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FULL PAPER

Incorporation of *Neolamarckia cadamba* leaf extract into chitosan coating for maintaining the quality of white shrimp (*Litopenaeus vannamei*)

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Abstract

Neolamarckia cadamba or locally known as kelampayan or laran is traditionally used for treatments of illnesses such as diabetes, anaemia and cancer. This plant has been reported for its phytochemical compounds which include alkaloids, phenolics and flavonoids as well as various bioactivities such as antioxidant, antibacterial and anti-inflammatory activity. Despite its use as a medicinal plant, information on the possible utilization for controlling the quality of food is scarce. This study aimed at exploring the efficacy of *N. cadamba* leaf extract incorporated into a coating solution at different concentrations (1%, 2% and 3%) for retaining the quality of white shrimp in chilled storage. Assessment of physical, chemical, microbial quality, melanosis formation and sensory analysis of the white shrimp was performed during 9 days of storage at 4°C. Following treatment of the coating solution on white shrimp, weight loss and pH values were recorded. Total volatile base nitrogen was assessed using the distillation method, and shrimp quality and melanosis formation were scored through visual inspection. In the sensory acceptance test, panellists assessed the appearance, colour, odour, texture and overall acceptance of shrimp using a nine-point hedonic scale. This study reported that the coating solution incorporated with *N. cadamba* leaf extract at all tested concentrations significantly ($p<0.05$) retarded the percentage of weight loss, decreased total volatile base nitrogen and delayed melanosis formation in white shrimp. The application of the coating with the incorporation of *N. cadamba* leaf extract also showed a higher score in quality assessment and better sensory acceptance of white shrimp. *Neolamarckia cadamba* leaf extract in the coating also presented a positive implication towards reduced growth of microorganisms in the white shrimp. This study, therefore, showed the potential of *N. cadamba* leaf extract as an agent for postharvest treatment to retain the quality of white shrimp.

1. Introduction

Pacific white shrimp (*Litopenaeus vannamei*) receives continuous demand in Malaysia. It is one of the most dominant shrimp species produced globally which accounts for up to 4.9 million tonnes produced in 2018 (52.9% of all crustaceans) (FAO, 2020). Shrimp is a good source of protein, vitamins and minerals (Dayal *et al.*, 2013). Despite the high demand, shrimp is immensely prone to deterioration caused by biochemical and microbial activities (Dong *et al.*, 2018). The decomposition of shrimp may be triggered by various factors including enzymes. The presence of polyunsaturated fatty acids in white shrimp can enhance autoxidation which encourages the production of an unpleasant odour or taste (Okpala, 2016). Lipid autoxidation thus reduces the postharvest quality and shelf life of white shrimp. Furthermore, the shelf life of white shrimp during refrigerated storage and shipping is greatly influenced by microbial spoilage caused by pathogenic bacteria such as *Pseudomonas* spp. (Gokoglu and Yerlikaya, 2008; Na *et al.*, 2018). Another great concern related to the quality loss of shrimp is melanosis, which is a natural post-mortem enzymatic oxidation of phenols caused by the polyphenol oxidase that forms

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dark pigments (Nirmal and Benjakul, 2011a; Gonçalves and de Oliveira, 2016). These deteriorations in shrimp affect consumer acceptability and cause enhanced economic losses (Martínez-Alvarez *et al.*, 2005).

Preservation methods used to prevent melanosis and quality losses of white shrimp includes chemical treatment that poses a health risk to consumers (Sae-leaw and Benjakul, 2019a). The interest in the exploration of natural alternatives to delay deterioration and extend the shelf life of shrimp is thus increased. Application of coating and treatments with phenolic compounds, essential oil and plant extracts on seafood are among the preservative methods that have been investigated (Nirmal and Benjakul, 2009; Yuan, Zhang, Tang and Sun, 2016; Mubarak *et al.*, 2019). The coating acts as an inert barrier and can be an efficient carrier for additives such as natural antioxidants that helps to maintain the quality of shrimp (Sae-leaw and Benjakul, 2019a). The incorporation of plants extracts such as green tea extract (Yuan, Zhang, Tang and Sun, 2016), pomegranate peel extract (Yuan *et al.,* 2016) and lime peel extract (Khaledian *et al.*, 2021) into coating solutions has been previously reported to be efficient for preventing melanosis and delaying the deterioration of shrimp.

Neolamarckia cadamba, or locally known as kelampayan is one of the underexplored medicinal plants that is grown mainly in South Asia and Southeast Asia, including Malaysia (Pandey and Negi, 2016). It is commonly used as a traditional remedy to treat various illnesses such as diabetes, anaemia, cancer and others (Pandey and Negi, 2016). Previous studies have noted a potent antioxidant and antimicrobial activity in the leaf extract of this plant (Mishra *et al.*, 2011; Gupta *et al.*, 2013). These activities are often attributed to phenolics such as chlorogenic acid, as well as flavonoids such as catechin and rutin which have been reported to be present in the leaf extract of *N. cadamba* (Ganjewala *et al.*, 2013; Pandey and Negi, 2016). Nevertheless, *N. cadamba* leaf has not been explored towards application in sustaining quality of food particularly seafood. This study, therefore, aimed to investigate the potential of *N. cadamba* leaf extract in delaying deterioration and extending the shelf life of white shrimp.

2. Materials and methods

2.1 Preparation of Neolamarckia cadamba leaf extract and coating solution

Neolamarckia cadamba leaf was obtained from Mentakab, Pahang and transported to the laboratory at Universiti Malaysia Terengganu. The leaf was oven-dried at 40°C overnight and thereafter grounded into fine powder. The powdered *N. cadamba* leaf was then added to methanol (1:3, w/v) and extracted for 24 hrs at room temperature in a shaking incubator (100 rpm speed rate). The extract was then filtered with Whatman No. 1 filter paper, and the residue was extracted twice more (1 hr each time with 1:1 ratio of sample to methanol). All filtrates were combined and evaporated using a rotary evaporator to remove the methanol (Ahmed *et al.,* 2011; Zayed *et al*., 2014).

Coating solutions were prepared following Yang *et al.* (2014) and Sabaghi *et al.* (2015). Chitosan coating solution (2%, w/v) was prepared by dissolving chitosan (low molecular weight, deacetylation degree ≥ 75%) (Sigma–Aldrich, Iceland) into distilled water. Acetic acid (1%) and glycerol (1:4 ratio with chitosan) was added to the solution as a plasticizer (Moslem *et al.*, 2015). Depending on the treatment, a specific amount of NLE was added to prepare for different concentrations (1, 2 and 3%). Chitosan 2% solution without incorporation of NLE was regarded as the positive control in this study, and uncoated shrimp represents negative control. The formulation for the coating developed in this study was filed for Utility Innovation in Malaysia (Utility Innovation Application Number: UI2022004924).

2.2 Shrimp collection and treatment

White shrimp was purchased from Marang, Terengganu and immediately transported to the laboratory in an insulated polyethylene box filled with ice within 2 hrs. Upon arrival, shrimp were subdivided into 5 groups of treatments which includes control (C): 2% chitosan solution; 2% chitosan + 1% NLE; 2% chitosan + 2% NLE; and 2% chitosan + 3% NLE. The shrimp were completely immersed in respective coating solutions for 3 mins to achieve uniform surface coating and then drained and dried at room temperature for 10 mins. Shrimp without coating is prepared to represent negative control. Each group was triplicated and packed inside covered polyethylene containers and stored at 4 ± 1°C for 9 days. Analyses were performed at 3 days intervals until the end of the storage period (Yuan, Zhang, Tang and Sun, 2016).

2.3 Weight loss

The percentage of weight loss in shrimp was determined using the following equation (Suresh, 2015):

$$\text{Percentage of weight loss} = \frac{\text{Initial weight (g)} - \text{Weight after storage (g)}}{\text{Initial weight (g)}} \times 100$$

2.4 Total volatile base nitrogen

Total volatile base nitrogen (TVB-N) was determined following the method described by Sewwandi *et al.* (2016) with some modifications. White shrimp samples (25 g) were homogenized with 50 mL of

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7.5% trichloroacetic acid (TCA) in a homogenizer for 1 min and filtered with Whatman No. 2 filter paper. The filtrate (25 mL) was loaded into a distillation tube and added with 10 mL of 10% (w/v) aqueous NaOH solution and sealed prior to performing steam distillation. The distillate was collected in a flask containing 4% boric acid and a 2:1 methyl red /methylene blue (mixed indicator). The distillate was then titrated against 0.025M H_2SO_4 to the endpoint which was indicated by the change of colour from green to pink. The TVB-N content was then calculated after blank correction by steam distillation with 25 mL of the distilled water sample.

2.5 Quality assessment, melanosis assessment and acceptance test

Quality assessment, melanosis assessment and acceptance test for white shrimp samples were evaluated by thirty panellists on day-9 of storage. Quality assessment was performed in accordance with Ainaz (2019) following the criteria outlined in Table 1.

Melanosis assessment on white shrimp was conducted through visual inspection using a ten-point scoring method as stated by Nirmal and Benjakul (2009). Panellists were asked to give the melanosis score 0 to 10 for shrimp, where 0 = absent; 2 = slight (up to 20% of shrimp's surface affected); 4 = moderate (20% to 40% of shrimp's surface affected); 6 = notable (40% to 60% of shrimp's surface affected); 8 = severe (60% to 80% of shrimps' surface affected); 10 = extremely heavy (80% to 100% of shrimp's surface affected).

Panellists gave scores to the appearance, colour, odour, texture and overall acceptance of the shrimp sample using a 9-point hedonic scale (Nirmal and Benjakul, 2011b), where 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much and 1 = dislike extremely.

2.6 Microbial quality evaluation

White shrimp (25 g) was homogenized in a stomacher with 225 mL of 0.85% sterile saline water at 8000 rpm for 1 min. Then, the samples were serially diluted and plated on nutrient agar and incubated at 36°C for 48 hrs. Microbial colonies were recorded and converted into $\log_{10}$ CFU/mL.

2.7 Statistical analyses

Statistical analyses were performed using the IBM SPSS Statistics package version 24. Analysis of variance (ANOVA) was performed followed by Tukey's multiple comparison test. P values of less than 0.05 were considered statistically significant.

3. Results and discussion

3.1 Percentage weight loss of white shrimp

Figure 1 shows the percentage weight loss of shrimp throughout the storage period. Weight loss in shrimp during storage is caused by the loss of water through the shrimp's surface layer which leads to dehydration (Liu *et al.*, 2020). All white shrimp samples showed increased weight loss throughout the storage period. Uncoated shrimp had the highest rate of increase when compared to the coated ones. White shrimp coated with chitosan incorporated with the NLE at different concentrations significantly delayed the increase in weight loss ($p<0.05$). In a previous study, chitosan coating was suggested to act as a moisture-sacrificing agent which delayed the evaporation of moisture from coated fish fillets (Mohan *et al.*, 2012). Farajzadeh *et al.* (2016) also reported the efficiency of gelatin-chitosan coating in

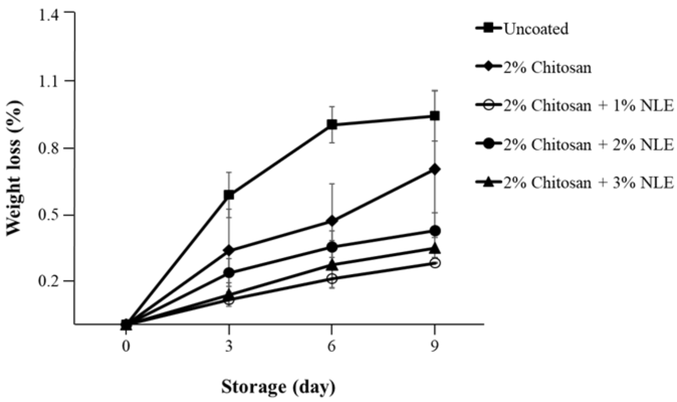

Figure 1. Effect of different coatings on the percentage of weight loss of white shrimp during storage at 4°C. Data are represented as mean ± SE.

Table 1. Quality assessment for white shrimp

Attributes	Category			
	0 (Excellent)	1 (A)	2 (B)	3 (Rejected)
Head	Bright without black	Bright without black	Slightly dark with	Dark
Eyes	Black and bright eyes	Black and lustreless eye	Slightly grey eyes	Grey eyes
Odour	Fresh to sea	Natural	Weak ammonia	Sour and putrid
Flesh	Transparent and shiny	Lime colour	Opaque and lustreless (dull)	Opaque and incipient browning

Source: Ainaz (2019).
Note: Higher scores reflect lower white shrimp quality

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reducing the weight loss of coated shrimp. In the present study, it was observed that the incorporation of NLE further enhanced the potential of this coating in reducing the weight loss of shrimp. The presence of antioxidant activity in the NLE as reported by Gupta *et al.* (2013) may help in enhancing the sealing property of the coating to prevent the evaporation of moisture and gas from the shrimp (Umachigi *et al.*, 2007). Other plant extracts such as grape seed extract was also previously reported to improve the effectiveness of coating which allowed the retention of moisture of white shrimp during storage (Baek *et al.*, 2021).

3.2 Total volatile base nitrogen

TVB-N is a typical parameter of spoilage for seafood that is commonly attributed to ammonia produced from the activity of spoilage bacteria (Arancibia *et al.*, 2015). Figure 2 illustrates the increase of TVB-N value in shrimp at the end of the storage period (day-9) from the initial time point of the experiment (day-0). The highest increase in TVB-N value was recorded in uncoated shrimp (31.4 mg/100 g). The increase of TVB-N value for shrimp coated with chitosan combined with 1%, 2% and 3% NLE were significantly lower ($p<0.05$) when compared to uncoated shrimp. The positive impact of coating treatment in delaying TVB-N formation in shrimp was also reported with the use of gelatine-chitosan coating (Farajzadeh *et al.*, 2016). Khaledian *et al.* (2021) also reported a slower increase in TVB-N formation in white shrimp when applied with edible coating incorporated with lime peel. A similar observation in white shrimp was also seen after treatment with cashew leaf extract as reported by Sae-leaw and Benjakul (2019b). There have been previous reports on the phytochemical constituents of *N. cadamba* including polyphenols (Zayed *et al.,* 2014; Pandey and Negi, 2016). Polyphenols are highly attributed for antimicrobial activity (Nirmal and Bejakul, 2009; Besbes *et al.*, 2016) which potentially explains the effectiveness in lowering the rate of increase of TVB-N values in treated white shrimp.

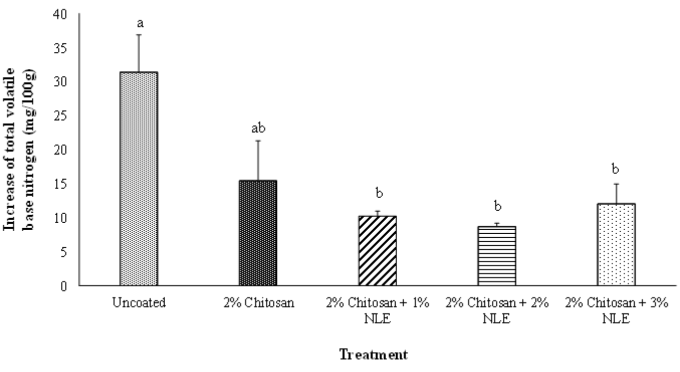

Figure 2. Effect of different coatings on the increase of total volatile base nitrogen (TVB-N) in white shrimp during storage at 4°C. Data are represented as mean ± SE. Different letters on bars indicate significant differences, $p<0.05$.

3.3 Quality assessment

Spoilage of shrimp enhances subsequent production of strongly rancid, and putrid odours which are rejected for consumption by consumers. Table 2 shows that coating with chitosan + NLE at all tested concentrations provides a better score on quality parameters when compared to uncoated and shrimp coated with chitosan only. Chitosan with 2% NLE showed the best total score compared to other treatments. The presence of compounds with potent bioactivities such as antioxidants and antimicrobials in *N. cadamba* leaf (Mishra *et al.*, 2011; Gupta *et al.*, 2013) gives an advantage in preventing spoilage. This, therefore, aids in a better quality of the tested shrimp, thus a better score of the quality parameters.

3.4 Melanosis assessment

The assessment of melanosis is illustrated in Figures 3A and 3B. The formation of melanosis in crustaceans affects the market value and reduces the profit from the seafood industry. Chitosan with the incorporation of NLE was found to be significantly effective in lowering the development of melanosis when compared to uncoated and shrimp coated with chitosan only. Phenolic compounds in the coating solutions were able to retard the polyphenol oxidase and could reduce the quinone formed in white shrimp (Nirmal and Benjakul, 2011a). Reduced formation of melanosis in shrimp due to the presence of phenolics in other plant extracts was also discussed in Sae-leaw and Benjakul (2019b) and Nagarajan *et al.* (2021).

Table 2. Effect of different coatings on the score of white shrimp quality assessment after 9 days of storage at 4°C

Treatment	Score of white shrimp quality assessment				
	Head	Eyes	Odour	Flesh	Total
Uncoated	2.7	1.9	2.1	2.2	8.9
2% Chitosan	2.4	1.7	2.0	2.1	8.2
2% Chitosan + 1% NLE	1.7	1.5	1.4	1.6	6.2
2% Chitosan + 2% NLE	1.4	1.4	1.2	1.5	5.5
2% Chitosan + 3% NLE	1.7	1.5	1.3	1.4	5.9

Note: Higher scores reflect lower white shrimp quality

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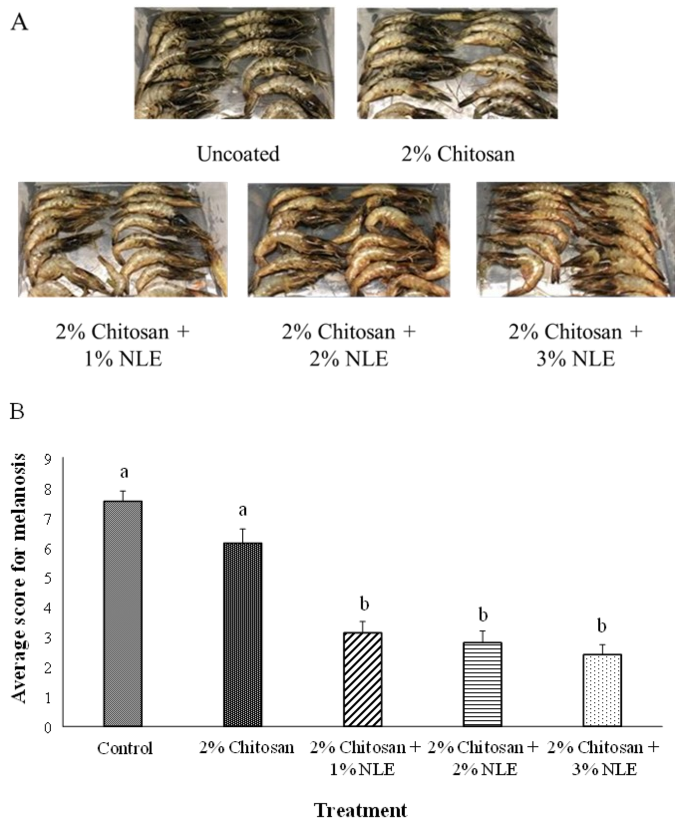

Figure 3. Effect of different coatings on the melanosis in white shrimp. (A) Images of uncoated and coated white shrimp after 9 days of storage at 4°C, (B) Score of the melanosis for white shrimp after 9 days of storage at 4°C. Bars with different notations are significantly different ($p<0.05$).

3.5 Acceptance test

The results of attributes of the sensory acceptance test are illustrated in Figure 4. A significant difference was observed for all the attributes between uncoated white shrimp and white shrimp coated with chitosan + NLE at different concentrations. Chitosan + 2% NLE was the most favourable compared to the uncoated and chitosan alone. According to the Chinese National Standard (1994), the shrimp that obtained scores less than 5 were classified as unacceptable. This is because of the putrid odour, no shiny colour, bad appearance, mushy texture and decline in overall acceptance. Farajzadeh *et al.* (2016) also stated that the sensory score was associated with the results of total bacteria count. The results from this study showed shrimp coated with chitosan + NLE at all tested concentrations were acceptable to the panellists.

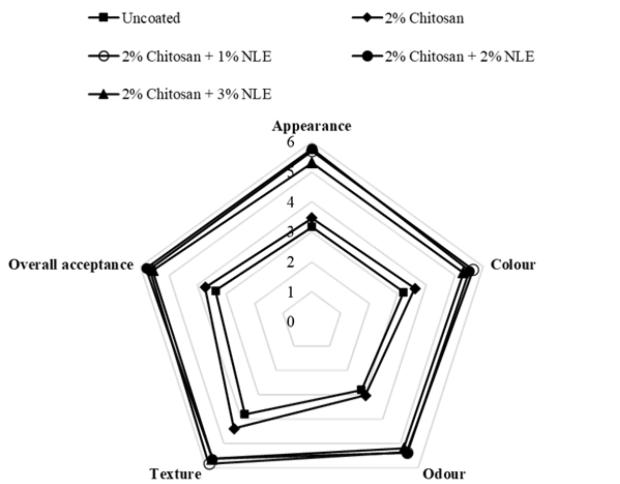

Figure 4. Effect of different coatings on the average score of different attributes in the acceptance test on white shrimp after 9 days of storage at 4°C.

3.6 Assessment of microbial quality

This study also reports on the effect of chitosan coating combined with NLE on the microbial quality evaluation of white shrimp. After 9 days of storage, coating treatment of chitosan and chitosan with the incorporation of 1% NLE did not provide a positive impact on microbial growth in shrimp samples. In this study, all white shrimp samples showed increased microbial load at day-9 of storage from the initial point of the experiment (day-0) (Table 3). Coating treatment of chitosan and chitosan with the incorporation of 1% NLE did not provide a positive impact in terms of delaying microbial growth during the storage period when compared to the control. However, chitosan coating incorporated with 2% and 3% NLE showed a lower microbial load on day-9 when compared to control and chitosan-coated shrimp. This shows the potential of NLE with the two concentrations to delay the increase in microbial load over the duration of storage. NLE at 2% concentration was observed to be the most efficient in delaying the increase of microbial load in shrimp. The antimicrobial activity can be attributed to the phenolic compounds present in *N. cadamba* leaf (Dubey *et al.*, 2011). Nirmal and Benjakul (2011a) also reported that phenolic compounds can reduce microbial growth as well as improve the quality of shrimp. *Neolamarckia cadamba* leaf has also been previously reported for its potent antibacterial and antifungal activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus*, *Escherichia coli*, *Aspergillus niger, Candida albicans* and *Aspergillus fumigatus* (Khandelwal *et al.*, 2016; Pandey and Negi, 2016).

Table 3. Effect of different coatings on the microbial load ($\log_{10}$ CFU/mL) of white shrimp during storage at 4°C

Treatment	Microbial load ($\log_{10}$ CFU/mL)	
	Day 0	Day 9
Control	4.4	>2000 CFU/mL
2% Chitosan	4.4	>2000 CFU/mL
2% Chitosan + 1% NLE	4.4	>2000 CFU/mL
2% Chitosan + 2% NLE	4.4	5.1
2% Chitosan + 3% NLE	4.4	5.7

4. Conclusion

This study revealed the potential of *N. cadamba* leaf

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extract (NLE) as an additive in coating for retaining postharvest quality factors of white shrimp. Chitosan added with NLE at all tested concentrations (1%, 2% and 3%) delayed the increase of weight loss and total volatile base nitrogen values in white shrimp during cold storage. NLE also helped retain the quality parameters of the shrimp and lowered the development of melanosis which is a crucial factor for the acceptance of this seafood. The incorporation of this plant extract was not only found to have a synergistic effect on the chitosan coating in the preservation of shrimp quality, but it showed positive scores in acceptability by the sensory panellists. The increase of total microbial load in white shrimp samples at the end of the storage period was also retarded particularly by the addition of 2% NLE in the chitosan coating. NLE thus shows potential as a supplementary agent to chitosan coating in retaining the postharvest quality of white shrimp.

Conflict of interest

The authors declare no conflict of interest.

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