A SEMINAR PAPER ON

MELANOSIS INHIBITION OF SHRIMP USING NATURAL EXTRACTS DURING ICE STORAGE

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A SEMINAR PAPER

ON

MELANOSIS INHIBITION OF SHRIMP USING NATURAL EXTRACTS DURING ICE STORAGE[1]

BY

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ABSTRACT

The subject of the present study was to see the effectiveness of natural extracts to inhibit melanosis and overall quality changes of shrimp during ice storage. The melanosis of deepwater pink shrimp (*Parapenaeus longirostris*) and Pacific white shrimp (*Litopenaeus vannamei*) was evaluated. Deepwater pink shrimp was treated with grape seed extract (GSE) and on the other hand Pacific white shrimp was treated with lead seed extract powder (LSEP), grape seed extract (GSE), pomegranate peel extract (PPE), green tea extract (GTE), GTE in combination with Ascorbic Acid (AA), chitosan and chitosan in combination with PPE as well as GTE extract during ice storage. And all these treatment are compared with control and Sodium sodium metabisulfite (SMS) treatment. The melanosis score, sensory score and other chemical changes was significantly improved in shrimp treated with natural extracts during ice storage for 9-12 days. These results suggested that use of natural extracts could be an effective alternative to synthetic antimelanosic agents to inhibit post-mortem melanosis and improve the quality of shrimp during storage in ice.

Keywords: Melanosis, Quality Change, Shrimp, Natural Extract

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CHAPTER I
INTRODUCTION

Fish and fishery products are one of the most traded food commodities in the world. Though capture fisheries remain unchanged since the end of the 80's, aquaculture production is on an increasing trend. In the early 90's production was around 10 million tons; in 2014, it reached an all-time high of 73.7 million tons of aquatic foods (FAO, 2016). Among these products, crustaceans most importantly shrimp is the leading seafood consumed in many countries over the world because of their delicacy. In 2014, farmed crustaceans accounted for only 8.2% (6.9 million tons) of the total volume of aquaculture production; nonetheless, this commodity was responsible for 21.7% (US$ 30.9 billion) of the total value (FAO, 2016).

However, melanosis or blackening, the formation of black spots in crustaceans during postmortem storage, severely damage the market value and leads to huge economical loss (Kim et al., 2002). It is due to a biochemical mechanism which oxidizes phenols to quinones by polyphenoloxidase (PPO). This is followed by non-enzymatic polymerization and auto-oxidation of the quinones, giving rise to dark pigments of high molecular weight (Benjakul et al., 2005). Melanosis causes no direct harm to consumers but it damages the sensory features of crustaceans, decreasing their quality, shelf life, and subsequently, their commercial value (Gomez-Guillen et al., 2005).

In order to reduce great economic losses, iced or refrigerated storage is usually routinely used to preserve the quality of the shrimp. However, in refrigerated or iced condition PPO remains active and so melanosis still takes place in iced or refrigerated storage (Gokoglu and Yerlikaya, 2008; Nirmal and Benjakul, 2010). To alleviate or retard the melanosis in shrimp, and assure a extended shelf life, antimelanosic agents, such as 4-hexyl-1,3- benzenediol (4-hexylresorcinol), sulphite-based compounds, and phosphates, have been experimented in conjunction with iced storage and proved to be effective to inhibit melanosis (Thepnuan et al., 2008). However, using synthetic compounds to hinder melanosis in seafood is limited due to increasing regulatory attention and food safety concerns. For instance, using sodium metabisulphite (SMS) to retard melanosis of shrimp could cause the exceed the level of sulfur dioxide residue.(Gomez-Guillen et al., 2005). Moreover, sulphiting agents are well-known to produce allergic reactions and serious disturbances in asthmatic subjects (Thepnuan et al., 2008).

Due to the potential health hazards of chemical additives, natural products, especially those containing natural antioxidants and antimicrobial properties, have been intensively examined as safe alternatives to synthetic compounds (Encarnacion et al., 2010; Maqsood et al., 2013). phenolic compounds from natural products were also used as a good alternative for sulphiting agent for retarding melanosis in crustaceans (Maqsood et al., 2013). Recently, a series of studies conducted on the utilization of natural extracts to retard melanosis formation and extend the shelf life of shrimp. Melanosis formation was inhibited using grape seed extract on deepwater pink shrimp (*Parapenaeus longirostris*) (Gokoglu and Yerlikaya, 2008); pacific white shrimp treated with catechin during iced storage (Nirmal and Benjakul, 2009); pacific white shrimp by the extract of lead (*Leucaena leucocephala*) seed (Nirmal and Benjakul, 2011a). Moreover, green tea extracts (Nirmal and Benjakul, 2011b); using green tea extract in combination with ascorbic acid (Sun et al., 2014); using chitosan coating combined with pomegranate peel extract (PPE) (Yuan et al., 2016a); chitosan coating combined with green tea extract (GTE) (Yuan et al., 2016b) was used for inhibition of polyphenoloxidase and retardation of quality loss of pacific white shrimp during iced storage.

Although the presence of black spot on shrimp is not harmful to human health, however, it drastically reduces the consumer's acceptability and the market value because of their appearance (Kim et al., 2002). Therefore, the aim of the present study was to determine the application of natural extracts as an alternative to synthetic compounds to inhibit the melanosis formation and improve the quality of shrimp during ice storage.

The present study was conducted with the following objectives:

a) To determine the natural extracts that are used to inhibit melanosis of shrimp,
b) To study the overall quality pattern of shrimp during ice storage due to the use of natural extracts, and
c) To determine the effectiveness of natural extract as a better alternative to the synthetic compounds in improving the quality of shrimp during ice storage.

CHAPTER II
MATERIALS AND METHODS

This seminar paper is exclusively a review paper. It has been prepared by reviewing the various articles published in different Books, Proceedings, Abstracts, Review papers, Journals, MS thesis, Ph.D. Dissertation etc. available in the library of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, including Bangladesh Fish Research Institute, Bangladesh Fisheries Development Corporation, DoF, etc. or anywhere else. I prepared this paper in consultation with my learned major professor, and other concerned experts. The necessary thoughts, ideas, facts and findings has been collected through internet searching and incorporated with the body of the seminar. In addition to that constructive and valuable suggestions of the experts were included, as and when necessary, in preparing this paper. Mostly secondary data have been adopted.

CHAPTER III

REVIEW OF MAJOR FINDINGS AND DISCUSSION

Melanosis Formation Score

The scale to evaluate melanosis formation is shown in table 1. A Score of four or less indicated high- quality product with minimal melanosis. A score between four and eight were considered moderarte. A score of eight or greater represented severe defects, approaching unacceptable.

Table 1. Scale for melanosis evaluation

Melanosis scores	Description
0	Absent
2	Slight, noticeable on some shrimp
4	Slight, noticeable on most shrimp
6	Moderate, noticeable on most shrimp
8	Heavy, noticeable on most shrimp
10	Heavy, totally unacceptable

Source: Montero et al. (2001)

Melanosis Analysis:

The score for melanosis of deepwater pink shrimp (*Parapenaeus longirostris)* in the concentrations of 0, 2.5, 5.0, 10 and 15 g L^{-1} grape seed extract (GSE) is shown in table 2. The highest score for melanosis was obtained in control treatment and the lowest score for melanosis was obtained in treatment with grape seed extract of 15 g L^{-1}. Omar (2005) found high melanosis in untreated shrimps during the storage, whereas the samples treated with sodium metabisulphite had slight melanosis. So, it can be said that GSE extract can be used instead of sodium metabisulphite to inhibit melanosis.

Table 2. Mean melanosis scores of deepwater pink shrimp treated with grape seed extract and stored at 4 °C

Storage days	Treatment				
	Control	2.5 g L^{-1}	5 g L^{-1}	10 g L^{-1}	15 g L^{-1}
0	0	0	0	0	0
1	4.0 ± 1.41^{ax}	1.6 ± 0.89^{bx}	2.0 ± 1.41^{bx}	2.0 ± 0^{bx}	0^{cx}
2	8.0 ± 1.41^{ay}	4.0 ± 1.41^{bcy}	3.2 ± 1.09^{bcx}	4.4 ± 0.89^{by}	2.4 ± 0.89^{cy}
3	10.0 ± 0^{az}	8.0 ± 1.41^{bcz}	10.0 ± 1.09^{aby}	8.0 ± 0.89^{cdz}	6.0 ± 0.89^{dz}

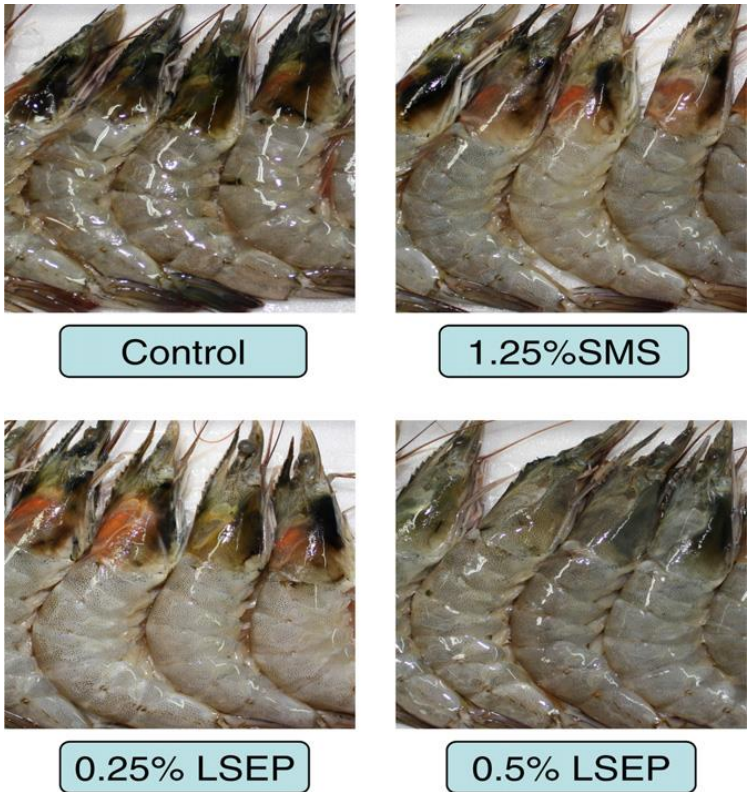
Source: Gokoglu and Yerlikaya (2008)

*All values reflect the mean values of scores given by panellists (n ¼ 5) ± standard deviation.

[a–d] Values in the same row, followed by different letters, are significantly different ($P < 0.01$).

[x–z] Values in the same column, followed by different letters, are significantly different ($P < 0.01$).

A photograph of Pacific white shrimp treated without and with 0.25% and 0.5% lead seed extract powder (LSEP) and those treated with 1.25% SMS after 12 days of iced storage is represented in Fig. 1. In general, similar melanosis score was found between samples treated with 1.25% SMS and 0.25% LSEP throughout the storage time of 12 days in ice ($P > 0.05$). Overall, shrimp treated with 0.5% LSEP had a lowered melanosis formation, compared to other treatments after 12 days of iced storage ($P < 0.05$). Echeverria et al. (2002) reported that *Leucaena leucocephala c*ontains mimosine and it is an analogue of tyrosine. Therefore, treatment of shrimp with LSEP could retard the melanosis formation of shrimp.

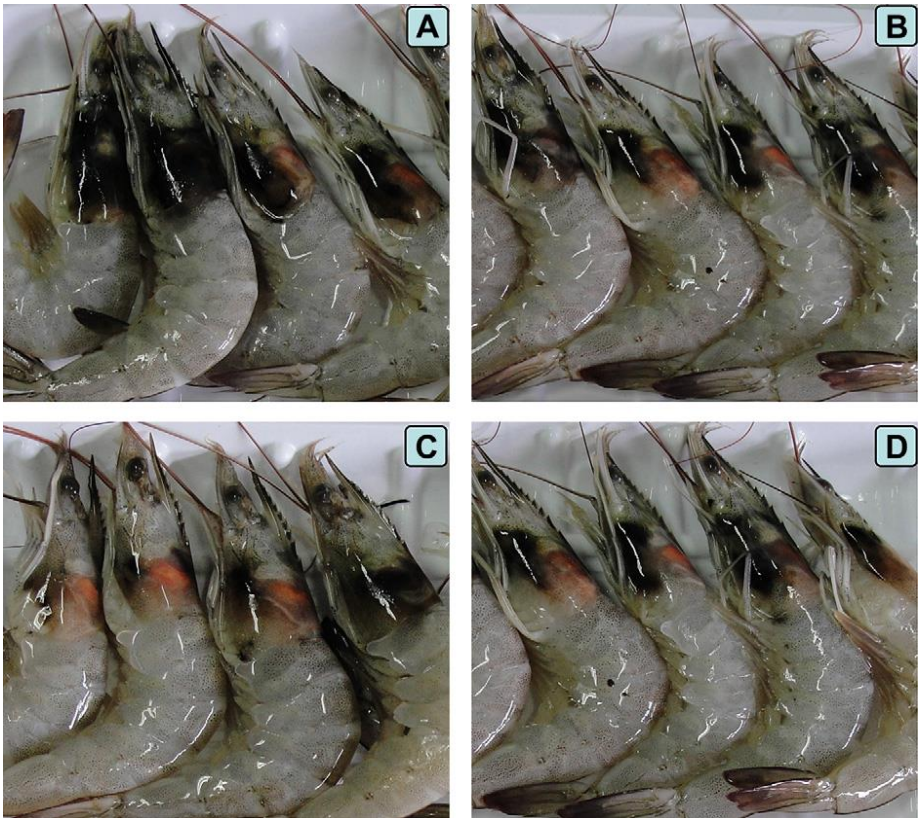


Source: Nirmal and Benjakul (2011a)

Fig. 1. Photographs of Pacific white shrimp without and with treatment of LSEP at different concentrations after 12 days of iced storage.

A Photograph of melanosis formation of Pacific white shrimp without and with different treatments at day 12 of iced storage is shown in Fig. 2. Green tea extract at a level of 10 g L^{-1} was less effective in controlling the formation of melanosis in Pacific white shrimp, as compared to the extract at 5 g L^{-1}, during iced storage. Soysal (2008) reported that the green tea extract at 15 g L^{-1} was effectively retarded the browning of apple slices. This result reveals that green tea

extract at the level of 5 g L^{-1} had the inhibitory effect on melanosis formation of Pacific white shrimp during iced storage.



Source: Nirmal and Benjakul (2011b)

Fig. 2. Photographs of Pacific white shrimp treated without and with green tea extract after 12 days of iced storage. A: Control; B: 12.5 g L^{-1} sodium metabisulphite; C: 5 g L^{-1} green tea extract; D: 10 g L^{-1} green tea extract.

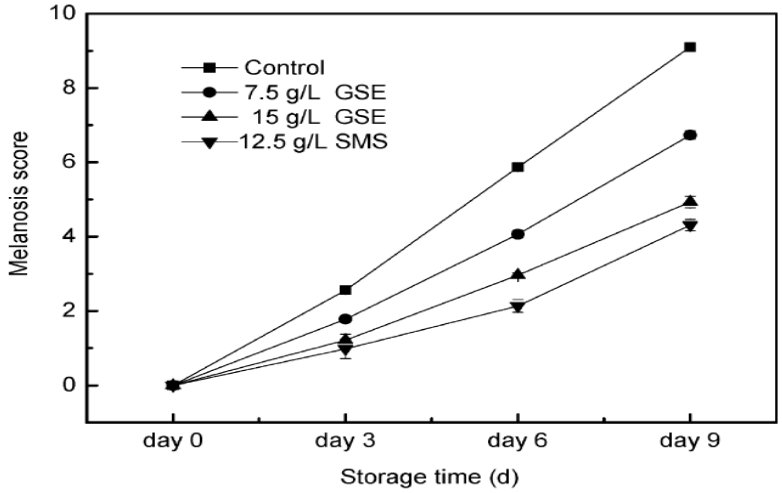
Melanosis score of Pacific white shrimp soaked with 0.1% green tea extract (GTE) without and with (0.005% and 0.01%) Ascorbic Acid (AA) for different times during iced storage of 12 days is represented in table 3. The increase in melanosis score was lowered in shrimp treated with GTE or GTE + AA in comparison with the control and SMS treated samples. Phenolic compound could act as reducing agent, metal chelator, or oxygen scavenger, which inactive PPO, thereby retarding the melanosis formation. AA could provide hydrogen molecule to catechin or could directly involve in the reduction of quinone to hydroquinone. (Nirmal and Benjakul, 2010)

Table 3. Melanosis score of Pacific white shrimp treated with 0.1% GTE or 0.1% GTE combined with 0.005% or 0.01%) AA during 12 days of iced storage

Immersion time (min)	Treatment	Storage time (days)						
		0	2	4	6	8	10	12
	Control	0 ± 0.0^{aG}	1.6 ± 0.3^{aF}	2.8 ± 0.3^{aE}	4.3 ± 0.6^{aD}	6.6 ± 0.6^{aC}	8.3 ± 0.5^{aB}	10 ± 0.0^{aA}
5	0.1% GTE	0 ± 0.0^{aG}	1 ± 0.0^{bF}	1.6 ± 0.2^{bE}	2.6 ± 0.3^{bD}	4 ± 0.0^{bcC}	5.6 ± 0.6^{bB}	6.6 ± 0.6^{bA}
	0.1% GTE+0.005% AA	0 ± 0.0^{aF}	0.8 ± 0.3^{bE}	1 ± 0.0^{cE}	2 ± 0.0^{cdD}	3.3 ± 0.3^{cdeC}	4.6 ± 0.5^{cdeB}	6 ± 0.0^{bcdA}
	0.1% GTE+0.01% AA	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.5 ± 0.0^{cdE}	2 ± 0.0^{cdD}	3.1 ± 0.3^{deC}	4.6 ± 0.6^{cdeB}	6 ± 0.0^{bcdA}
15	0.1% GTE	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.6 ± 0.3^{cdE}	2.3 ± 0.6^{bcD}	4 ± 0.0^{bcC}	5.5 ± 0.5^{bcB}	6.5 ± 0.5^{bcA}
	0.1% GTE+0.005% AA	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.6 ± 0.3^{cdE}	1.6 ± 0.3^{dD}	2.6 ± 0.5^{eC}	4.5 ± 0.5^{deB}	5.3 ± 0.6^{deA}
	0.1% GTE+0.01% AA	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.6 ± 0.4^{cdE}	1.3 ± 0.3^{dD}	2.8 ± 0.3^{eC}	4.3 ± 0.3^{eB}	5 ± 0.0^{eA}
30	0.1% GTE	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.6 ± 0.5^{cdE}	1.6 ± 0.3^{dD}	3.6 ± 0.6^{bcdC}	5.3 ± 0.3^{bcdB}	6.5 ± 0.5^{bcA}
	0.1% GTE+0.005% AA	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.5 ± 0.0^{cdE}	1.3 ± 0.3^{dD}	3.3 ± 0.2^{cdeC}	4.6 ± 0.3^{cdeB}	5.6 ± 0.3^{deA}
	0.1% GTE+0.01% AA	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.6 ± 0.3^{cdE}	1.3 ± 0.6^{dD}	3 ± 0.0^{deC}	5.6 ± 0.6^{bB}	5.3 ± 0.3^{deA}
60	0.1% GTE	0 ± 0.0^{aF}	0 ± 0.0^{cF}	1 ± 0.0^{cE}	2.6 ± 0.3^{bD}	4.3 ± 0.3^{bC}	5.3 ± 0.6^{bcdB}	6.6 ± 0.6^{bA}
	0.1% GTE+0.005% AA	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.3 ± 0.3^{eE}	1.3 ± 0.3^{dD}	3.3 ± 0.3^{cdeC}	5 ± 0.0^{bcdeB}	6 ± 0.0^{bcdA}
	0.1% GTE+0.01% AA	0 ± 0.0^{aF}	0 ± 0.0^{cF}	0.5 ± 0.0^{cdE}	1.5 ± 0.0^{dD}	3.6 ± 0.6^{bcdC}	5 ± 0.3^{bcdeB}	5.8 ± 0.3^{cdA}

Source : Nirmal and Benjakul (2012)

The melanosis score of Pacific white shrimp treated with 7.5 g/L of grape seed extract (GSE), 15 g/L of GSE, 12.5 g/L SMS and control treatment are shown in Fig.3. The score of melanosis of Pacific white shrimp treated by 15 g/L of GSE was less than that treated by 7.5 g/L of GSE. Gokoglu and Yerlikaya (2008) revealed that GSE could delay the formation of melanosis of *Parapenaeus longirostris* during ice storage for 3 days.

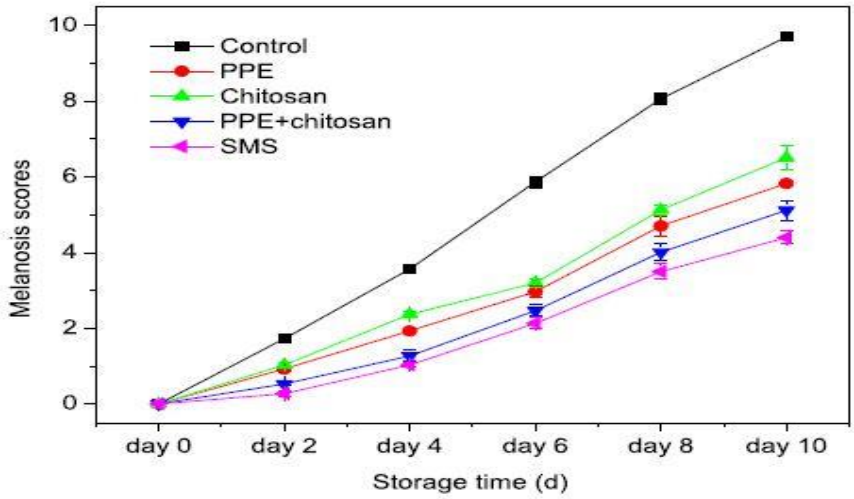


Source: Sun et al. (2014)

Fig. 3. Effect of grape seed extracts on melanosis score of Pacific white shrimp during iced storage.

Phenolic compounds seems to inhibit the PPO of shrimp and be a good substitute for sulphiting agent for retarding melanosis in crustaceans (Fang et al., 2013). So, it may be possible that GSE which is rich in polyphenol could also inhibit the melanosis formation of Pacific white shrimp by inhibition of the PPO activity.

The melanosis scores of Pacific white shrimp treated with pomegranate peel extract (PPE), chitosan, chitosan combined with PPE extract, SMS and control treatment are shown in Fig. 4. The score of melanosis of Pacific white shrimp treated by chitosan coating and PPE was less than those treated by PPE or chitosan coating alone. Basiri et al. (2015) observed that PPE-treated shrimp obtained lower melanosis score during 6 days of storage under refrigeration. It can be stated that PPE had inhibition effect on the melanosis of Pacific white shrimp.

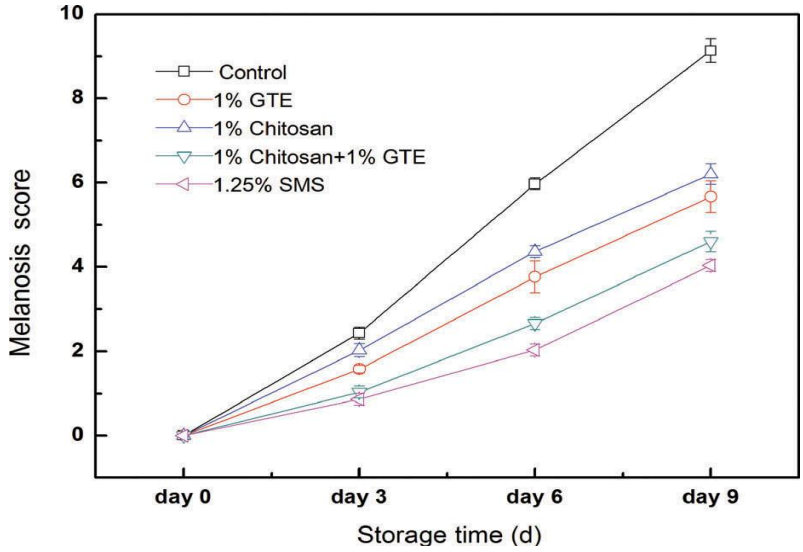


Source: Yuan et al. (2016a)

Fig. 4. Combined effect of chitosan and pomegranate peel extract on melanosis scores of Pacific white shrimp during iced storage

Combined effect of chitosan and green tea extract (GTE) on melanosis score of Pacific white shrimp during storage in ice for 9 days is shown in Fig. 5. The melanosis formation in Pacific white shrimp treated by chitosan coating, GTE, and GTE + chitosan coating was significantly inhibited ($p < 0.05$), compared with the control. GTE had an inhibition effect on the formation of melanosis of Pacific white shrimp. Phenolic compounds in the GTE could significantly inhibit polyphenoloxidase from cephalothorax of Pacific white shrimp (Nirmal & Benjakul, 2011b). Additionally, GTE + chitosan coating showed more effectiveness than chitosan coating or GTE

alone in retarding of melanosis in shrimp which suggests that there was a synergistic effect between chitosan coating and GTE.



Source: Yuan et al. (2016b)

Fig. 5. Combined effect of chitosan and green tea extract on melanosis score of Pacific white shrimp during storage in ice.

Sensory Analysis:

Sensory score for Pacific white shrimp treated with 0.1% GTE or 0.1% GTE + 0.005% or 0.01% AA is shown in Table 4. Shrimp treated with GTE + AA showed the higher sensory score in comparison with other treatments ($P<0.05$). Nirmal and Benjakul (2009) reported that shrimp treated with 2% ferulic acid had higher score for color, taste, and flavor likeness after 10 days of iced storage, compared with the control and those treated with 1.25% sodium metabisulfite. So, the treatment of Pacific white shrimp using GTE + AA yielded the shrimp with the higher sensory property during ice storage.

The changes in sensory quality of Pacific white shrimp treated by GSE and SMS in comparison with the control are shown in Fig. 6. The decrease of sensory score in Pacific white shrimp treated by GSE and SMS were drastically inhibited, compared with the control and the decrease rate of sensory scores in Pacific white shrimp treated by 15 g/L of GSE was significantly less than that treated by 7.5 g/L of GSE. The sensory score of Pacific white shrimp without treatment was 5.4 at the 6th day of iced storage, which reached unacceptable level according to the Chinese National Standard (GB2741-94) (Chinese National Standard, 1994).

Table 4. Sensory score of Pacific white shrimp treated with 0.1% GTE or 0.1% GTE + AA (0.005% or 0.01%) before and after 12 days of iced storage

Storage time (days)	Treatments	Color	Odor	Taste	Flavor	Overall
0	Control	7.4 ± 1.34^{aA}	7.3 ± 0.74^{aA}	7.5 ± 1.16^{aA}	7.4 ± 1.08^{aA}	7.7 ± 0.57^{aA}
	1.25% SMS	7.3 ± 0.84^{aA}	7.4 ± 0.87^{aA}	7.3 ± 0.99^{aA}	7.3 ± 1.09^{aA}	7.6 ± 1.01^{aA}
	0.1% GTE	7.2 ± 1.31^{aA}	7.5 ± 0.64^{aA}	7.8 ± 0.86^{aA}	7.4 ± 0.85^{aA}	7.6 ± 0.75^{aA}
	0.1% GTE+0.005% AA	7.5 ± 0.85^{aA}	7.5 ± 0.63^{aA}	7.6 ± 1.15^{aA}	7.6 ± 1.01^{aA}	8.0 ± 0.55^{aA}
	0.1% GTE+0.01% AA	7.5 ± 1.22^{aA}	7.6 ± 0.49^{aA}	7.7 ± 0.61^{aA}	7.4 ± 0.85^{aA}	8.0 ± 0.55^{aA}
12	Cont.	4.9 ± 0.82^{cB}	5.5 ± 0.51^{cB}	5.3 ± 0.92^{cB}	5.2 ± 0.89^{bB}	5.5 ± 0.65^{cB}
	1.25% SMS	5.4 ± 0.51^{cB}	6.1 ± 0.77^{bB}	5.6 ± 0.75^{bcB}	5.5 ± 0.85^{bB}	5.5 ± 0.65^{cB}
	0.1% GTE	6.4 ± 0.51^{bB}	6.5 ± 0.51^{bB}	6.0 ± 0.91^{bB}	5.8 ± 0.69^{bB}	6.2 ± 0.72^{bB}
	0.1% GTE+0.005% AA	6.7 ± 0.46^{abB}	6.9 ± 0.47^{abB}	6.6 ± 0.74^{aB}	6.6 ± 0.84^{aB}	6.8 ± 0.69^{aB}
	0.1% GTE+0.01% AA	7.1 ± 0.66^{aA}	7.1 ± 0.66^{aA}	6.6 ± 0.75^{aB}	6.6 ± 0.84^{aB}	6.9 ± 0.82^{aB}

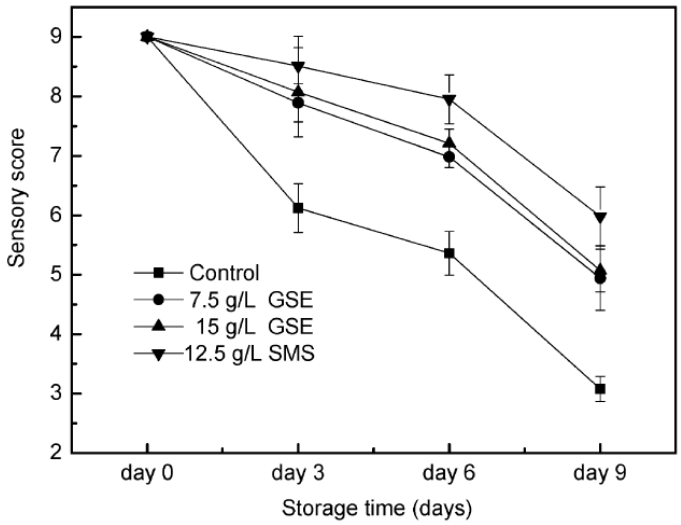
Source: Nirmal and Benjakul (2012)

Different capital letters in the same column within the same treatment indicate the significant differences ($p<0.05$)

The different letters in the same column within the same storage time indicate significant differences ($p<0.05$)

Values are mean ± standard deviation (n=3)

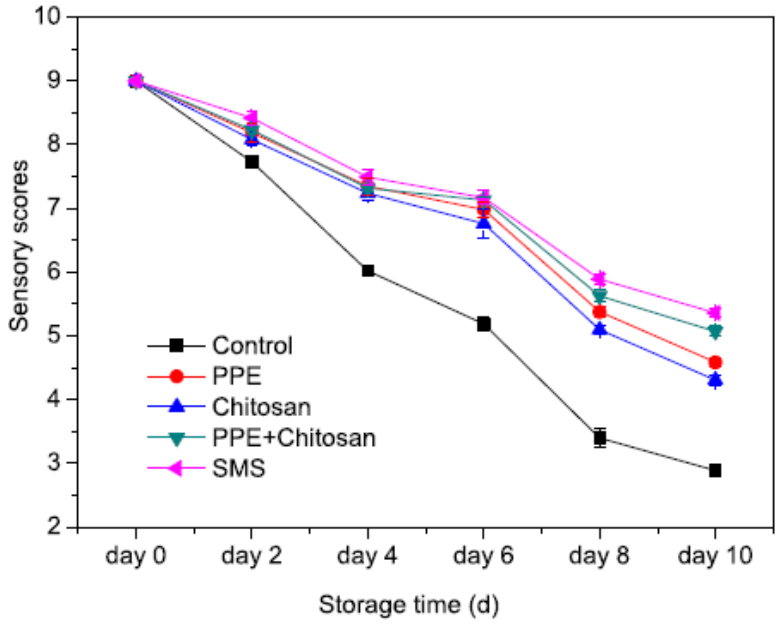
In contrast the sensory score of Pacific white shrimp treated by 15 g/L of GSE reached to 5.1 at the 9th day of iced storage. These results suggest that the treatment with GSE could develop the sensory properties of Pacific white shrimp.



Source: Sun et al. (2014)

Fig. 6. Effect of grape seed extracts on sensory score of Pacific white shrimp during iced storage.

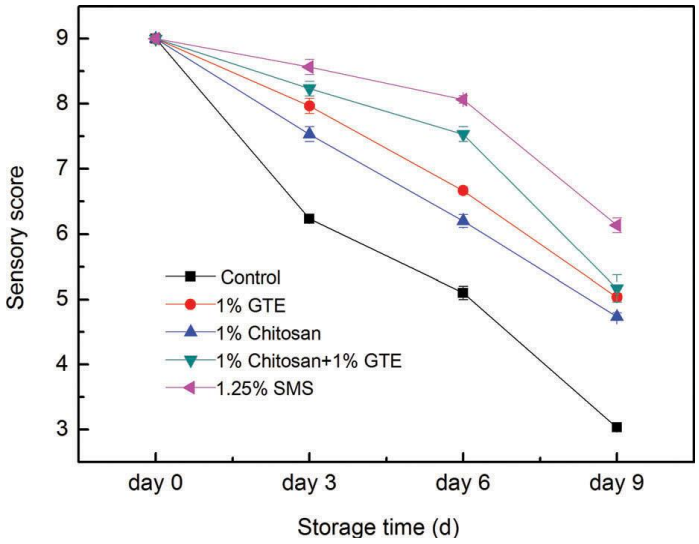
The combined effect of chitosan and pomegranate peel extract (PPE) on sensory scores of Pacific white shrimp during iced storage is shown in Fig. 7. In general, the sensory scores of shrimp shows a tendency to decrease during iced storage. The sensory score of control shrimp was 5.19 at the 6th day of iced storage, which reached unacceptable level according to the Chinese National Standard (GB2741-94) (Chinese National Standard, 1994). The decrease of sensory scores in shrimp treated by PPE, chitosan coating, chitosan coating + PPE were significantly lower than that of the control shrimp. These results recommend that treatment with chitosan coating + PPE could advance the sensory properties of shrimp.



Source: Yuan et al. (2016a)

Fig. 7. Combined effect of chitosan and pomegranate peel extract on sensory scores of Pacific white shrimp during iced storage

The sensory score of Pacific white shrimp (*Litopenaeus vannamei*) treated with green tea extract (GTE), chitosan coating, chitosan coating combined with GTE, sodium metabisulfite (SMS) and control treatment during storage of ice for 9 days is shown in Fig.8. The decrease rate of sensory score in shrimp treated by GTE + chitosan coating was significantly lower than that treated by GTE or chitosan coating alone ($p < 0.05$). These results suggest that treatment with GTE + chitosan coating could improve the sensory properties of shrimp.

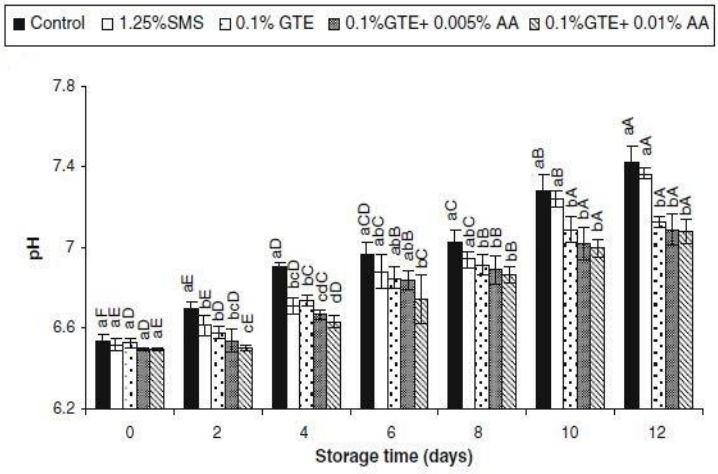


Source: Yuan et al. (2016b)

Fig. 8. Combined effect of chitosan and green tea extract on sensory score of Pacific white shrimp during storage in ice

pH Analysis:

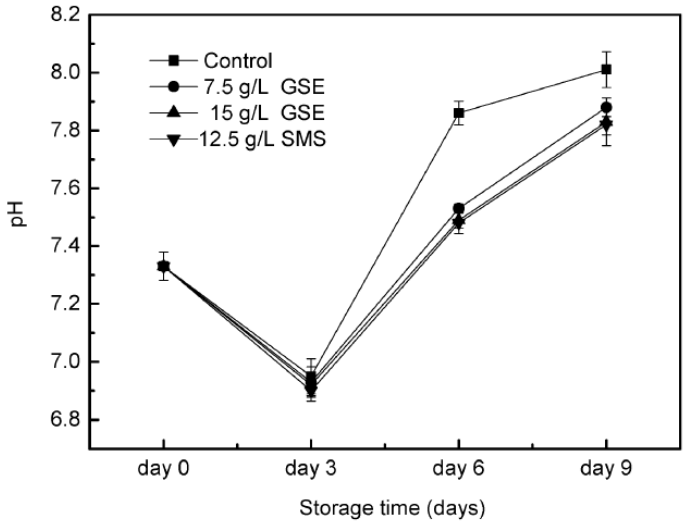
The changes in pH of Pacific white shrimp treated with GTE alone or GTE + AA during iced storage is depicted in Fig. 9. The pH change in shrimp was probably due to the accumulation of basic compounds caused by the activity of bacteria or enzymatic actions (López- Caballero et al., 2007).



Source: Nirmal and Benjakul (2012)

Fig. 9. pH of Pacific white shrimp treated with 0.1% GTE or 0.1% GTE + AA (0.005% or 0.01%) during 12 days of iced storage. Different capital letters on the bars within the same treatment indicate the significant differences ($P<0.05$). The different letters on the bars within the same storage time indicate significant differences ($P<0.05$).

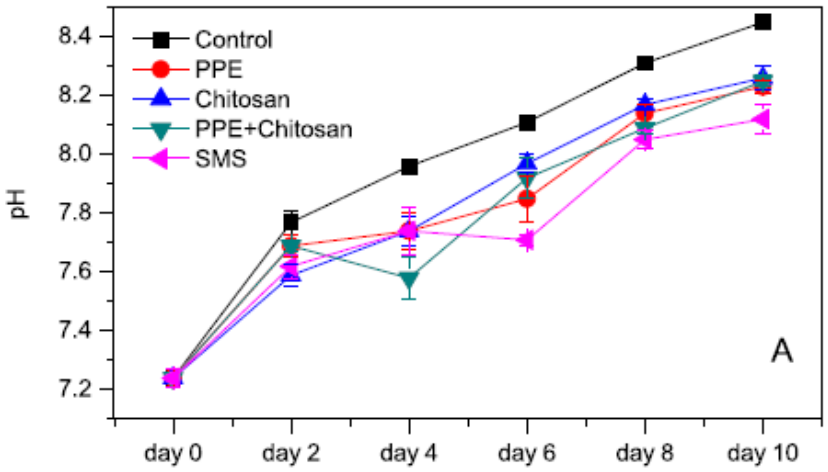
The increase in pH value was lowered in shrimp treated with GTE and GTE + AA during iced storage ($P<0.05$). Shrimp treated with SMS showed the higher pH value, compared with those treated with GTE or GTE + AA. It suggests that GTE+ chitosan shows higher quality than SMS. The changes in pH of Pacific white shrimp treated by GSE and SMS, compared with the control is showed in Fig. 10. The increase of pH was lower in the GSE treated group than control group for all the sampling days. The rate of increase of pH treated by 15 g/L of GSE was less than that treated by 7.5 g/L of GSE. Shamshad et al. (1990) reported that shrimp (*Penaeus merguiensis*) was not acceptable when the pH was greater than 7.6. It suggests that GSE might play a role in retarding quality loss of shrimp, in which the spoilage or decomposition could be lowered.



Source: Sun et al. (2014)

Fig. 10. Effect of grape seed extracts on pH of Pacific white shrimp during iced storage

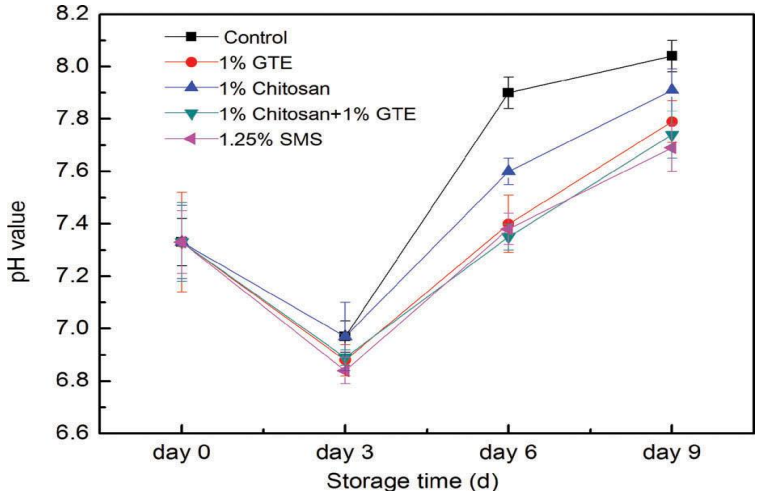
The combined effect of chitosan and PPE on pH of Pacific white shrimp during iced storage is depicted in Fig. 11. The increase of pH in shrimp was significantly inhibited by chitosan coating + PPE or chitosan coating alone. These results recommend that chitosan coating + PPE might play a role in reducing quality loss of shrimp.



Source: Yuan et al. (2016a)

Fig. 11. Combined effect of chitosan and pomegranate peel extract on pH of Pacific white shrimp during iced storage.

The combined effect of chitosan and green tea extract on pH of Pacific white shrimp during storage in ice for 9 days is shown in Fig. 12. The rate of pH rise varied with different treatments. The rise of pH in Pacific white shrimp treated by GTE, chitosan coating, GTE + chitosan coating was considerably lower than the control shrimp ($p < 0.05$). It suggest that GTE + chitosan coating might contribute in reducing quality loss of shrimp.

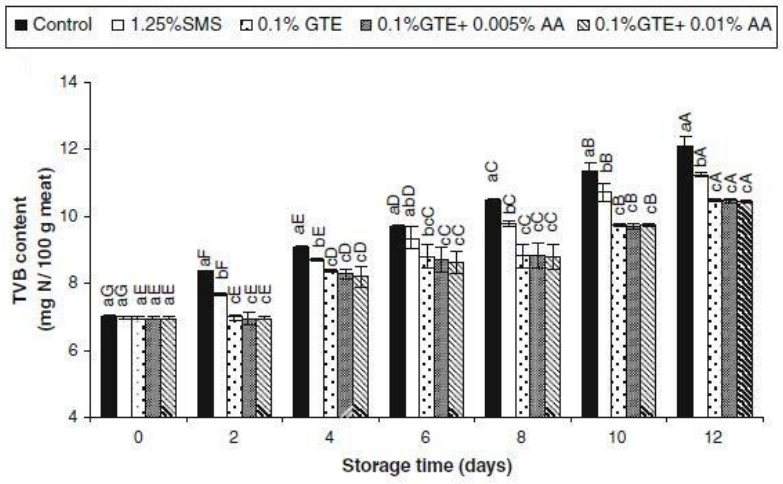


Source: Yuan et al. (2016b)

Fig. 12. Combined effect of chitosan and green tea extract on pH of Pacific white shrimp during storage in ice

TVB-N Analysis:

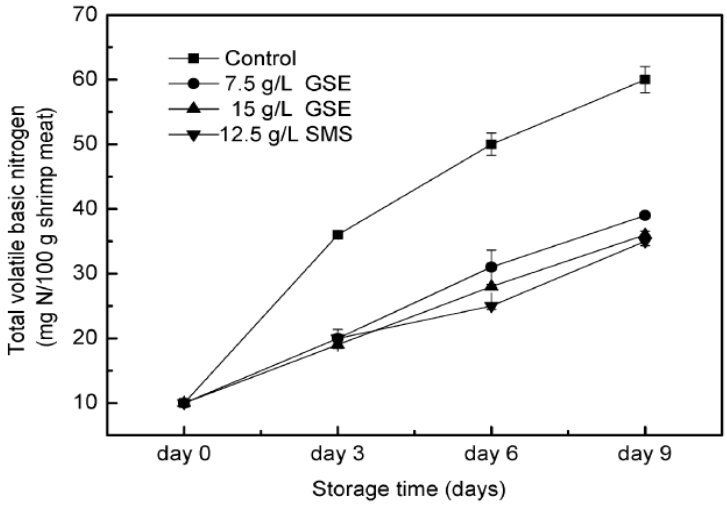
Total volatile basic nitrogen (TVB-N) is a common and important indicator of the quality of seafood because the rise of TVB-N value in seafood is related to microbial and chemical spoilage. The total volatile base content of Pacific white shrimp treated with 0.1% GTE or 0.1% GTE + AA (0.005% or 0.01%) during 12 days of iced storage is showed in Fig. 13. The increase in TVB content was lower in the shrimp treated with GTE or GTE + AA in comparison with the control and those treated with SMS throughout the storage of 12 days ($P<0.05$). The result recommends that GTE had antimicrobial effect in shrimp during iced storage



Source: Nirmal and Benjakul (2012)

Fig. 13. Total volatile base content (b) of Pacific white shrimp treated with 0.1% GTE or 0.1% GTE + AA (0.005% or 0.01%) during 12 days of iced storage. Different capital letters on the bars within the same treatment indicate the significant differences ($P<0.05$). The different letters on the bars within the same storage time indicate significant differences ($P<0.05$).

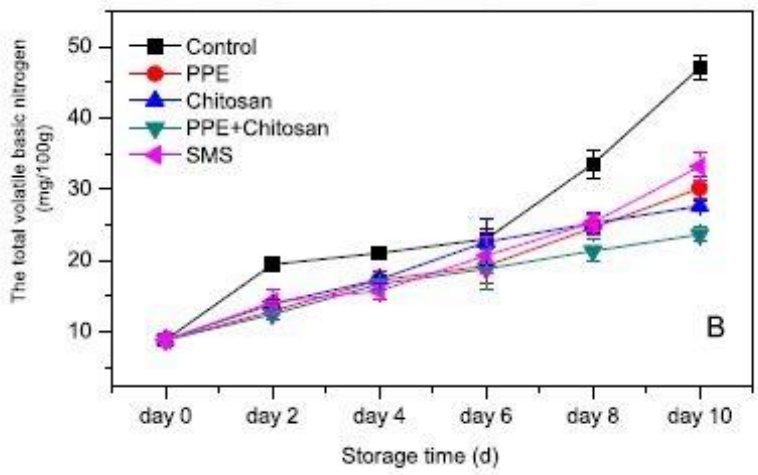
The TVB-N value of Pacific white shrimp treated by GSE and SMS, compared with the control is depicted in Fig. 14. The increase in TVB-N value was highest in the control group for all the sampling days and was significantly inhibited after treatment with SMS and GSE. On the other hand, the rise rate of TVB-N value of Pacific white shrimp treated by 15 g/L of GSE was less than that treated by 7.5 g/L of GSE. According to the Chinese National shrimp sanitary standard, the TVB-N value of fresh shrimp should be < 300 mg/100 g (GB2741-94) (Chinese National Standard, 1994). The TVB-N value of shrimp treated by 15 g/L GSE was 36.7 mg/100 g at the 9th day of iced storage. So, it can be said that shrimp quality can be better if it is treated with GSE extract.



Source: Sun et al. (2014)

Fig. 14. Effect of grape seed extracts on the total volatile basic nitrogen content of Pacific white shrimp during iced storage.

The combined effect of chitosan and PPE on the total volatile basic nitrogen Content of Pacific white shrimp during iced storage the changes in TVB-N values of Pacific white shrimp is depicted in Fig. 15. The TVB-N values in shrimp treated by chitosan coating + PPE were significantly less than those treated by PPE or chitosan coating alone at the 8th and 10th day of storage ($p < 0.05$). According to the Chinese National standard (GB2741-94), the TVB-N value of fresh shrimp should be < 300 mg/100 g. (Chinese National Standard, 1994).

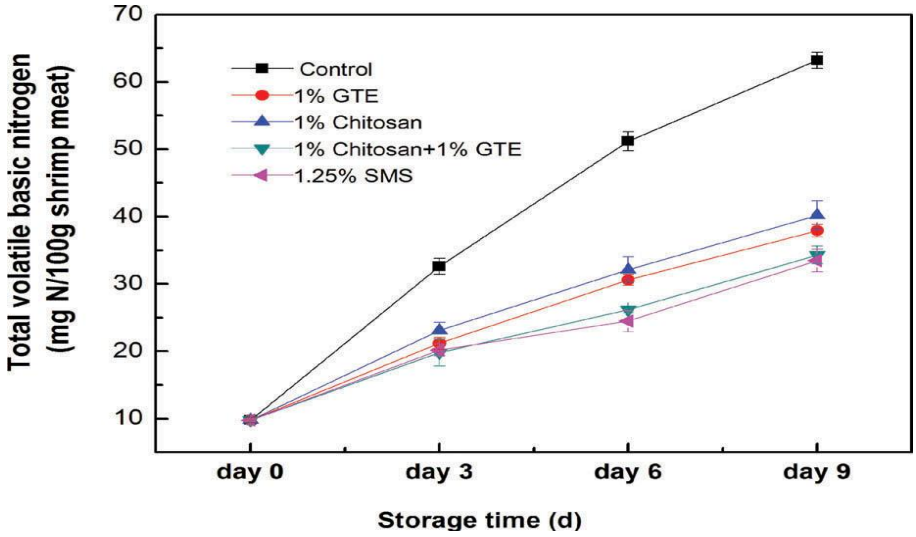


Source: Yuan et al. (2016a).

Fig. 15. Combined effect of chitosan and pomegranate peel extract on the total volatile basic nitrogen Content of Pacific white shrimp during iced storage

However, the TVB-N value of shrimp treated by was 23.66 mg/100 g at the end of iced storage (10th day). So, it can be said that shrimp quality can be better if it is treated with chitosan coating + PPE.

The changes of values of Pacific white shrimp treated by GTE, chitosan coating, GTE + chitosan coating in comparison with the control is showed in Fig. 16. The rise rate of TVB-N values in Pacific white shrimp varied with different treatments, which was highest in the control shrimp for all the sampling days. The rise of TVB-N values in Pacific white shrimp treated by GTE, chitosan coating, GTE + chitosan coating was notably inhibited, compared with the control and that treated by GTE + chitosan coating was significantly less than those treated by GTE or chitosan coating alone ($p < 0.05$).



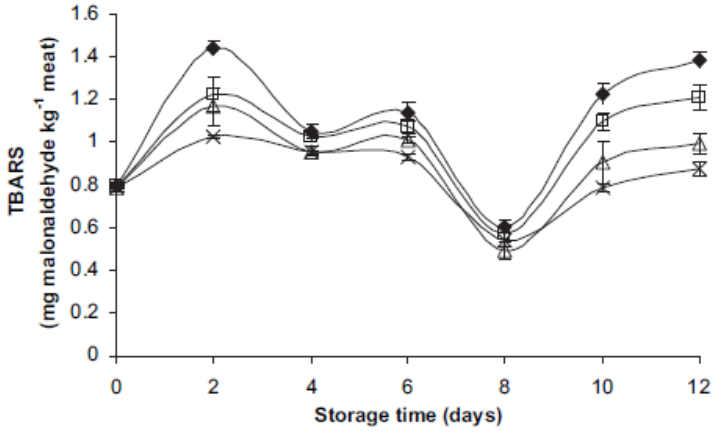
Source: Yuan et al. (2016b).

Fig. 16. Combined effect of chitosan and green tea extract on the total volatile basic nitrogen content of Pacific white shrimp during storage in ice.

TBARS Analysis:

Thiobarbituric acid reactive substances (TBARS) value of Pacific white shrimp treated without and with green tea extract during iced storage is shown in Fig. 17. Higher TBARS value was observed in the control, compared with shrimp treated with green tea extract and those treated with SMS during iced storage of 12 days ($P < 0.05$). At day 12 of storage, shrimp treated with 10 gL^{-1} green tea extract showed the lowest TBARS values ($P < 0.05$), followed by those treated with 5 gL^{-1} extract and SMS, respectively. Overall, shrimp treated with green tea extract at both levels, had the lowered lipid oxidation, especially after 8 days of storage, compared to shrimp

treated with SMS and the control ($P < 0.05$). Tang et al. (2001) reported that he addition of green tea extract retarded the oxidation in mackerel patties during refrigerated and illuminated storage. So, shrimp treated with GTE will provide low TBARS value.



Source: Nirmal and Benjakul (2011b)

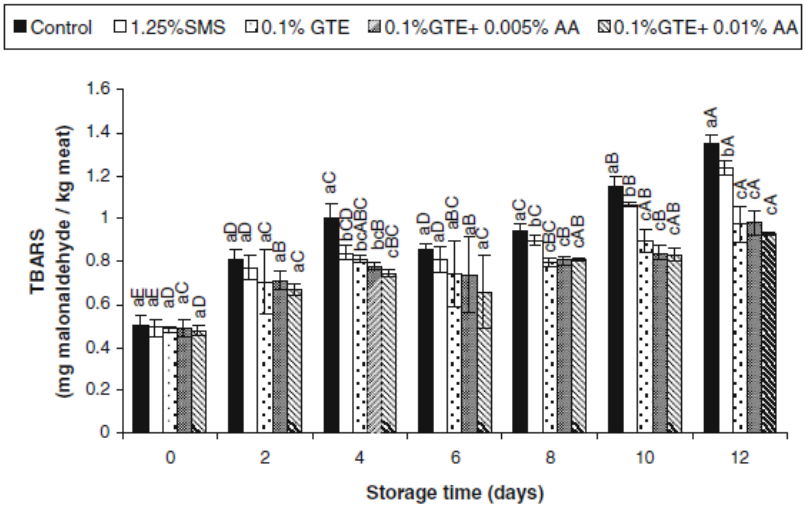
Fig. 17. TBARS of Pacific white shrimp treated with ethanolic green tea extract at different levels during 12 days of iced storage.

Thiobarbituric acid reactive substances (TBARS) value of Pacific white shrimp without and with treatment of GTE or GTE + AA during iced storage is shown in Fig. 18. TBARS value of all samples increased with increasing storage time ($P<0.05$). Shrimp treated with GTE depicted the lower TBARS during 4–12 days, compared with the control and those treated with SMS ($P<0.05$). From the result, AA had no combination effect on antioxidative activity of GTE, regardless of concentrations used. Majchrzak et al. (2004) reported that incorporation of AA up to 30 mg into 100 mL of green tea extract solution showed the linear increase in total antioxidant activity of green tea extract. So, lipid oxidation in shrimp treated with GTE could be prevented to some degree without the incorporation of AA during extended storage.

Microbiological Analysis:

The effect of GSE on total aerobic plate count (TPC) of Pacific white shrimp during iced storage is depicted in Fig.19. The raise of total aerobic plate count of Pacific white shrimp treated by SMS and GSE was significantly restrained, compared with the control. Several studies have proved that grape seed extracts by different solvents possessed antimicrobial activity. Jayaprakasha et al. (2003) observed defatted grape seed powder extracts prepared by

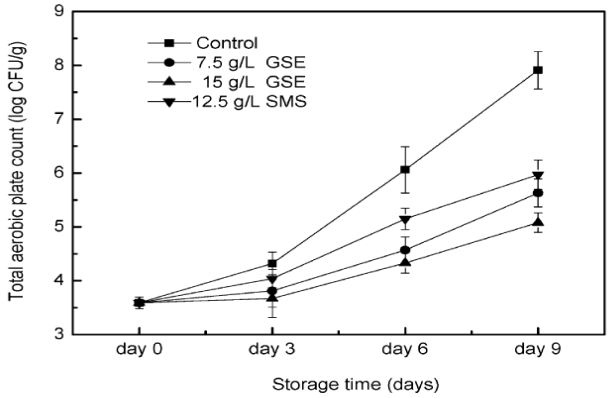
acetone:water:acetic acid (90:9.5:0.5) and methanol:water:acetic acid (90:9.5:0.5) could completely inhibited gram-positive bacteria at 850 _ 1000 ppm and gram-negative bacteria at 1250 _ 1500 ppm concentration.



Source: Nirmal and Benjakul (2012)

Fig. 18. TBARS of Pacific white shrimp treated with 0.1% GTE or 0.1% GTE + AA (0.005% or 0.01%) during 12 days of iced storage. Different capital letters on the bars within the same treatment indicate the significant differences (P<0.05). The different letters on the bars within the same storage time indicate significant differences (P<0.05).

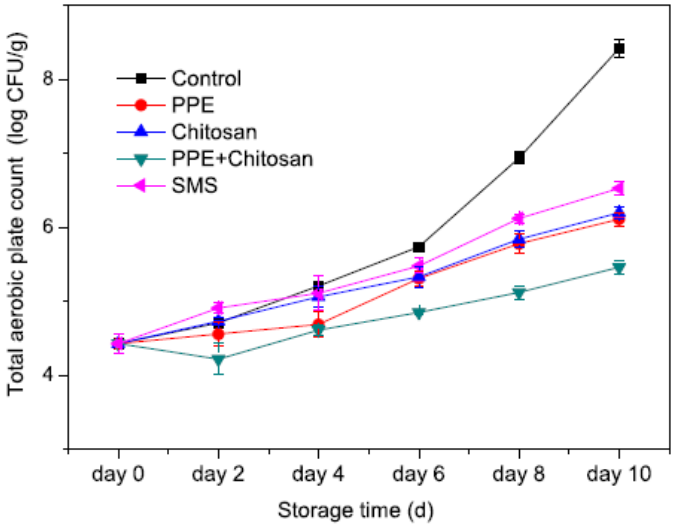
Adamez et al. (2012) found that grape seeds aqueous extracts had antimicrobial activity and that the inhibitory effect of phenolic compounds from seeds extracts was more potent against gram-positive bacteria than gram-negative. Abovementioned result also indicates that GSE could exert the antimicrobial activity in Pacific white shrimps during iced storage.



Source: Sun et al. (2014)

Fig. 19. Effect of grape seed extracts on total aerobic plate count of Pacific white shrimp during iced storage.

The TPC of Pacific white shrimp during iced storage are shown in Fig. 6. The increase in TPC was highest in control shrimp for all the sampling days, whereas that was significantly inhibited in shrimp treated by PPE, chitosan coating, chitosan coating + PPE, respectively. The increase in TPC of shrimp treated by PPE + chitosan was significantly less than that treated by PPE or chitosan coating alone at the 6th, 8th and 10^{th} day of storage ($p < 0.05$). Chitosan coatings can inhibit the growth of aerobic bacteria (Devlieghere et al., 2004). This finding further assured that chitosan coating could inhibit the microbial growth in shrimp. Moreover, the efficacy of chitosan coating to reduce microbial growth was increased, when chitosan coating was used in combination with PPE. The result suggests that chitosan coating showed synergism in antimicrobial activity when used in combination with PPE.

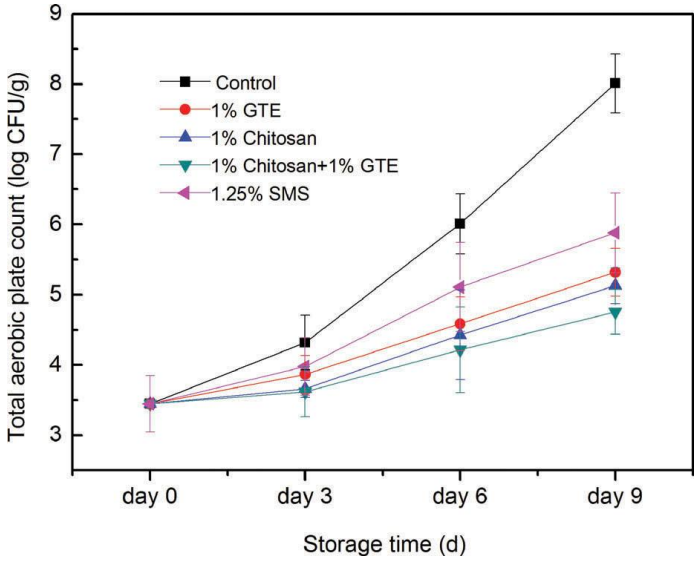


Source: Yuan et al. (2016a).

Fig. 20. Combined effect of chitosan and pomegranate peel extract on total aerobic plate counts of Pacific white shrimp during iced storage.

The combined effect of chitosan and green tea extract on the TPC of Pacific white shrimp during storage in ice is showed in Fig.16. The increase in TPC of Pacific white shrimp treated by GTE, chitosan coating, GTE + chitosan coating was significantly inhibited, compared with the control and that treated by GTE + chitosan coating was significantly less than that treated by GTE or chitosan coating alone ($p < 0.05$). Chitosan coatings can retard the growth of aerobic bacteria (Devlieghere et al., 2004). This finding further assured that chitosan coating could inhibit the microbial growth in shrimp. Moreover, the efficacy of chitosan coating to reduce microbial growth was increased, when chitosan coating was used in combination with GTE. The result

suggests that chitosan coating showed synergism in antimicrobial activity when used in combination with GTE.



Source: Yuan et al. (2016b)

Fig. 21. Effect of chitosan combined with green tea extract on the TPC of Pacific white shrimp during storage in ice.

CHAPTER IV
CONCLUSION

Grape seed extract, lead seed extract powder, pomegranate peel extract, and green tea extract can be used to retard melanosis of shrimp during ice storage. The melanosis was significantly retarded, the sensory scores were significantly improved and the increase in total aerobic plate counts, pH, total volatile basic nitrogen and thiobarbituric acid reactive substances values were significantly inhibited in shrimp during storage in ice for 9-12 days. Finally, we can say that use of natural extracts could be an effective alternative to synthetic antimelanosic agents to inhibit post-mortem melanosis and improve the quality of shrimp during storage in ice.

CHAPTER V

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