The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2022), 46(1), 108-124

ORIGINAL RESEARCH PAPER

EFFECT OF CHITOSAN COATING COMBINED WITH CUSTARD APPLE (*ANNONA SQUAMOSA L.*) PEEL EXTRACT ON THE QUALITY OF PACIFIC WHITE SHRIMP DURING COLD STORAGE

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Received on 27 September 2021
Revised on 11 May 2022

Abstract

This study examined the changes in the quality of the Pacific white shrimps coated with chitosan incorporated with custard apple peel extract (ASE). Generally, there was a positive effect on texture, color, and biochemical characteristics of shrimp samples treated with either chitosan coating or chitosan coating combined with ASE as against the control after 12 days of storage at 4°C. The increases in the values of weight loss, pH, peroxide values, total volatile basic nitrogen, and thiobarbituric acid reactive substances of shrimps were significantly inhibited in shrimps treated with a chitosan-ASE coating. Coating with chitosan combined with ASE at a concentration of 300 mg GAE/L was proven as the most effective preservation method for whiteleg shrimp during cold storage. Thereby, it may suggest that the combination of chitosan and ASE could be used as an effective alternative for the natural coating to extend the shelf-life and maintain the shrimp quality.

Keywords: pacific white shrimp, chitosan coating, *Annona squamosa* L., preservation, seafood quality

Introduction

Pacific white shrimp, or whiteleg shrimp (*Litopenaeus vannamei*), is one of the widely cultivated aquatic species in Vietnam. Shrimps are high in protein, low in fat, and especially low in cholesterol, making them a good source of nutrition (Pan *et al.*, 2019). However, depending on storage conditions, postharvest shrimps are susceptible to rapid change and spoilage due to enzyme proteolytic activities, lipid

https://doi.org/10.35219/foodtechnology.2022.1.09

oxidation, and microbial degradation. Furthermore, the color change caused by the formation of melanosis (black spot) degrades shrimp quality. Melanosis develops as a result of a biological process in which polyphenol oxidase oxidizes phenol to quinone (Nirmal and Benjakul, 2009a). This is followed by non-enzymatic quinone polymerization resulting in high-molecular-weight dark pigments (Benjakul *et al.*, 2005). Although the development of black spots appears to be innocuous to consumers, it has a significant impact on the market value and customer acceptance of the goods, leading to possible financial loss (Montero *et al.*, 2001). Several preservation methods such as temperature control, humidity management, and inhibitory action were used during storage and distribution to preserve shrimps' quality for domestic consumption and export, as well as to maintain and improve their shelf - life (Pan *et al.*, 2019).

Several chemicals such as ethylenediaminetetraacetic acid, benzoic acid, polyphosphate, ascorbic acid, and sodium chloride have been used as preservatives to extend the shelf-life of shrimp. Among them, sulfites are preservatives used to control melanosis formation in shrimp that can cause allergic reactions, even severe disorders in people with asthma (Pardio *et al.*, 2011). As a result, efforts to develop novel and safe storage methods are ongoing, allowing the product's taste, color, and texture to be retained for an extended time. Antimicrobial agents, particularly substances with natural antibacterial characteristics, have been developed as a major strategy for extending the shelf-life of shrimp. Among them, phenolic compounds from plants are getting more and more attention as natural additives with antioxidant and antimicrobial activities (Nirmal and Benjakul, 2009b).

Custard apple, *Annona squamosa* L. (AS), belongs to the genus *Annona*. In postharvest processing, the fruit pulp is mostly used, while the peel and seeds are considered to be discarded by-products. It has been reported that custard apple peel contains many phenolic compounds, mostly alkaloids, flavonoids, and terpenoids, with antibacterial and antioxidant characteristics (Nguyen *et al.*, 2021; Sidhu and Zafar, 2020). Therefore, the combination of this custard apple peel extract with chitosan coating can be highly potential for shrimp preservation as well. Even though antioxidant and antibacterial properties are widely known biological features of chitosan, supporting prolonging the shelf life and improving the quality of chitosan-coated food (Fernández-Saiz *et al.*, 2013; Qiu *et al.*, 2016), there is no research in the literature reported about the impact of chitosan coating in combination with ASE on the quality of applied seafood products. Accordingly, the objective of this work was to look into the details of the changes in physicochemical properties of whiteleg shrimp coated with chitosan combined with ASE at different concentrations during the retention period of 12 days at cold temperature (4±1^{o}C).

Materials and methods

Materials

The custard apples were obtained from Tay Ninh Province in Vietnam. The peel of custard apples was separated and rinsed with water to remove fleshy residues

before being dried by using hot air at 60°C until the moisture content reached 12%. The dried peel was powdered, then sieved through a 0.5 mm sieve, and stored in PE bags.

Chitosan (Food grade) was from Chitoworld Company (Vietnam). The degree of deacetylation of chitosan was ≥ 90%.

Alive shrimps (*Litopenaeus vannamei*) weighing 50-55 shrimps/kg were purchased from the local market in Ho Chi Minh City, Vietnam. Shrimps were placed in a styrofoam box with a shrimp-to-ice ratio of 1:2 (w/w) and delivered to the laboratory within 30 min.

Chemicals and reagents

Folin-Ciocalteu reagent (≥ 99.8%) and standard gallic acid (≥ 99.9%) were supplied by Merck (Germany). Thiobarbituric acid (≥ 98%) reagent was purchased from Sigma-Aldrich (USA). All other chemicals were analytical grade.

Preparation of Annona squamosa L. peel extract

The ethanolic extraction procedure was carried out by using a homemade modified microwave machine (Sanyo, Japan) with a previously developed procedure described by Nguyen *et al.* (2021). The custard apple peel powder was extracted by applying 60% ethanol, a solvent/material ratio of 25/1 (v/w) for 5 min with a microwave power of 214 W. The collected extract was passed through Whatman paper No. 4 to filter, and concentrated under a vacuum with a rotary evaporator (Bibby RE-301, UK) until the solvent was evaporated.

Preparation of chitosan-ASE solution and shrimp samples

For chitosan solution preparation, 15 g of chitosan (food grade) was mixed with 900 ml of distilled water and stirred for 10 min, then 15 ml of glacial acetic acid was added into the mixture and stirred for 1 h at 40°C. After that, the solution was made to 1000 ml of volume with distilled water and kept at 4°C for 16 h. The custard peel extract was then put in the prepared chitosan solution with the amounts corresponding to concentrations of 100 (M1), 200 (M2), 300 (M3), and 400 (M4) mg gallic acid equivalents (GAE)/L of total polyphenol content. The shrimps were cleaned and divided randomly into six groups: the control group (uncoated) (M), the chitosan coating group (M0), and the treated groups (M1, M2, M3, and M4) by dipping into a coating solution of chitosan with the additions of ASE levels of 100, 200, 300, and 400 mg GAE/L, respectively. Shrimps of M0 and M1-4 groups were immersed in the solution of chitosan or chitosan-ASE for coating at 4°C for 20 min. Then, the treated shrimp were drained at room temperature for 10 min to produce the chitosan film. The samples were then packed and stored at a temperature of 4±1°C. On the 0^{th}, 3^{rd}, 6^{th}, 9^{th}, and 12^{th} day of storage, physicochemical parameters were measured.

Determination of total phenolic content

Determination of total phenolic content was done using the Folin-Ciocalteu method as described by Sripakdee *et al.* (2015) with modification. Sample (0.1 mL) has reacted with 1.8 mL Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and incubated at room temperature for 5 min. Next, 1.2 mL of

sodium carbonate (15%, w/v) was added to the mixture and allowed to stand for 90 min in darkness at room temperature. The absorbance was then determined at 765 nm using a UV-VIS spectrophotometer Genesys 20 (Thermo Fisher Scientific, USA). The results were expressed as mg gallic acid equivalents (mg GAE/L).

Determination of weight loss

The weight of shrimps stored in polyethylene (PE) bags was measured in the laboratory using an analytical balance with three replications per sample every three days. The percentage loss of shrimp weight before and after storage was used to calculate the weight loss (%) (Wang *et al.*, 2018).

pH determination

The shrimps' pH was determined by dissolving 1 g of the sample in 9 ml of distilled water (Mohammadalinejhad *et al.*, 2020). After that, a pH meter was used to determine the pH of the mixture in triplicates.

Determination of peroxide values

The peroxide values were estimated according to the Iodine titration method of Maghami *et al.* (2019) with modification. A filled centrifuge tube with 1 g of shrimp sample was added with 5 ml of chloroform and 10 ml of acetic acid and was shaken. Then 1 ml of saturated KI solution was put in, and the tube was left in the dark for 10 min to allow the reaction to proceed. After the reaction time, 75 ml of distilled water was added into the tube, and the peroxide value of the sample was quantified by titrating the mixture using $Na_2S_2O_3$ solution with a few drops of starch as an indicator until the blue-violet color disappeared. The above steps were repeated with the blank sample.

Determination of total volatile basic nitrogen values

Total volatile basic nitrogen (TVB-N) values were determined by applying the hot water distillation method described by Feng *et al.* (2016) with modifications. Weighing 5 g of sample and 2 g of MgO into a Kjeldahl tube, then 50 ml of distilled water was added. The conical flask containing 25 ml of 1% H_3BO_3 was inserted into the distillation system, then distillation was carried out for 10 min. After distillation, the solution obtained in the conical flask was titrated with 0.1N H_2SO_4 standard solution until the solution color changed from green to pink.

Thiobarbituric acid reactive substances (TBARs) determination

A sample of 5 g was weighed, ground, and added with 25 ml of 7.5% TCA solution. The mixture was then filtered and made up to 50 ml by the TCA solution (7.5%). After that, 5 ml of the extract was taken into a test tube, and added with 5 ml of 0.02 M thiobarbituric acid solution, then cooked in a boiling water bath for 40 min and cooled down to room temperature. The solution was incubated in the dark for 90 minutes and measured the absorbance at 532 nm using a spectrophotometer (Genesys 20, Thermo Fisher Scientific) (Liao *et al.*, 2018).

Color measurement

Color values were determined on the shrimp shell with the CIE L*a*b* system using a Minolta colorimeter (model CR-400) at three locations (head, body, and

tail). Triplicate measurements were taken at each shell site for every sample, and the average value was recorded. The total color difference (△E* value), which indicates the difference in color at the beginning and after the storage period, was calculated using the following formula: $\triangle \mathrm{E} * = \sqrt{(\triangle L)^2 + (\triangle a)^2 + (\triangle b)^2}$ (Yuan *et al.*, 2016).

Texture measurement

The texture profile analysis (TPA) of the shrimp sample was performed using a physicomechanical meter (Texture Analyzer, Brookfield CT3) according to Farajzadeh *et al.* (2016) with slight modification. Shrimp meat was cut in the middle of the body and placed on a flat plate. The measurement was carried out using a cylindrical plunger of 25 mm diameter (TA3/1000). The shrimp sample was compressed to 20% of its original height in two consecutive cycles, with a time interval of 1 s between each cycle, a deformation rate of 1 mm/s, and a compression force of 1 N. Values such as the hardness and springiness of white leg shrimp were determined.

Statistical analysis

All experiments were repeated three times, and the findings were given as the means with standard deviation values. Statgraphics Centurion XV program used analysis of variance (ANOVA) was applied to examine the difference in experimental data among samples for all parameters ($p<0.05$).

Results and discussion

Weight loss

Weight change is one of the physical properties that influence the food texture and sensory quality of shrimp (Wang *et al.*, 2018). Figure 1 depicts the weight loss of experimental Pacific white shrimp. It showed that a high concentration of incorporated ASE has positive effects on the water barrier properties of chitosan coating to prevent weight loss. The shrimp weight loss tended to increase gradually during the storage time, and there was a statistically significant difference in weight loss among tested samples ($p<0.05$). Those of the control (M) and chitosan coating (M0) samples increased at a faster rate than the chitosan-ASE coating samples after 12 days of cold storage. Furthermore, the higher amount of ASE added to the coating, the less weight loss of shrimp was observed during the storage. Shrimps coated with chitosan incorporated ASE at 300 mg GAE/L (M3) and 400 mg GAE/L (M4) had the least weight loss among the experimental samples because chitosan and ASE help to reduce weight loss in Pacific white shrimp during cold storage by forming a semi-permeable barrier around the product surface, thus preventing moisture loss.

The water vapor permeability of the blended film was lowered because the free volume of the network mesh of the polymeric matrix might decrease (Farajzadeh *et al.*, 2016). Similar results were reported by Sun *et al.* (2017), who found that through interactions with polyphenols via hydrogen bonding, increasing the concentration of incorporated extract from thinned young apple decreased the

water vapor permeability of chitosan film. It was also reported elsewhere that there was a significantly lower weight loss in the chitosan-nanoparticle coated shrimp as compared to the uncoating control (Wang *et al.*, 2018). Shrimp coated with chitosan incorporated ASE at 300 mg GAE/L had a weight loss of 37.44% at the end of storage time as compared to the control sample. However, the result was still higher than 32% reported by Jeon *et al.* (2002) when using a chitosan-gelatin coating to preserve cod for 12 days.

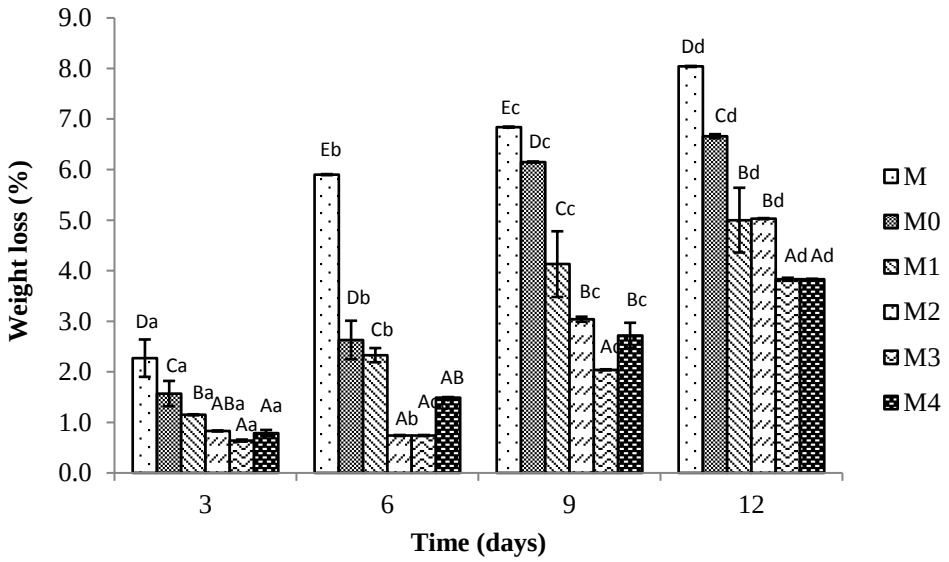

Figure 1. Changes in weight loss of shrimps during storage. M, M0, M1, M2, M3, and M4 are samples uncoated, coated with chitosan, coated with chitosan incorporated ASE at 100, 200, 300, and 400 mg GAE/L, respectively. Different letters of each bar indicate significant differences among storage times (lowercase) and differences among sample groups (uppercase) at $p<0.05$.

pH

The pH index is commonly used to assess the freshness of the seafood. Changes in pH reflect food quality during storage due to the activity of microorganisms or enzymes (Na *et al.*, 2018). Measured pH values of tested samples are presented in Figure 2. For the first 3 days of storage, the pH values of all samples were not significantly ($p>0.05$) different. From day 6, pH values started to increase gradually with the storage time and there was a statistically significant ($p<0.05$) difference among the samples.

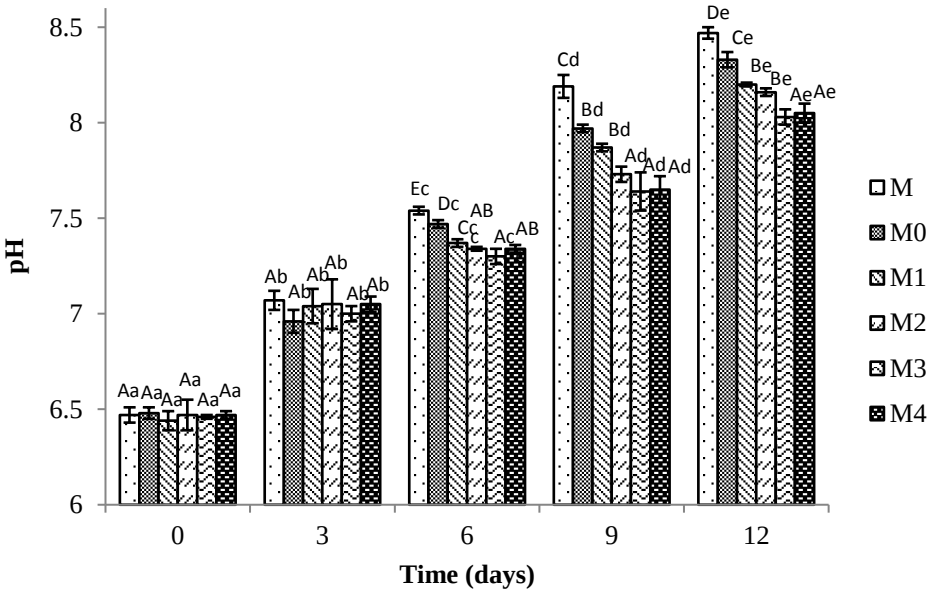

Figure 2. Changes in pH value of shrimps during storage. M, M0, M1, M2, M3, and M4 are samples uncoated, coated with chitosan, coated with chitosan incorporated ASE at 100, 200, 300, and 400 mg GAE/L, respectively. Different letters of each bar indicate significant differences among storage times (lowercase) and differences among sample groups (uppercase) at $p<0.05$.

During storage, the pH of all shrimp samples gradually increased from 6.44±0.05 (day 0) to 8.47±0.03 (day 12) due to trimethylamine and dimethylamine compounds produced by intrinsic enzymes and microorganisms, that change the pH of the shrimp from acidic to neutral or alkaline condition (Limbo *et al.*, 2009; Pacquit *et al.*, 2006). Among samples, the increase in pH values of the control is the most, followed by the chitosan coating sample without the addition of ASE. The pH increased more slowly in the chitosan-ASE coated samples, and the higher the concentration of ASE added to the coating, the slower the pH increased. The pH of the M3 and M4 samples increased more slowly than the other samples, reaching values of 8.03±0.04 and 8.05±0.05 (day 12), respectively. The obtained pH results were similar to that of Yuan *et al.* (2016), using chitosan coating combined with pomegranate peel extract to preserve whiteleg shrimp. The pH value of shrimp after 10 days of control and survey samples was 8.40 and 8.22 (day 10), respectively. After 6 days of storage, all shrimp samples still had good quality (pH<7.70). However, on day 9 of the storage, the pH value of the control (M) and sample coated only with chitosan (M0) exceeded the acceptable limit (pH>7.95). And on the 12th day, all the rest samples exceeded the pH limit, indicating spoiled shrimp. At that time, there was no significant difference in pH among all the samples (around 8.03 - 8.47), showing that the chitosan-ASE coating did not affect the pH value remarkably during storage. The reason is that the thick shell of shrimp

prevented the antioxidant and antibacterial ASE to penetrate and interact with shrimp meat, retarding the oxidation reactions and inhibiting the growth of microbiological (Wang *et al.*, 2015). The observation also agreed with the findings of Jiang *et al.* (2011) using an antibacterial coating from catfish skin gelatin to maintain quality and prolong the shelf-life of whiteleg shrimp.

Peroxide values

There are abundant polyunsaturated fatty acids in the tissue membranes of many crustaceans. Through auto-oxidation and enzyme-catalyzed reactions such as lipoxygenase and peroxidase, lipid oxidation is also one of the causes of deterioration of shrimp quality (Nirmal and Benjakul, 2009a). The peroxide value (PV) index, one of the most common quality indicators of fat during storage, indicates the number of primary oxidation products (Antonacopoulos and Vyncke, 1989; Okpala *et al.*, 2014).

During storage, the PV of shrimps in all treatments increased steadily, and there was a statistically significant difference among samples ($p<0.05$) (Figure 3). The control sample had the greatest increase in PV, followed by the sample coated only with chitosan. It can be explained that the increase in PV was caused by the oxidation of fatty acids in shrimp muscle, which resulted in the formation of hydroperoxides or peroxides (Nirmal and Benjakul, 2009a). However, because the hydroperoxide compounds formed by lipid oxidation are unstable and easily degraded to shorter-chain hydrocarbons such as aldehydes, the PV can be decreased further (Nirmal and Benjakul, 2011).

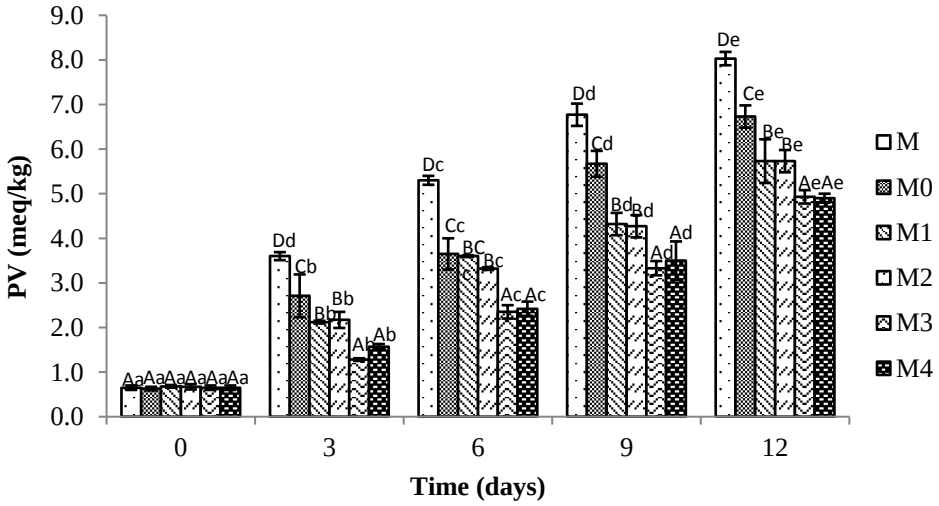

Figure 3. Changes in peroxide value of shrimps during storage. M, M0, M1, M2, M3, and M4 are samples uncoated, coated with chitosan, coated with chitosan incorporated ASE at 100, 200, 300, and 400 mg GAE/L, respectively. Different letters of each bar indicate significant differences among storage times (lowercase) and differences among sample groups (uppercase) at $p<0.05$.

The chitosan coating was shown to act as an excellent barrier in slowing down oxygen diffusion (Farajzadeh *et al.*, 2016; Sathivel *et al.*, 2007). The increasing rate of PV of the chitosan-ASE coating shrimps is slower and depended on the concentration of additional ASE. The values of samples coated with the additions of ASE levels of 300 and 400 mg GAE/L were the lowest, which are 4.93±0.15, 4.90±0.10 (meq/kg), respectively. It is obvious that the addition of ASE is effective in retarding the lipid oxidation of the shrimp samples. The results agree with the study of Sun *et al.* (2018) which showed that grass carp (*Ctenopharyngodon idellus*) fillets preserved with chitosan coating with the addition of 1% thinned young apple polyphenols showed remarkable inhibition of the proliferation of PV.

Total volatile basic nitrogen values

The total volatile basic nitrogen (TVB–N) is one of the important chemical indicators of deterioration in seafood (Wu, 2014). Figure 4 shows the change of TVB–N of the samples during storage. It is observed that the TVB–N increased progressively in all shrimp samples up to day 12. The difference is statistically significant among the samples ($p<0.05$). The TVB-N value of the control sample (M) increased rapidly from 5.88±0.28 (mg N/100g) to 49.94±0.43 (mg N/100g) on day 12. In addition, TVB–N values in shrimps coated with chitosan-ASE (M1-M4) were significantly lower than the values of sample coated only with chitosan (M0) on days 6, 9, and 12 of the storage.

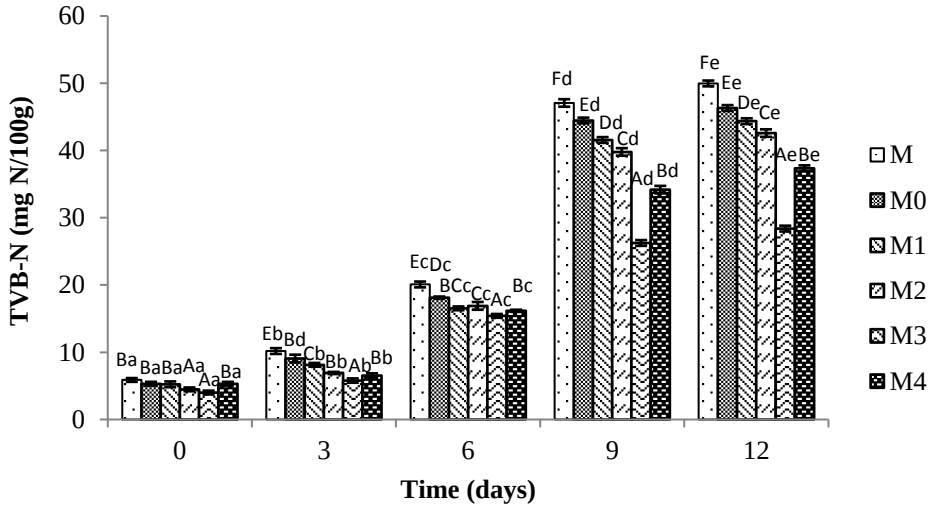

Figure 4. Changes in TVB-N value of shrimps during storage. M, M0, M1, M2, M3, and M4 are samples uncoated, coated with chitosan, coated with chitosan incorporated ASE at 100, 200, 300, and 400 mg GAE/L, respectively. Different letters of each bar indicate significant differences among storage times (lowercase) and differences among sample groups (uppercase) at $p<0.05$.

On day 6, the TVB–N values of all samples are less than 30 mg N/100g, which is compatible with the prawn sanitary standard (GB2741–94). However, on days 9 and 12, only the shrimp coated with chitosan and ASE (300 mg GAE/L) remained the TVB-N values at 26.25±0.43 mg N/100g and 28.39±0.43 mg N/100g, respectively. While the TVB-N values of the remaining samples exceeded the spoilage criteria for the fresh shrimp. The results are similar to the study of Jeon *et al.* (2002) on the storage of chitosan-coated Atlantic cod (*G. morhua*) and herring (*Clupea harengus*) for 12 days with the content of TVB–N decreased by 33-50% for the control. The increase in TVB-N is related to spoilage bacteria or endogenous enzymes (Arancibia *et al.*, 2015). This value is referred to as ammonia, primary, secondary, and tertiary amines, and proteolysis products which have been used as a frequent and significant index to assess seafood quality (Yuan *et al.*, 2016). A high value of TVB-N will cause an unpleasant and unacceptable odor (Lin *et al.*, 2013).

Thiobarbituric acid reactive substances

The TBARS index is widely used to evaluate lipid oxidation status and antioxidant activity in food products. The combined effect of chitosan and ASE concentration on the TBARs content of whiteleg shrimp during cold storage is shown in Figure 5.

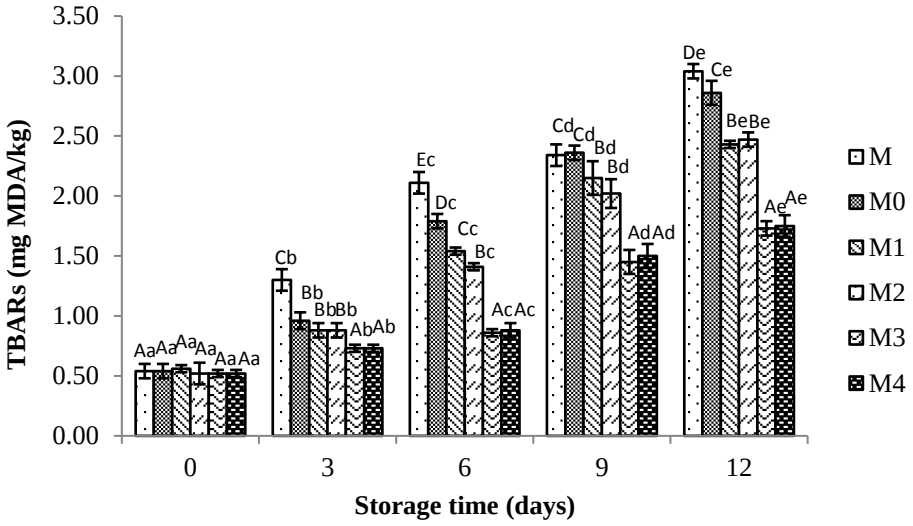

Figure 5. Changes in TBARs value of shrimps during storage. M, M0, M1, M2, M3, and M4 are samples uncoated, coated with chitosan, coated with chitosan incorporated ASE at 100, 200, 300, and 400 mg GAE/L, respectively. Different letters of each bar indicate significant differences among storage times (lowercase) and differences among sample groups (uppercase) at $p<0.05$.

As the storage time increased, a statistically significant increase in TBARs value was observed ($p<0.05$). However, the increasing rate varied among the treatments. TBARs value increased most in the control sample without coating, while samples coating only with chitosan and chitosan-ASE coated samples increased more slowly. Using chitosan coating in combination with ASE at 300 mg GAE/L the TBARs value increase was the slowest, from 0.52±0.03 (mg/kg) at day 0 to 1.73±0.06 (mg/kg) at day 12. There is no significant difference between ASE coating treatments at concentrations of 300 and 400 (mg GAE/L). It was also reported elsewhere that lipid oxidation in shrimp occurred more rapidly in the absence of antioxidant protection (Sun *et al.*, 2018). The ASE contains biological compounds such as flavonoids, saponins, and tannins with strong antioxidant effects (Nguyen *et al.*, 2021). In addition, chitosan also possesses antioxidant capacity due to its primary amine groups which can form a stable fluorosphere with volatile aldehydes such as malondialdehyde, a broken-down derivative from lipids during the oxidation (Alishahi and Aïder, 2012). Compared to control, shrimp samples coated with chitosan incorporated with ASE at a concentration of 300mg GAE/L showed a 43% decrease in TBARs value after 12 days of storage at 4^{o}C. This is better than the study of shrimp preservation with chitosan edible coating enriched with pomegranate peel extract, in which the TBARs value decreased by about 30% when compared to the control after 10 days of ice storage (Pan *et al.*, 2019).

Color

Color is among the most important quality parameters of seafood. Color indexes including L*, a*, b*, △E* are shown in Table 1. At the same storage time, all color indexes have statistically significant differences among samples ($p<0.05$). L* is the component that represents brightness on a scale of 0 (dark) to 100 (light) (Yuan *et al.*, 2016). Among the color, components are a*, which represents the difference between green and red, and b*, which represents the difference between blue and yellow (Farajzadeh *et al.*, 2016). The △E* value is a measure of the overall color difference. In general, the △E* values of the shrimps increased gradually as the storage time increased ($p<0.05$). During cold storage, the color change value of the control shrimp sample increased the most over time, from 5.24±0.06 (day 0) to 12.10±0.06 (day 12). It was found that the chitosan coating combined with ASE at a concentration of 300mg GAE/L effectively prevented color change during cold storage of whiteleg shrimp. Polyphenol oxidase is also a key enzyme in the synthesis of melanin, which is responsible for the development of melanosis. Therefore, ASE may play a role because it contains abundant polyphenols. Phenolic compounds were shown to inhibit the activity of polyphenol oxidase in shrimp, preventing melanogenesis during iced storage (Wang *et al.*, 2018). It was also reported that chitosan with its chelating action and coating-induced oxygen exclusion, prevented polyphenol oxidase enzyme activity and delayed the appearance of black spots in shrimp (*Pandalus borealis*) (Farajzadeh *et al.*, 2016).

Table 1. Changes in color attributes of shrimps during storage.

Attributes/ Treatments		**Storage time (days)**				
		0	3	6	9	12
L*	M	48.54^{Fe}±0.02	47.63^{Ed}±0.01	46.19^{Fc}±0.01	45.59^{Fb}±0.01	43.61^{Fa}±0.02
	M0	47.13^{Ee}±0.02	46.88^{Dd}±0.02	46.01^{Ec}±0.01	44.59^{Eb}±0.01	43.09^{Ea}±0,02
	M1	46.27^{De}±0.02	45.81^{Cd}±0.02	45.69^{Dc}±0.01	44.27^{Db}±0.02	42.79^{Da}±0.02
	M2	46.11^{Ce}±0.02	45.79^{Cd}±0.02	44.21^{Cc}±0.01	43.98^{Cb}±0.02	41.94^{Ca}±0.02
	M3	45.57^{Ae}±0.03	45.27^{Ad}±0.02	43.77^{Ac}±0.02	43.02^{Ab}±0.02	41.45^{Aa}±0.02
	M4	45.65^{Be}±0.01	45.45^{Bd}±0.02	43.96^{Bc}±0.02	43.48^{Bb}±0.02	41.84^{Ba}±0.02
a*	M	-0.67^{Da}±0.03	0.04^{Eb}±0.03	0.15^{Ec}±0.05	0.25^{Ed}±0.04	2.92^{Fe}±0.06
	M0	-0.82^{Ca}±0.04	-0.37^{Db}±0.04	-0.15^{Dc}±0.04	0.03^{Dd}±0.03	2.78^{Ee}±0.03
	M1	0.83^{Ca}±0.01	-0.65^{Cb}±0.03	-0.44^{Cc}±0.03	-0.23^{Cd}±0.05	2.62^{De}±0.03
	M2	-0.93^{Ba}±0.03	-0.70^{BCb}±0.04	-0.48^{Cc}±0.02	-0.45^{Bc}±0.03	1.34^{Cd}±0.03
	M3	-1.03^{Aa}±0.03	-0.87^{Ab}±0,03	-0.64^{Ac}±0.03	-0.55^{Ad}±0.04	0.50^{Ae}±0.03
	M4	-0.93^{Ba}±0.03	-0.71^{Bb}±0.03	-0.57^{Bc}±0.04	-0.46^{Bd}±0.04	0.77^{Be}±0.03
b*	M	3.60^{Ea}±0.03	4.40^{Eb}±0.05	5.34^{Ec}±0.05	9.60^{Fd}±0.06	14.04^{Fe}±0.08
	M0	3.25^{Da}±0.05	4.39^{Eb}±0.03	4.69^{Dc}±0.07	9.34^{Ed}±0.06	13.14^{Ee}±0.07
	M1	3.22^{Da}±0.07	4.21^{Db}±0.07	4.44^{Cc}±0.06	8.73^{Dd}±0.03	12.61^{De}±0.03
	M2	2.72^{Ca}±0.03	4.04^{Cb}±0.06	4.39^{Cc}±0.03	8.31^{Cd}±0.03	11.11^{Ce}±0.05
	M3	2.21^{Aa}±0.06	2.35^{Ab}±0.06	3.93^{Ac}±0.05	8.10^{Ad}±0.03	8.39^{Ae}±0.05
	M4	2.52^{Ba}±0.04	3.65^{Bb}±0.06	3.77^{Bc}±0.04	8.11^{Bd}±0.03	10.24^{Be}±0.07
△E*	M		5.24^{Da}±0.06	6.76^{Cb}±0.06	8.94^{Fc}±0.07	12.10^{Fd}±0.06
	M0		5.47^{Ea}±0.09	6.65^{Cb}±0.09	8.75^{Ec}±0.07	11.27^{Ed}±0.12
	M1		5.16^{CDa}±0.06	5.89^{Bb}±0.09	8.32^{Dc}±0.09	10.59^{Dd}±0.10
	M2		4.59^{Aa}±0.05	6.00^{Bb}±0.03	6.97^{Cc}±0.08	9.64^{Cd}±0.02
	M3		5.05^{Ca}±0.10	5.51^{Ab}±0.07	6.44^{Ac}±0.09	7.59^{Ad}±0.09
	M4		4.75^{Ba}±0.04	6.01^{Bb}±0.06	6.78^{Bc}±0.10	8.77^{Bd}±0.10

Note: M, M0, M1, M2, M3, and M4 are samples uncoated, coated with chitosan, coated with chitosan incorporated ASE at 100, 200, 300, and 400 mg GAE/L, respectively. Values reported are the mean±standard deviations. Mean within the same column (uppercase) and the same row (lowercase) with different letters are different ($p<0.05$).

Texture

The texture is also an important quality characteristic to determine the consumer acceptance of seafood products. The shrimps lose their firmness and springiness as storage time passes (Wang *et al.*, 2018). Table 2 shows how the texture of chitosan-ASE coated shrimps changed during cold storage.

Table 2. Changes in texture attributes of shrimps during storage.

Attributes/ Treatments		**Storage time (days)**				
		0	3	6	9	12
Hardness (N)	M	10.73^{Ad}±1.26	8.71^{Ac}±0.62	7.03^{Ab}±0.08	4.13^{Aa}±0.09	3.09^{Aa}±0.18
	M0	10.59^{Ad}±1.27	9.22^{Ac}±0.17	7.12^{Ab}±0.15	4.50^{Ba}±0.29	3.42^{Ba}±0.18
	M1	10.92^{Ae}±0.78	8.81^{Ad}±0.22	7.20^{Ac}±0.27	5.09^{Cb}±0.20	3.73^{Ca}±0.15
	M2	10.72^{Ad}±1.58	8.76^{Ac}±0.66	7.20^{Ab}±0.13	5.28^{Ca}±0.10	3.95^{Ca}±0.13
	M3	11.07^{Ad}±1.34	9.25^{Ac}±0.11	7.64^{Bb}±0.04	5.58^{Da}±0.13	5.00^{Ea}±0.12
	M4	11.21^{Ae}±0.63	8.95^{Ad}±0.35	7.28^{Ac}±0.23	5.35^{CDb}±0.06	4.39^{Da}±0.07
Springiness (mm)	M	0.74^{ABe}±0.02	0.62^{Ad}±0.03	0.49^{Ac}±0.02	0.34^{Ab}±0.02	0.24^{Aa}±0.04
	M0	0.75^{Ad}±0.03	0.63^{Ac}±0.05	0.51^{ABb}±0.01	0.37^{Aa}±0.03	0.32^{Ba}±0.02
	M1	0.69^{Abd}±0.02	$0.66^{Ad±}$0.02	0.56^{Bc}±0.05	0.45^{Bb}±0.04	0.35^{Ba}±0.03
	M2	0.74^{ABc}±0.06	0.69^{Abc}±0.07	0.56^{Bb}±0.03	0.51^{Cb}±0.04	0.36^{Ba}±0.03
	M3	0.76^{Bc}±0.02	0.71^{Abc}±0.07	0.65^{Cb}±0.01	0.55^{Ca}±0.03	0.52^{Ca}±0.02
	M4	0.74^{ABd}±0.04	0.71^{Ad}±0.05	0.63^{Cc}±0.04	0.55^{Cb}±0.01	0.47^{Da}±0.02

Note: M, M0, M1, M2, M3, and M4 are samples uncoated, coated with chitosan, coated with chitosan incorporated ASE at 100, 200, 300, and 400 mg GAE/L, respectively. Values reported are the mean± standard deviations. Mean within the same column (uppercase) and the same row (lowercase) with different letters are different ($p<0.05$).

The hardness and springiness of shrimp samples are similar at the beginning of storage ($p>0.05$). The trend of declining texture parameters was found on day 6, and the difference among the samples was statistically significant ($p<0.05$). Hardness is the most important textural property of meat or seafood (Yuan *et al.*, 2016). In all treatments during cold storage, the firmness index of shrimps was significantly reduced. The hardness of the control sample decreased more quickly than values obtained from the chitosan coating and chitosan-ASE coating samples. The hardness of the control uncoated sample dropped from 10.73±1.26 N (day 0) to 3.09±0.18N (on day 12), while, at the same storage time the shrimp sample coated with chitosan reached the hardness of 3.42±0.18 N. The maximum hardness (day 12) was observed at 5.00±0.12 N with the stiffer texture of shrimp samples coated with chitosan incorporated ASE at a concentration of 300mg GAE/L (day

12). The same phenomenon was observed with the results of the springiness of whiteleg shrimp. The springiness of shrimp in all treatments decreased during storage. However, on days 9 and 12 of storage, the springiness of shrimp treated by chitosan- ASE coating was significantly improved ($p<0.05$) as compared with those of the control shrimp sample. Compared with all other samples, the highest springiness value was observed in the shrimp treated with chitosan in combination with 300mg GAE/L of ASE. The findings are similar to the study results of Yuan *et al.* (2016) who reported that the hardness and springiness of ice-preserved Pacific white shrimp treated with chitosan coating in combination with pomegranate peel extract were also significantly improved when compared with the control uncoated sample. In another research, (Farajzadeh *et al.*, 2016) showed that the chitosan-gelatin coating improved the texture property of shrimps in cold conditions. Li *et al.* (2013) also used chitosan coating incorporated with grape seed extract and tea polyphenols to extend shelf-life and improve texture attributes of red drum (*Sciaenops ocellatus*) fillets during cold storage.

Conclusions

This study shows the potential of chitosan combined with custard apple (*Annona squamosa* L.) peel extract as a natural coating for Pacific white shrimps (*Litopenaeus vannamei*) during 12 days of cold storage. Shrimps treated with a chitosan-ASE coating had lower increases in weight loss, pH, PV, TBARs, TVB-N, and improved color and texture properties, compared with the control samples. The best quality was obtained from shrimps coated with chitosan incorporated ASE at a concentration of 300 mg GAE/L. Therefore, chitosan combined with ASE can be used as promising preservation coatings to maintain the quality of whiteleg shrimp.

Author Contributions: Conceptualization, H.P.T and T.N.T; methodology H.P.T and P.N.T.T; formal analysis, Q.H.T.T and P.N.T.T; investigation, T.N.T and Q.H.T.T; resources, T.N.T and H.P.T; writing-original draft preparation, T.N.T and Q.H.T.T; writing-review and editing, H.P.T; visualization, T.N.T and Q.H.T.T; supervision, H.P.T and T.N.T; project administration, T.N.T and P.N.T.T.

Acknowledgments: This study was supported by the Institute of Biotechnology and Food Technology - Industrial University of Ho Chi Minh City.

Conflicts of Interest: The authors declare no conflict of interest.

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