (2022). Effect of multiple freeze–thaw cycles on the quality of Russian sturgeon (*Acipenser gueldenstaedtii*) determined by traditional and emerging techniques. *European Food Research and Technology*, 248, 95-107. https://doi.org/10.1007/s00217-021-03859-y

Wei, P., Zhu, K., Cao, J., Dong, Y., Li, M., Shen, X., Duan, Z., & Li, C. (2021). The inhibition mechanism of the texture deterioration of tilapia fillets during partial freezing after treatment with polyphenols. *Food Chemistry*, 335, 127647.
https://doi.org/10.1016/ j.foodchem.2020.127647

Yarnpakdee, S., Kaewprachu, P., Jaisan, C., Senphan, T., Nagarajan, M., & Wangtueai, S. (2022). Extraction and physico–chemical characterization of chitosan from Mantis shrimp (*Oratosquilla nepa*) shell and the development of bio-composite film with agarose. *Polymers*, 14, 3983.
https://doi.org/10.3390/polym14193983.

Yasemi, M. (2017). Prevention of denaturation of freshwater crayfish muscle subjected to different freeze-thaw cycles by gelatin hydrolysate. *Food Chemistry*, 234, 199-204. https://doi.org/10.1016/j.foodchem.2017.04.183

Ye, T., Chen, X., Zhu, Y., Chen, Z., Wang, Y., Lin, L., Zheng, Z., & Lu, J. (2022). Freeze-thawing treatment as a simple way to tune the gel property and digestibility of minced meat from red swamp crayfish (*Procambarus clarkiix*). *Foods*, 11, 837. https://doi.org/10.3390/foods 11060837

Zhang, Y., Li, F., Yao, Y., He, J., Tang, J., & Jiao, Y. (2021). Effects of freeze-thaw cycles of Pacific white shrimp (*Litopenaeus vannamei*) subjected to radio frequency tempering on melanosis and quality. *Innovative Food Science & Emerging Technologies*, 74, 102860. https://doi.org/10.1016/j.ifset.2021.102860