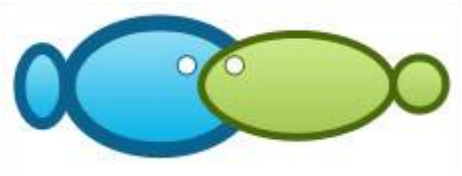

The changes in quality of nanocomposite-packed peeled white leg shrimp (*Litopenaeus vannamei*) during chilled storage

[1]Le T. Minh-Thuy, [1]Nguyen V. Thom, [2,3]Duc-N. Lam, [3]Dan-T. Van-Pham

[1] College of Aquaculture and Fisheries, Can Tho University, 94000, Can Tho, Vietnam; [2] Department of Chemistry, School of Education, Can Tho University, 94000, Can Tho, Vietnam; [3] Polymer Material Lab, College of Engineering, Can Tho University, 94000, Can Tho, Vietnam. Corresponding author: L. T. M. Thuy, ltmthuy@ctu.edu.vn

Abstract. This study investigated the quality changes of peeled white leg shrimp (*Litopenaeus vannamei*) packed with polyvinyl alcohol (PVA), PVA/nanocrystalline cellulose/graphene oxide (PVA/CNC/GO), or PVA/GO films during chilled preservation. The DPPH radical scavenging activity was the highest for the PVA/CNC/GO film. Therefore, the PVA/CNC/GO film-packed shrimp had the lowest peroxide value change, indicating lipid oxidation over the ninth day of storage. The bacterial analysis confirmed that PVA/GO film substantially inhibited microbial deterioration after storage for 18 days. Findings revealed that PVA/CNC/GO film significantly contributed to retaining the peeled shrimp properties, such as texture profile, pH, water holding capacity, and sensory attributes.
Key Words: cold storage, nanocomposite film, peeled white leg shrimp, peroxide value.

Introduction. White leg shrimp (*Litopenaeus vannamei*) is one of the most internationally traded fishery commodities as well as an economically representative aquatic resource in Vietnam with exports reaching 3.23 billion USD in 2022 (VASEP 2022). Generally, shrimp rapidly perish after harvesting and processing due to microbial deterioration and autolytic enzyme-mediated protein denaturation and lipid oxidation, resulting in nutrition and sensory quality deterioration (Dong et al 2018). Hence, preservation techniques for suppressing several unexpectable changes in shrimp quality have always been important. The importance of packing film type in short-term shrimp preservation under chilled conditions is noteworthy. Traditionally, polyethylene (PE) has been the common material for package preparation conveniently, although they have poor oxidation resistance and are decomposed slowly, leading to the negative environmental influence. Subsequently, clean and bioactive film processing has been widely explored. Several studies have suggested that films prepared using chitosan incorporated with green tea extract (Yuan et al 2016), PE containing rosemary and cinnamon essential oils (Dong et al 2018) and chitosan/clove essential oil/kojic acid (Liu et al 2020) could positively promote ice-stored shrimp shelf life and muscle properties. Recently, innovative nanocomposite materials, such as graphene oxide (GO) and nanocrystalline cellulose (CNC), have been promising for generating films due to their thermal ability, water vapor permeability, UV absorbability, gas-barrier characteristic, and biodegradability (Hu et al 2017; Sung et al 2017). Polyvinyl alcohol (PVA), a water-soluble polyhydroxy polymer, has received attention for its potential applications in food processing based on its easy preparation; nontoxic, noncarcinogenic, biodegradable and bioadhesive characteristics; excellent chemical resistance; and physical properties. PVA, also considered a binder, is an eco-friendly and hydrophilic synthetic polymer that may form films, although its mechanical durability is low in high ambient humidity (Halima 2006; Zhang et al 2020). The mechanical strength and water vapor barrier performance of the PVA film were enhanced by supplementing GO and CNC into PVA due to the water resistance of GO (El Miri et al 2016; Jia et al 2020). Therefore, we aimed to thoroughly

investigate the effects of nanocomposite compound-based films on peeled white leg shrimp quality during chilled preservation.

Material and method

Time and location. The study was conducted from January to May 2023 at the College of Aquaculture and Fisheries, Can Tho University and Polymer Material Lab, College of Engineering, Can Tho University.

Materials. The live white leg shrimps (40-50 shrimp kg^{-1}) were purchased from a traditional market in Can Tho city, Vietnam and transported to the laboratory within 25 min. Upon receiving, the shrimp were washed thoroughly before beheading, manual peeling, and deveining. The shrimps were then rewashed in cold water (6-8°C), drained for 1 min, and prepared for wrapping. Nanocrystalline cellulose (CNC) and graphene oxide (GO) were provided by Polymer Material Lab, College of Engineering, Can Tho University, Vietnam. Polyvinyl alcohol (PVA) was purchased from Tianjin Fu Chemical Reagents Factory (China), acetic acid, methanol, chloroform, potassium iodine, and 0.1 N $Na_2S_2O_3$ were procured from Merck (Germany). The plate count agar was purchased from HiMedia Laboratories (India). The reagents used were of the highest grade.

Experimental design

Packing film preparation. The PVA, PVA/CNC/GO (98.62 PVA: 0.59 CNC: 0.79 GO), and PVA/GO (99.21 PVA: 0.79 GO) films were prepared using a modified version of the solvent casting method reported by Morimune et al (2012) and Jia et al (2020). First, the components were vigorously stirred at 90ºC for 2 hours to obtain a homogeneous aqueous suspension. Then, the suspension was poured into a plastic tray (50 × 35 cm^2) and dried at 60ºC in an oven for 24 hours. After being meticulously removed from the tray, the 0.05 mm thick films without air bubbles were used for measuring the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity before packing peeled white leg shrimp.

The peeled white leg shrimp packing. The peeled white leg shrimps (20 bodies each) were packed in different films (20 × 30 cm^2), placed in plastic containers, and stored at 0-4°C for 18 days. The shrimp quality (texture, pH, water holding capacity (WHC), lipid oxidation, color, sensory evaluation and total aerobic bacterial count) was assessed every three days during storage.

Sample analysis. Determination of DPPH radical scavenging activity of film: a 0.1 g film fraction was mixed with 15 mL methanol and stirred for 3 hours. Then, 2 mL solution was added to 2 mL 0.1 mM DPPH solution in methanol and incubated in dark for 30 min before measuring the absorbance (A) at 517 nm (Wu et al 2003). DPPH radical scavenging activity was calculated as:

$$DPPH(\%) = \left[\frac{(A_{sample} - A_{blank})}{A_{blank}}\right] \times 100$$

Blank: distilled water was used instead of the sample solution.

pH measurement: the 1:5 (w/v) shrimp and distilled water mixture was homogenized before measuring the pH using CONSORT C1020P Multi-parameter analyzer, calibrated with pH 4.0 and 9.0 buffer solutions.

Determination of water holding capacity (WHC): a syringe containing Whatman paper No. 4 at the bottom was first weighed (M_1). Then, 1.5 g shrimp sample was added to this syringe (M_2 = M_1+1.5 g). It was then placed in a 10 mL centrifuge tube, centrifuged for 10 min at 5,000 rpm using a Mikro 22-R centrifuge (Hettich centrifuge, Germany), and reweighed (M_3). The WHC was calculated as reported by Ofstad et al (1993):

$$\text{WHC } (\%) = 100 - \left\{\left[\frac{(M_2 - M_3)}{(M_2 - M_1)}\right] \times 100\right\}$$

Color assessment: colorimeter PCE-CSM 2 (China) was used for color measurement. The surface color of shrimps was determined as L*, a*, and b* representing lightness, green (-) to red (+), and blueness (-) to yellowness (+), respectively.

Texture profile analysis: the TA.Xt2i Texture Analyzer (Stable Micro Systems, YL, UK) was used for analyzing the shrimp texture (Annamalai et al 2015). The parameters for texture analysis were: 0.1 cm s^{-1} constant test speed of P/57 probe, 50% sample deformation, 3 second hold period between cycles, and 10× g trigger force. Hardness and springiness of individual samples were calculated based on the force–time curves.

Microbiological analysis: a total of 10 g minced shrimp was transferred into 90 mL sterile 0.85% (w/v) saline solution. It was serially diluted in 1:10 ratio using 0.85% (w/v) sterile saline solution. Then, 1 mL appropriate dilution was added to the Petri dish before adding 15 mL nutrient agar. The Petri dishes were slightly rotated, rested to completely dry the medium, and incubated at 37ºC for 24 hours. The microbial colonies formed were counted and expressed as colony-forming units per gram sample (CFU g^{-1}) according to the Nordic Committee for Food Analyses (NCFA 2013).

Peroxide value (PV) was determined as described by American Oil Chemists' Society (AOCS 1997). Briefly, 5 g sample was mixed with 20 mL 3:2 acetic acid-chloroform solution, swirled for 2 hours to extract the lipid, and then incubated with 0.5 mL saturated KI solution for 5 min in dark space before adding 30 mL distilled water and 0.5 mL 0.1% starch solution. The prepared solution was titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) until the blue color disappeared. A control blank (5 mL distilled water used) was also analyzed. The PV was calculated as:

$$\mathrm{PV\ (meq/kg)} = \frac{[(\mathrm{A} - \mathrm{B}) \times \mathrm{N} \times 1000]}{\mathrm{M}}$$

where: A and B are the $Na_2S_2O_3$ volume (mL) consumed by the sample and blank, respectively; N is the $Na_2S_2O_3$ concentration (N); and M is the sample weight (g).

Sensory evaluation: shrimp sensory properties (color, odor, texture, and taste) were evaluated by five panelists trained in sensory assessment based on the detailed description according to TCVN 3215-79 (1979). Using a 5-point hedonic scale for each characteristic, high score meant shrimp with excellent quality, with the maximum score for the four characteristics being 20 points.

Statistical analysis. All obtained data were presented as means±standard deviation (SD) of triplicate measurements. The SPSS package (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Duncan's test was used to determine the significant difference among treatments ($p < 0.05$).

Results and Discussion

DPPH radical scavenging activity (%) of the packaging films. The analysis results reported in Table 1 indicated that the GO/CNC-supplemented PVA film had improved antioxidant activity in comparison to that of PVA film.

Table 1

DPPH radical scavenging activity (%) of the different film types

Film types	*PVA*	*PVA/CNC/GO*	*PVA/GO*
DPPH (%)	20.62 ± 0.652^{a}	44.12 ± 1.192^{b}	36.41 ± 0.959^{c}

Results are means±standard deviation (n = 3). Different superscripts (a-c) within a row are significant differences among samples ($p < 0.05$).

The PVA/CNC/GO film possessed the highest DPPH radical scavenging activity (44.12%). Nasution et al (2020) reported that the DPPH antioxidant activity of CNC film in conjunction with basil extract was 57.95%. Moreover, Baali et al (2019) showed that GO had an effective antioxidant property, with 40% DPPH radical scavenging activity. Through electron transfer and dehydrogenation, GO ionizes transition metal ions and destroys free radicals (Suresh et al 2015; Baali et al 2019). The increased antioxidant activity in this result was in accordance with the report of Cheng et al (2021) that

PVA/corn starch and PVA/chitosan films had 31.16% and 41.10% DPPH scavenging activities, respectively (Annu et al 2021).

The effect of film types on peeled shrimp pH level during cold storage. The pH values of peeled shrimp during cold storage are presented in Table 2. The pH values slightly increased from 6.40 to 7.13 in all samples after cold storage for 18 days. This is because the microbes present and endogenous enzymes hydrolyzed shrimp meat, which generates NH_3 (Hultmann et al 2012). The pH of PVA/CNC/GO and PVA/GO film-packed shrimp were 6.83 and 6.77, respectively, which are lower than that of PVA film-packed shrimp (pH = 7.13). Alparslan & Baygar (2017) reported that the pH of pink shrimp (*Pandalus borealis*) packed in a chitosan and orange peel oil-based film changed from 6.44 to 7.94 after cold storage for 15 days. The shrimp meat maintained good quality if its pH was < 7.7 (Çolakoğlu et al 2006).

WHC of the peeled shrimp. The high WHC demonstrated that the protein conformation and water-protein binding are maintained. The WHC decreased due to the loss in shrimp muscle texture owing to the impact of enzymes and microbes on shrimp muscle, particularly protein denaturation (Table 2). In general, after prolonged storage for 18 days, WHC of PVA/CNC/GO, PVA/GO, and PVA film-packed shrimp reduced from 98.35 to 95.67%, from 98.75 to 95.78%, and 98.63 to 93.75%, respectively. Therefore, PVA/CNC/GO and PVA/GO films assisted the peeled shrimp in retaining water. The WHC change in this study followed the pattern reported by Farajzadeh et al (2016), with the water loss for chitosan-gelatin film-packed white leg shrimp increasing from 1.16 to 5.09% during cold preservation for 14 days.

Table 2

Change in pH and water holding capacity (WHC) of peeled shrimp

Days	*pH*			*WHC (%)*		
	PVA	*PVA/CNC/GO*	*PVA/GO*	*PVA*	*PVA/CNC/GO*	*PVA/GO*
0	6.49±0.31^{a}	6.43±0.09^{a}	6.40±0.23^{a}	98.63±0.50^{e}	98.35±1.41^{d}	98.75±0.41^{d}
3	6.52±0.04^{a}	6.56±0.04^{ab}	6.56±0.02^{ab}	97.14±0.13^{d}	97.53±0.65^{cd}	97.38±0.16^{c}
6	6.67±0.26^{ab}	6.60±0.20^{abc}	6.47±0.20^{ab}	97.34±0.21^{d}	97.54±0.07^{c}	97.47±0.52^{c}
9	6.99±0.15^{bc}	6.70±0.20^{bc}	6.57±0.20^{ab}	96.44±0.39^{c}	96.60±0.14^{b}	97.54±0.12^{c}
12	6.80±0.12^{bc}	6.71±0.06^{bc}	6.60±0.06^{ab}	94.43±0.32^{ab}	96.61±0.09^{b}	96.59±0.18^{b}
15	6.90±0.13^{bc}	6.73±0.09^{bc}	6.61±0.11^{ab}	93.71±0.20^{a}	96.32±0.33^{b}	95.71±0.04^{a}
18	7.13±0.10^{c}	6.83±0.09^{c}	6.77±0.20^{b}	93.75±0.09^{a}	95.67±0.07^{a}	95.78±0.19^{a}

Results are means±standard deviation (n = 3). Different superscripts (a-e) within a column are significantly different among samples ($p < 0.05$).

Textural properties. Texture is the crucial shrimp meat-related parameter for consumer acceptance (Annamalai et al 2015). The textural change of shrimp meat stored in cold (Table 3) might be influenced by many factors, such as size, pH, and myofibrillar protein or connective tissue degradation (Yuan et al 2016).

Table 3

The hardness and springiness of the peeled shrimp

Days	*Hardness (g)*			*Springiness (%)*		
	PVA	*PVA/CNC/GO*	*PVA/GO*	*PVA*	*PVA/CNC/GO*	*PVA/GO*
0	12051±267^{e}	10826±228^{e}	11235±722^{e}	0.79±0.05^{c}	0.84±0.11^{c}	0.78±0.04^{c}
3	11045±830^{d}	10529±204^{d}	11214±56^{e}	0.75±0.03^{bc}	0.77±0.01^{bc}	0.75±0.01^{bc}
6	10544±212^{d}	9957±583^{cd}	9987±951^{d}	0.74±0.03^{bc}	0.68±0.01^{ab}	0.73±0.02^{bc}
9	9038±518^{c}	9445±195^{c}	9325±462^{cd}	0.72±0.02^{bc}	0.67±0.03^{ab}	0.74±0.01^{abc}
12	9266±470^{c}	9340±138^{bc}	8607±310^{bc}	0.68±0.03^{ab}	0.66±0.03^{a}	0.70±0.01^{ab}
15	8252±276^{b}	8970±312^{b}	8294±282^{ab}	0.64±0.04^{a}	0.65±0.05^{a}	0.69±0.04^{ab}
18	7765±581^{a}	7942±761^{a}	7936±449^{a}	0.65±0.05^{a}	0.64±0.02^{a}	0.67±0.02^{a}

Results are means±standard deviation (n = 3). Different superscripts (a-e) within a column are significantly different among samples ($p < 0.05$).

Muscle hardness declined gradually and the muscle texture softened due to spoilage (Dong et al 2018). Although the texture did not change among shrimp packed with various films, the hardness of PVA/CNC/GO-based film-packed shrimp was 7942× g higher than that of the PVA- and PVA/CNC/GO-based film-packed shrimp (7936× g and 7765× g, respectively) at the end of storage, indicating that PVA/CNC/GO-based packing film suppressed muscle texture change. Moreover, Zhang et al (2015) reported that cold-stored white leg shrimp hardness was reduced by 40.23% after 16 days.

Color change of peeled shrimp. Seafood market value and consumer perception are evaluated through its color, which reflects freshness and aesthetics. The L*, a*, and b* values of peeled shrimp in all packing films changed substantially.

Lipid oxidation and astaxanthin pigment degradation during cold storage forms yellow and black pigments, which darkens the muscle color (Qian et al 2013). The lightness of PVA film-packed shrimp decreased faster than that of others, as indicated by the L* values (50.94, 52.83, and 52.20 for the shrimp packed in PVA, PVA/CNC/GO, and PVA/GO films, respectively). Additionally, the shrimp packed in PVA film was more yellow, as indicated by the higher b* value than that of the others. Thus, the GO or CNC-supplemented films retarded color variation. Li et al (2016) reported that the color of frozen-stored shrimp (*Fenneropenaeus chinensis*) changed unexpectedly, as the L* value decreased from 54.52 to 44.28 and b* value increased from 17.79 to 31.24.

Change of total aerobic microorganisms of peeled shrimp. The change of total aerobic microorganisms (CFU g^{-1}) of peeled shrimp is given in Table 4.

The total aerobic bacterial count gradually increased during storage in the samples packed with different films. The microbial count in GO-supplemented PVA film-packed shrimp reached 2.70×10^3 CFU g^{-1} over the storage period of 18 days, which was retarded significantly in comparison to that in shrimp packed in PVA/CNC/GO and PVA films (5.27×10^3 and 7.01×10^3 CFU g^{-1}, respectively). The total number of aerobic microorganisms in this study was lower than that reported by Yuan et al (2016), who used a chitosan–green tea extract film for white leg shrimp preservation (3.45×10^3 and 8.01×10^3 CFU g^{-1} on the first and ninth days, respectively). According to Dong et al (2018), the microbial counts in white shrimp packed in an activated polyethylene film containing rosemary and cinnamon essential oils after 10 days were 4.15-6.03×10^3 CFU g^{-1}. Similarly, Liu et al (2020) reported that the microbial count in white shrimp packed in a chitosan film containing clove essential oil after cold storage for 15 days was 8.58×10^3 CFU g^{-1}.

Table 4

Total aerobic microorganism count and peroxide value (PV) of peeled shrimp

	Total aerobic microorganisms (CFU g^{-1})			*Peroxide value (PV, meq kg^{-1})*		
Days	*PVA*	*PVA/CNC/GO*	*PVA/GO*	*PVA*	*PVA/CNC/GO*	*PVA/GO*
0	1.29×10^3	1.31×10^3	1.20×10^3	1.08±0.10^{a}	1.01±0.08^{a}	1.13±0.03^{a}
3	1.98×10^3	1.63×10^3	1.30×10^3	2.06±0.03^{c}	1.84±0.14^{b}	2.30±0.13^{d}
6	3.27×10^3	1.83×10^3	1.39×10^3	3.50±0.10^{e}	2.44±0.14^{c}	2.68±0.22^{e}
9	3.47×10^3	2.60×10^3	1.42×10^3	3.55±0.13^{e}	2.34±0.20^{c}	2.93±0.27^{e}
12	4.40×10^3	3.55×10^3	1.81×10^3	2.77±0.13^{d}	2.04±0.19^{bc}	2.30±0.10^{d}
15	4.90×10^3	4.24×10^3	1.92x10^3	2.62±0.10^{d}	1.79±0.03^{b}	1.84±0.13^{c}
18	7.01×10^3	5.27×10^3	2.70×10^3	1.71±0.10^{b}	1.23±0.07^{a}	1.55±0.06^{a}

Results are means±standard deviation (n = 3). Different superscripts (a-e) within a column are significantly different among samples ($p < 0.05$).

Peroxide value (PV). The lipid oxidation was measured as the PV expressing primary oxidation product formation (Table 4). The PV of the PVA film-packed shrimp increased from 1.08 to 3.55 (meq kg^{-1}) during the first nine days, while the PVA/CNC/GO-packed

shrimp had the lowest PV (2.34 meq kg^{-1}). This indicates that packing shrimp in CNC and GO-supplemented films inhibited lipid oxidation. Since the unstable primary product was transformed into the secondary products, the PV decreased for the remaining storage period (Benjakul et al 2005; Boselli et al 2005). Seafood still guaranteed the desirable properties when its PV did not exceed 5 meq kg^{-1} (Okpala 2014). This result was similar to those reported by Alparslan & Baygar (2017) and Farajzadeh et al (2016), which used bioactive orange leaf essential oil-gelatin and chitosan-gelatin films, respectively, for packing white leg shrimp to prevent lipid oxidation.

Sensory evaluation. Overall, the sensory acceptability of shrimp packed with various films was non-significantly different up to the ninth day (Table 5). Oxidative deterioration, enzyme action, and microbial propagation are the fundamental causes that decrease the organoleptic quality (Abdollahi et al 2014). The sensorial characteristics of PVA film-packed shrimp remained lower than those of PVA/GO/CNC and PVA/GO film-packed shrimp because the barrier properties of nanocomposite compound-based films have contributed to shrimp quality stability during storage.

Table 5

Effect of packing films on sensory attributes of peeled shrimp during cold storage

Days	*Sensory score*		
	PVA	*PVA/CNC/GO*	*PVA/GO*
0	18.30 ± 0.06^{d}	18.27 ± 0.10^{e}	18.23 ± 0.15^{f}
3	18.16 ± 0.10^{d}	18.09 ± 0.12^{e}	17.91 ± 0.06^{e}
6	17.32 ± 0.37^{c}	17.60 ± 0.26^{d}	17.41 ± 0.08^{d}
9	17.10 ± 0.25^{c}	17.19 ± 0.09^{c}	17.10 ± 0.07^{c}
12	16.46 ± 0.62^{b}	17.03 ± 0.58^{c}	17.10 ± 0.15^{c}
15	14.70 ± 0.58^{a}	16.10 ± 0.15^{b}	16.70 ± 0.30^{b}
18	14.43 ± 0.15^{a}	15.17 ± 0.05^{a}	15.13 ± 0.10^{a}

Results are means±standard deviation (n = 3). Different superscripts (a-f) within a column are significantly different among samples ($p < 0.05$).

Conclusions. We conclude that shrimp preservation in nanocomposite films at cold maintained the muscle stability and integrity as well as shrimp shelf life. PVA/CNC/GO film noticeably retarded lipid oxidation as shown by the lowest PV, whereas PVA/GO film positively impacted microbial inhibition during shrimp storage compared to PVA film. Consequently, nanocomposite application in seafood packaging should be further studied.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Le-Thi Minh-Thuy, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, 94000, Can Tho, Vietnam, e-mail: ltmthuy@ctu.edu.vn
Nguyen-Van Thom, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, 94000, Can Tho, Vietnam, e-mail: thomb1409156@gmail.com
Duc-Ninh Lam, Department of Chemistry, School of Education, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, 94000, Can Tho, Vietnam; Polymer Material Lab, College of Engineering, Can Tho University, 3/2 Street, Ninh Kieu District, 94000, Can Tho, Vietnam, e-mail: lamducninh2@gstudent.ctu.edu.vn
Dan-Thuy Van-Pham, Polymer Material Lab, College of Engineering, Can Tho University, 3/2 Street, Ninh Kieu District, 94000, Can Tho, Vietnam, e-mail: vpdthuy@ctu.edu.vn

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