

Inhibition of melanosis in white shrimp *Penaeus vannamei* using beetroot extract during iced and room temperature storage

S. J. Laly^{*1}, A. Jeyakumari¹, Abhay Kumar², L. N. Murthy³ and George Ninan¹

¹ICAR-Central Institute of Fisheries Technology, Matsyapuri P. O., Willingdon Island, Kochi - 682 029, Kerala, India

²Mumbai Research Centre of ICAR-Central Institute of Fisheries Technology, Vashi - 400 703, Navi Mumbai, Maharashtra, India

³National Fisheries Development Board, Hyderabad - 500 052, Telangana, India



Abstract

Effect of beetroot extract in inhibition of melanosis formation and quality of white shrimp *Penaeus vannamei* was evaluated in iced and room temperature storage. Shrimps were treated with beetroot extract (BT) at 1 and 4% and compared with control and commercial anti-melanosis agent, 1.25% sodium metabisulfite (SMS) and stored in ice. The effect of BT4% treatment at room temperature was also evaluated. There was no significant difference ($p > 0.05$) in the melanosis score of SMS and BT4% till 7th day of iced storage while BT1% was not effective in retarding the melanosis from 5th day of storage. In room temperature storage, melanosis formation was similar both in the case of SMS and BT4% till 6th hour of storage. Total volatile base nitrogen (TVBN) value and aerobic plate count (APC) of BT treated samples were significantly lower than control and SMS ($p < 0.05$) in both storage conditions and lower thiobarbituric acid reactive substances (TBARS) values indicated the antioxidant activity of beetroot extract. BT4% treatment inhibited growth of H_2S producing organisms (1.3-2.1 log cycles) and *Pseudomonas* spp. (0.3-1 log cycles) compared to control and SMS. BT4% treatment resulted in a storage life of 9 days in iced condition and eight hours at room temperature. Beetroot extract treatment at 4% level was found as effective as sodium metabisulfite in preventing melanosis till 7th day of iced storage and 6th hour of room temperature storage.

Introduction

Shrimps are highly preferred seafood delicacy all over the world due to outstanding taste and nutritional quality. The white shrimp *Penaeus vannamei* contributes to 90% of global aquaculture production (Nirmal and Benjakul, 2009a) and in India, this species is farmed commercially primarily for export. Shrimps are considered highly perishable as the quality deteriorates quickly during storage due to melanosis and microbial spoilage (Gokoglu and Yerlikaya, 2008). The post-mortem melanosis or blackening of shrimp reduces the market value and leads to economic loss. Melanosis is a biochemical process in which phenols are oxidised to quinones by enzymes called polyphenol oxidase (PPO) or tyrosinase which are synthesised in the hepatopancreas of shrimp. The colourless

quinones undergoes non-enzymatic polymerisation and auto-oxidation, giving rise to high molecular weight dark pigments or black spots (Benjakul *et al.*, 2005). These dark pigments, called melanin, form and accumulate mainly beneath the carapace of cephalothorax (Nirmal and Benjakul, 2011a). Although these black spots are harmless in nature, they can affect consumer acceptance and reduce market value. In addition to melanosis, lipid oxidation initiated by autoxidation and reactions mediated by lipoxygenase, peroxidase and microbial enzymes also harm the quality.

In order to prevent melanosis formation, the shrimp industry has been depending upon treatment using sulfite for many years. In addition to sulfite, treatment using an enzyme called 4-hexylresorcinol has also



*Correspondence e-mail:
lalyjawahar@gmail.com

Keywords:

Beetroot extract, Melanosis, Shrimp, Sodium metabisulfite, Storage

Received : 14.06.2022

Accepted : 02.05.2024

been reported (Martinez-Alvarez *et al.*, 2008). Sulphite containing compounds can irreversibly react with quinines and prevent polymerisation and resulting in colourless compounds (Montero *et al.*, 2001). But application of sodium metabisulfite (SMS) at higher levels can lead to the retention of sulfite residue in the meat, which is a known allergen to many consumers. It can also lead to severe disturbances in asthmatic persons (Dewitt, 1998). Labeling of shrimp with sulfite residues greater than 10 mg kg⁻¹ is mandated by the European Union (Edmonds, 2006). Hence many researchers have investigated safe and effective alternatives to tackle the issue of melanosis using natural sources such as grape seed extract (Gokoglu and Yerlikaya, 2008), green tea extract (Nirmal and Benjakul, 2011b), lead seed extract (Nirmal and Benjakul, 2011c), green tea extract with chitosan coating (Yuan *et al.*, 2016) and pomegranate peel extract with other additives (Viji *et al.*, 2018).

Utilisation of phenolic compounds can prevent melanosis in crustaceans (Maqsood *et al.*, 2013). Beetroot is a vegetable with high antioxidant capacity reported with a total phenolic content of 50-60 µmol g⁻¹ dry weight (Vinson *et al.*, 1998; Kahkonen *et al.*, 1999). It is rich in water-soluble nitrogenous pigments, called betalains, composed of the red betacyanins and the yellow betaxanthins which are free radical scavengers and can prevent oxidation of biological molecules (Pedreno and Escribano, 2001). Hence studies were conducted to investigate melanosis inhibition effect of beetroot extract in *Penaeus vannamei* during iced and room temperature storage, as well as the associated changes in biochemical, microbial and sensory characteristics.

Materials and methods

Preparation of beetroot extract and collection of shrimp

Beetroot extract was prepared from fresh beetroot (*Beta vulgaris*) pomaces. The pomaces were peeled, chopped into small pieces and ground using a mixer. It was then extracted using a solvent mixture of 95% ethanol in an aqueous solution containing 0.5% acetic acid. The extraction was carried out at a ratio of 1:1 (ground beetroot to solvent mixture), mixed using a vortex mixer overnight. The liquid portion or the extract was collected by sieving through a muslin cloth. The extract was concentrated by evaporation using a rotary vacuum drier at a temperature of 65°C. The concentrated beetroot extract was dried overnight in a hot air oven at a temperature of 60°C till it became thick. The dried extract was collected and stored at refrigerated condition until use. Dipping solutions of beetroot extract for shrimp treatment was prepared at 4 and 1% using distilled water.

Fresh white shrimp, *Penaeus vannamei* (Average weight 41±2.8 g) was collected from a farm connected to VKM seafoods, Navi Mumbai and transported to the laboratory in iced condition (at 1:1 ratio), in insulated ice boxes.

Beetroot extract treatment during iced storage

The shrimp were deiced and divided into four lots. Lot 1 was taken as control, added with distilled water at a ratio of 1:2 (wt/v) for 1 min. Lot 2 was treated with 1.25% sodium metabisulfite (SMS) dissolved in distilled water at a ratio of 1:2 (w/v) for 1 min at 4°C. Lots 3 and 4 were treated with 4% (BT 4%) and 1% (BT 1%) solution of beetroot

extract respectively at a ratio of 1:1 (w/v) for 15 min at 4°C. After treatment, the shrimps were drained and small lots of shrimps were packed in plastic bags and sealed. These were then packed in ice at a ratio of 1:1 in an insulated box. Samples were drawn on alternative days for further analysis of melanosis score, sensory characteristics, biochemical and microbiology parameters.

Beetroot extract treatment during room temperature storage

Fresh shrimp sample was divided into three lots. Lot 1 was taken as control, added with distilled water at a ratio of 1:2 (wt/v) for 1 min. Lot 2 was treated with 1.25% SMS dissolved in distilled water at a ratio of 1:2 (w/v) for 1 min at 4°C. Lot 3 was treated with 4% solution of beetroot extract at a ratio of 1:1 (w/v) for 15 min at 4°C. After treatment, the shrimps were drained and stored at room temperature. The samples were monitored at an interval of 2 h for melanosis formation and further analysis.

Biochemical analysis

The shrimps were beheaded and peeled to collect the meat for biochemical analysis. The collected meat was homogenised using a mixer grinder. Proximate composition of shrimp meat was analysed as per AOAC method (AOAC, 2005). The samples were subjected to analysis in triplicate. pH of homogenised shrimp meat was evaluated using digital pH meter (EcoScan pH 5, EUTECH Instrument) (AOAC, 2005) after mixing 4 g of sample with 40 ml of distilled water. Total volatile base nitrogen (TVBN) content in shrimp meat was analysed after extraction with 10% trichloroacetic acid (TCA) using the micro diffusion method (Conway, 1950). Oxidative stability of the shrimp meat was determined by evaluating thio barbituric acid reactive substances (TBARS) using spectrophotometer (Cole-Palmer, USA) (Tarladgis *et al.*, 1960).

Microbiological analysis

A sample of 25 g of shrimp meat was collected aseptically and homogenised with 225 ml of phosphate buffer in a stomacher bag using a Stomacher® 400 Lab system (Seward Limited, UK) for 2 min. Serially diluted samples of three consecutive dilutions with 9 ml sterile phosphate buffer (pH 7.2) were plated in plate count agar for mesophilic count in duplicates (AOAC, 2005). H₂S producing bacteria were evaluated using 1 ml of phosphate buffer homogenate by plating on peptone iron agar (HiMedia, Mumbai) as per Ginson *et al.* (2015). *Pseudomonas* bacteria were isolated using *Pseudomonas* agar (PA) base (HiMedia, M085) and tested as per Mead and Adams (1977).

Melanosis assessment

Melanosis assessment of white shrimp was carried out through visual inspection by five experienced evaluators using ten-point scoring as per the method of Montero *et al.* (2001). Ten samples from each lot were selected randomly for assessment. Panelists were asked to give the melanosis score (0 to 10) for shrimp, where 0 = absent; 2 = slight (up to 20% of shrimps' surface affected); 4 = moderate (20 to 40% of shrimps' surface affected); 6 = notable (40 to 60% of shrimps' surface affected); 8 = severe (60 to 80% of

shrimps' surface affected) and 10 = extremely heavy (80 to 100% of shrimps' surface affected).

Sensory analysis

Sensory evaluation of samples was done by five experienced sensory evaluation panel members in the laboratory, separately for raw and cooked shrimp. Sensory scoring was carried out as per the method of Amerine *et al.* (1965) on attributes such as appearance, colour, odour, taste and texture using score ranging from 9 (like extremely) to 1 (dislike extremely). An overall acceptability score was calculated based on individual scoring. An overall score below four was considered rejected.

Statistical analysis

Each of the analyses were carried out in triplicate (n = 3) and the statistical software SPSS 16 (SPSS Inc., Chicago, IL, USA) was used for Analysis of Variance (ANOVA). The statistical significance was determined at 95% confidence level (p<0.05).

Results and discussion

Proximate composition

Analysis of proximate composition of fresh Indian white shrimp showed 78.39±0.34% moisture, 20.45±0.4% protein, 0.31±0.002 fat and 1.18±0.02% ash content. This is similar to the nutrient composition in this species previously reported by Jeyakumari *et al.* (2020).

Melanosis

There was no sign of melanosis in the shrimp samples on the day 0 of iced storage. The beetroot extract treated shrimp was red in colour due to the rich content of betalain pigments of beetroot, which imparted a pinkish colour to the treated shrimp (Fig. 1) and did not have a negative effect on sensory acceptability. During storage, a pronounced appearance of melanosis (p<0.05) was observed in the control shrimp from 3rd day onwards while there were no signs of melanosis in other treatment samples. The melanosis score of control shrimps increased rapidly and resulted in a rapid loss in visual quality which was similar to the previous reports by Nirmal and Benjakul (2011a), Fang *et al.* (2013) and Sun *et al.* (2014). Signs of melanosis started appearing in SMS and beetroot extract treated samples from 5th day of iced storage. There was no significant difference (p>0.05) in the melanosis score of shrimps treated with SMS and beetroot extract at 4% level till 7th day of ice storage. The beetroot extract treatment at lower level or 1% was not effective in retarding the melanosis formation from 5th day of storage. Phenolic compounds in the beetroot extract strongly inhibited polyphenol oxidase from the cephalothorax of white shrimp, reducing quinone formation and there by decreasing melanosis. In the later stages of storage from 9th day onwards, SMS treated samples showed pronounced formation of melanosis. Normally bisulfite inhibit melanosis by its action with intermediate quinone, forming sulfoquinone (Ferrer *et al.*, 1989). Sulfur dioxide formed from SMS can evaporate during extended storage or dissolve in molten ice, resulting in reduced amount of SMS remaining in the sample (Nirmal and Benjakul, 2009b).

The changes in melanosis formation during room temperature storage is shown in Fig. 2. There were no signs of melanosis

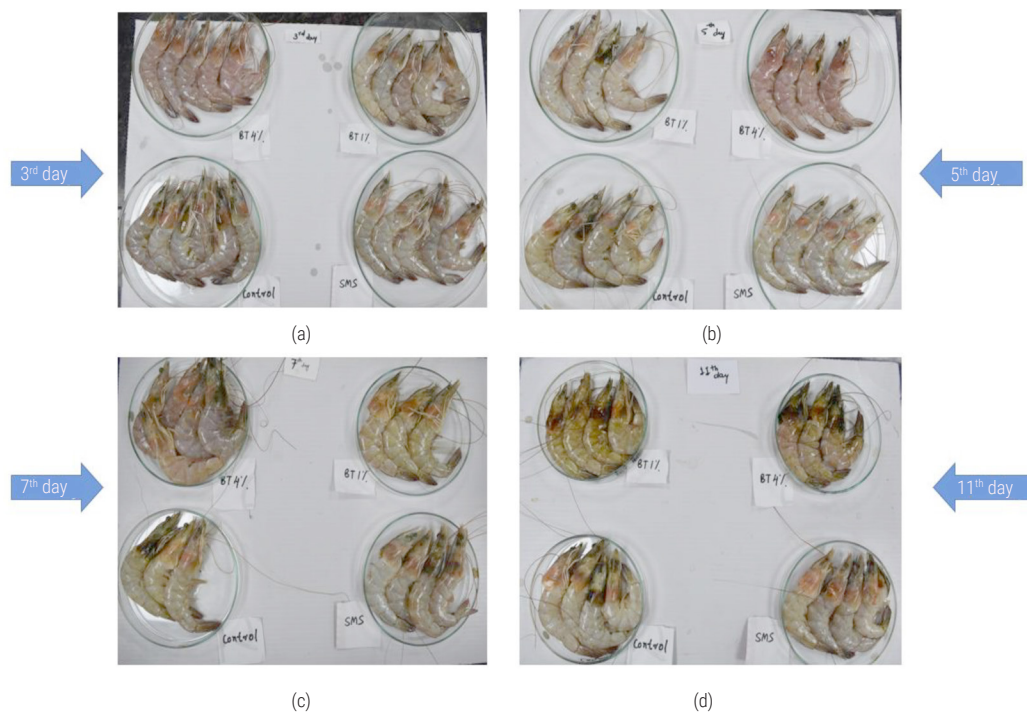


Fig. 1. Treated shrimp samples during iced storage on (a) Day 3; (b) Day 5; (c) Day 7 and (d) Day 11 . Control, SMS – Treated with sodium metabisulfite, BT1% - Beetroot extract treated at 1% level, BT4% - Beetroot extract treated at 4% level

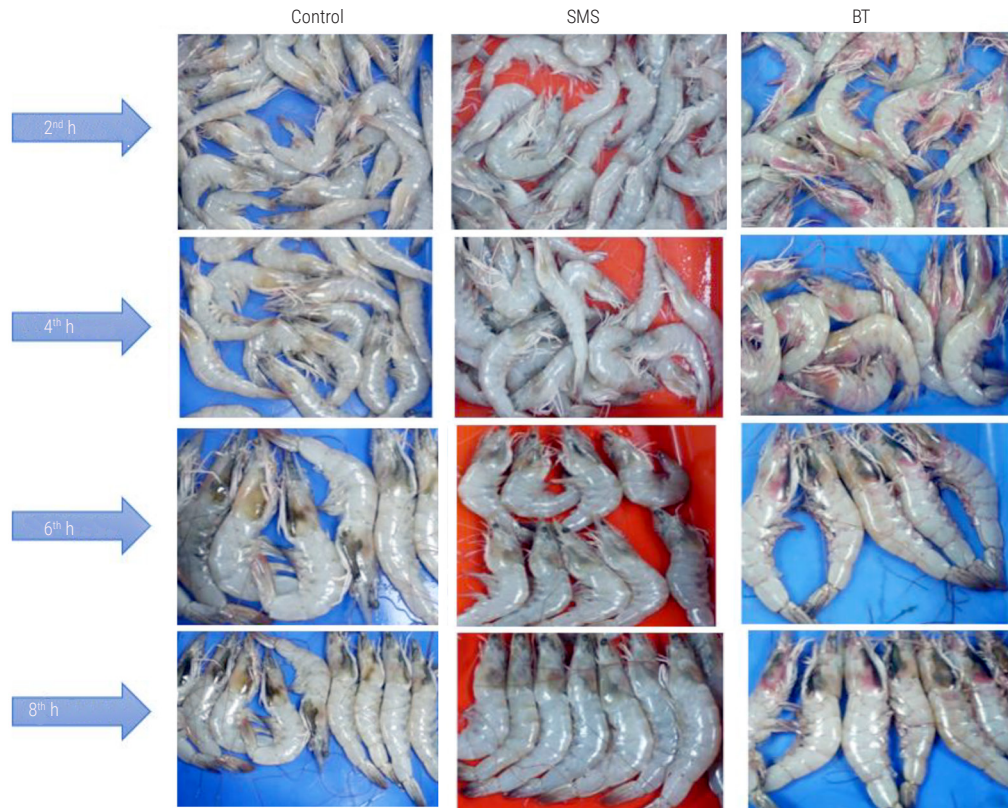


Fig. 2. Treated shrimp samples during room temperature storage. Control, SMS – Treated with sodium metabisulfite, BT - Beetroot extract treated at 4% level

formation in both treatments (SMS and BT 4%) till 4th hour of storage in comparison to control. From 6th hour of storage, formation of melanosis in the cephalothorax regions of shrimp was observed clearly both in the case of SMS and BT 4%. Melanosis formation was more pronounced in the case of beetroot extract treated sample from 8th hour of storage than the SMS treated shrimp. Melanosis developed in the cephalothorax and pleopods of shrimp due to the activity of polyphenol oxidase (PPO) enzyme (Diaz-Tenorio *et al.*, 2007).

The present study indicates the efficacy of beetroot extract containing phenolic compounds in preventing melanosis formation of shrimp during the initial hours of storage similar to that of SMS without any residual effects. Inhibition of melanosis due to interaction of phenolic compounds to the active sites of PPO was reported by Kubo and Kinst-Hori (1998). Manheem *et al.* (2012) reported the effect of precooking on development of melanosis and observed lowering of PPO activity at higher core temperature with higher cooking losses.

Biochemical quality

PH

The change in pH of shrimp samples with or without beetroot extract treatment is given in Table 1. The initial pH of white shrimp on day 0 was 6.51 ± 0.03 . Even though the pH of control and SMS treated shrimp did not show any significant difference ($p > 0.05$),

the pH of beetroot extract treated samples at 4% level showed significant increase ($p < 0.05$) to a pH of 6.71 ± 0.01 . In all the samples, the pH increased during storage and reached 7.5, 7.47, 7.75 and 7.64 respectively. This can be due to the accumulation of basic compounds like ammonia and volatile bases produced by the action of bacteria and enzymes (Lopez Caballero *et al.*, 2007). On each storage day, the pH of control and treatments were significantly different and the pH of SMS treated shrimps was significantly lower than BT4% during initial days of storage from day 0 to day 5. The pH of SMS was observed to be significantly higher during the later days from day 7 to day 9 of storage, in comparison to BT4%. Higher content of non-protein nitrogenous compounds in crustaceans contributes towards the increment of pH during storage (Shahidi, 1994).

The changes in pH of shrimp samples stored at room temperature are presented in Table 2. The initial pH of the samples was in the range of 6.6 to 6.71. Rich content of non-protein nitrogenous compounds contributes towards a higher pH value in the case of crustaceans. In all the samples, the pH increased significantly during storage at room temperature and reached values of 7.0, 6.82 and 7.08 in case of control, SMS and BT4% respectively. The liberation of basic compounds like ammonia and volatile compounds during proteolysis by bacterial enzymes can increase the pH during spoilage of shrimp. pH of control samples reached value of 7.0 by 8th hour of storage. The higher temperature of storage in room condition aggravated the spoilage process through bacterial and enzymic activity.

Table 1. Changes in pH, TVBN and TBARS during iced storage of beetroot extract treated samples along with SMS treated and control shrimps

Parameters	Samples	Storage days					
		0	3	5	7	9	11
pH	Control	6.51±0.03 ^{aA}	6.85±0.02 ^{bB}	7.18±0.0 ^{cC}	7.32±0.04 ^{dA}	7.33±0.02 ^{dA}	7.5±0.01 ^{eA}
	SMS	6.55±0.01 ^{aB}	6.81±0 ^{bA}	7.23±0.01 ^{cD}	7.41±0.01 ^{dB}	7.47±0.03 ^{eC}	7.47±0.01 ^{eB}
	BT1%	6.52±0.01 ^{aA}	6.87±0 ^{bC}	6.9±0.01 ^{cA}	7.6±0.02 ^{dC}	7.46±0.01 ^{eC}	7.75±0.02 ^{fC}
	BT4%	6.71±0.01 ^{aC}	7.08±0.01 ^{aD}	7.08±0.01 ^{aB}	7.32±0.02 ^{bA}	7.36±0.03 ^{cB}	7.64±0.01 ^{dD}
TVBN (mg%)	Control	10.2±0 ^{aA}	25.2±0.3 ^{bD}	30.8±0.2 ^{cB}	39.2±0.2 ^{dC}	40.6±0 ^{eC}	43.4±0 ^{fB}
	SMS	10.2±0 ^{aA}	20.2±0.2 ^{bC}	25.2±0.2 ^{cA}	37.8±0 ^{dB}	39.2±0 ^{eB}	40.6±0 ^{fA}
	BT1%	10.2±0 ^{aA}	19.6±0.2 ^{bB}	25.2±0.1 ^{cA}	36.4±0.3 ^{dA}	39.2±0.2 ^{eB}	43.4±0 ^{fB}
	BT4%	10.2±0 ^{aA}	18.2±0 ^{bA}	25.2±0.2 ^{cA}	36.4±0.3 ^{dA}	36.4±0.2 ^{dA}	40.6±0 ^{eA}
TBARS (mg malonaldehyde kg ⁻¹)	Control	0.03±0 ^{aA}	0.07±0.005 ^{bD}	0.09±0 ^{cD}	0.09±0 ^{cC}	0.09±0 ^{cC}	0.11±0.005 ^{dD}
	SMS	0.03±0 ^{aA}	0.03±0 ^{bA}	0.07±0 ^{dC}	0.07±0.005 ^{eB}	0.06±0.01 ^{cB}	0.07±0 ^{dC}
	BT1%	0.03±0 ^{aA}	0.06±0.01 ^{fC}	0.04±0.005 ^{bB}	0.04±0 ^{eA}	0.04±0.01 ^{dA}	0.04±0.005 ^{cA}
	BT4%	0.03±0 ^{bA}	0.04±0.005 ^{cB}	0.03±0.005 ^{aA}	0.05±0 ^{eA}	0.04±0.005 ^{dA}	0.05±0.005 ^{fB}

Values are given as mean ± standard deviation, n=3, Values in a column with different superscript letters of A to D indicate significant difference between treatments (p<0.05) for each parameter and values in a row with different superscript letters from a to f indicate significant difference between storage periods (p<0.05).

Table 2. Changes in pH and TVBN during room temperature storage of beetroot extract treated samples along with control and SMS treated shrimps

Parameters	Samples	Storage hours					
		0	2	4	6	8	10
pH	Control	6.6±0 ^{aA}	6.71±0.02 ^{bA}	6.78±0.02 ^{cB}	6.85±0.03 ^d	7.09±0.01 ^{fC}	7±0.01 ^{eB}
	SMS	6.71±0.01 ^{aC}	6.88±0.01 ^{bB}	6.79±0.04 ^{bB}	6.86±0 ^d	6.95±0.01 ^{eB}	6.82±0.01 ^{cA}
	BT4%	6.65±0.01 ^{aB}	6.7±0 ^{bA}	6.64±0.01 ^{aA}	6.89±0.01 ^c	6.72±0.03 ^{bA}	7.08±0.02 ^{dC}
TVBN (mg%)	Control	18.8±0 ^{aA}	26.6±0.1 ^{bB}	32.2±0.02 ^{cB}	37.8±0 ^{dB}	40.6±0.1 ^{eC}	42.8±0.01 ^{fB}
	SMS	18.8±0 ^{aA}	25.2±0.005 ^{bA}	32.2±0.1 ^{cB}	36.4±0.1 ^{dA}	36.4±0.1 ^{dA}	39.2±0.02 ^{eA}
	BT4%	18.8±0 ^{aA}	25.2±0.1 ^{bA}	30.8±0.1 ^{cA}	36.4±0.02 ^{dA}	39.2±0.02 ^{eB}	39.2±0.02 ^{eA}

Values are given as mean±standard deviation, n=3, Values in a column with different superscript letters of A to C indicate significant difference between treatments (p<0.05) for each parameter and values in a row with different superscript letters from a to f indicate significant difference between storage periods (p<0.05).

TVBN

TVBN is an important index for measuring seafood quality, which includes the content of ammonia and primary, secondary and tertiary amines. The changes in TVBN values of ice stored white shrimp are presented in Table 1. Overall, the TVBN values of ice stored samples increased significantly (p<0.05) during 11 days of ice storage both in the case of control and treatment samples. But the increase in TVBN showed differences with respect to the treatments, where the control sample showed significantly higher value (p<0.05) throughout the storage period. The TVBN value of beetroot extract treated samples were significantly lower than that of control and SMS (p<0.05) indicating the efficiency of beetroot extract in reducing the spoilage of white shrimp. The generally considered maximum limit of acceptability of TVBN is 30-35 (Connell, 1995). As per European Commission Regulation No 2074/2005, TVBN limit for fishery products (EC 2005) is 25-35 mg 100 g⁻¹. The initial TVBN value of control was 10.2 mg 100 g⁻¹ and it reached 39.2 mg 100 g⁻¹ on 7th day of ice storage while in the case of treatments, it was significantly lower. In the present study, TVBN crossed 40 mg 100 g⁻¹ on the day of sensory rejection.

Progress of TVBN formation during room temperature storage of shrimp is given in Table 2. Significant (p<0.05) increase in TVBN during storage was observed where the control group showed the maximum TVBN value followed by SMS and BT4%. The TVBN content in all the cases increased significantly (p<0.05) at 2 h

interval of room temperature storage. The increase in TVBN can be related to bacterial growth and endogenous enzyme activity (Jeyakumari *et al.*, 2018). All the groups crossed 35 mg 100 g⁻¹ at 6th hour of storage while control reached 40 mg 100 g⁻¹ by 8th hour of storage. The lower content of TVBN in the SMS and BT4% compared to control indicates the effect of the treatment on spoilage bacteria.

TBARS

Lipid oxidation is a form of deterioration that can render the fish and shrimp unacceptable. This process can be initiated by autoxidation and enzymatic activity from lipoxygenase, peroxidase and microbial enzymes (Nirmal and Benjakul, 2009b). TBARS is a commonly used indicator to measure the degree of lipid oxidation. The changes in TBARS values of Indian white shrimp samples with or without treatment are given in Table 1. The initial TBARS value was 0.03 mg malonaldehyde kg⁻¹ and it increased significantly (p<0.05) throughout the iced storage period of both control and treatments. The TBA values of shrimp samples treated with beetroot extract at 1 and 4% levels were significantly lower (p<0.05) in comparison to control and SMS treated shrimp, indicating the antioxidant effect of beetroot extract, which was previously reported by Vinson *et al.* (1998) and Kahkonen *et al.* (1999). Pigments in beetroot extract such as red betacyanins and the yellow betaxanthins are free radical scavengers which prevent free radical mediated oxidation of biological molecules (Pedreno and Escribano, 2001).

Microbial quality

Aerobic plate count (APC) of white shrimp treated with or without beetroot extract is shown in Fig. 3a. APC indicates the microbial spoilage of food especially important during prolonged storage period. In all the samples, APC increased significantly ($p < 0.05$) with increase of storage period in ice. APC of control was significantly ($p < 0.05$) higher than that of samples treated with SMS and beetroot extract at 1 and 4% levels. APC of beetroot extract treated samples were significantly lower ($p < 0.05$) than that of shrimp treated with SMS till 5th day of iced storage and the APC of beetroot extract treated at 1% level exceeded the APC of SMS treated shrimp from 7th day of iced storage onwards. The *in vitro* antibacterial activity of ethanol extract of beetroot pomace has been previously reported (Canadanovic-Brunet *et al.*, 2011) as also the high levels of antimicrobial activity of phenolic compounds (Baydar *et al.*, 2004). On 9th day of iced storage, the control reached 7 log CFU g⁻¹, the recommended limit of spoilage for fish and fishery products for human consumption (ICMSF, 2018). Shrimp treated with SMS and beetroot extract at 1 and 4% level reached 7 log CFU g⁻¹, 7.2 log CFU g⁻¹ and 7 log CFU g⁻¹ respectively on 11th day of iced storage. Yuan *et al.*, (2016) also reported APC level above the recommended limit in control samples of Pacific white shrimp on 9th day of iced storage. Phenolic compounds in the beetroot extract, may have chelated the ions required for microbial growth, thereby reducing microbial growth in beetroot extract treated shrimp to some extent.

Gram negative *Pseudomonas* spp. are reported as dominant microorganisms during spoilage of shrimp and prawn (Akitola and Bakare, 2012). In the present study, *Pseudomonas* count showed a similar trend as that of APC (Fig. 3d). The initial count of *Pseudomonas* was 2.3 log CFU g⁻¹ and it showed an increasing trend in all the samples with storage days. The count in BT4% was

significantly ($p < 0.05$) lower than the other samples indicating the activity of phenolic compounds in beetroot extract. The control samples recorded the highest count of 4.8 log CFU g⁻¹ on 11th day of storage. Phenolic compounds can form complexes with proteins in the cell wall of microbes, resulting in breakage of cell wall (Chanthachum and Beuchat, 1997). Similar to the previous report of Nirmal and Benjakul (2011b), SMS showed lower efficiency in controlling the psychrophilic *Pseudomonas* count.

H₂S producing organisms, like *Shewanella putrefaciens* produce offensive smell during spoilage of fish and shellfish due to the production of gases such as H₂S, formed from sulphur containing amino acids. H₂S producing organisms were observed to be absent in all the treatment groups till 3rd day of storage while in BT4% it was not observed till 5th day of iced storage, indicating the sensitivity of the organism to beetroot extract treatment (Fig. 3c). It showed an increasing trend along with the storage period with the highest count in control followed by SMS treated shrimps, while the count in both BT1% and BT4% was significantly lower. The control shrimps recorded the highest count of 4.7 log CFU g⁻¹, while it was significantly lower in all other samples. Similar delay in growth of H₂S producing bacteria in 0.1% catechin treated shrimp was reported by Nirmal and Benjakul (2009b).

The aerobic plate count (APC) of treated shrimps during room temperature storage is shown in Fig. 3b. The initial APC of shrimp was 5.1 log CFU g⁻¹ and it showed a significant decrease on 2nd and 4th hour of storage followed by a significant increase from 6th hour of storage. The lower APC values in the treatments compared to control also indicate the effectiveness of treatment against spoilage organisms. Gram negative bacteria namely *Pseudomonas* spp., *Achromobacter* spp., *Flavobacterium* spp. and Gram positive bacteria like *Micrococcus* spp. are aerobic spoilage

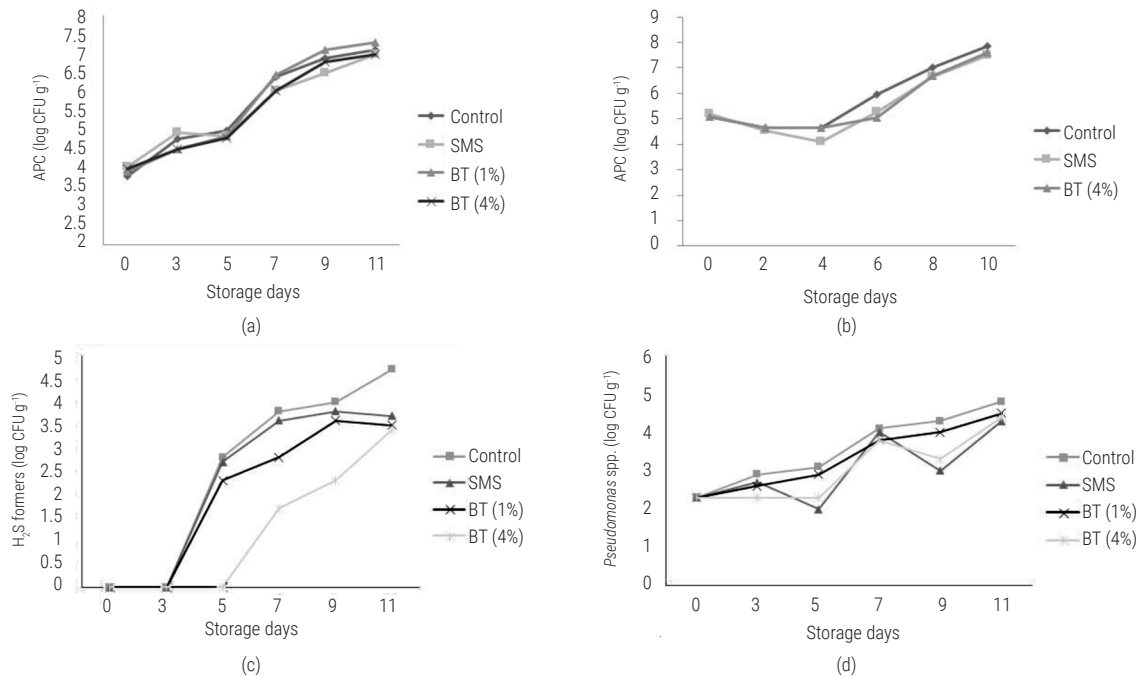


Fig. 3. Changes in APC of control and treatment samples during iced (a) and room temperature (b) storage. Changes in counts of (c) H₂S formers and (d) *Pseudomonas* spp. during iced storage

bacteria normally present in case of fresh shrimps (Lu, 2009). The control reached spoilage limit of 7 log CFU g⁻¹ (ICMSF, 2018) at 8th hour of storage while the treatments of SMS and beetroot extract reached spoilage limit at 10th hour of storage. Hence it again confirms the rapid spoilage of shrimp samples in ambient condition along with the rapid development of melanosis due to the activity of PPO at high temperature conditions. The study also indicates the antibacterial effect of phenolic compounds present in beetroot extract (Canadanovic-Brunet *et al.*, 2011) similar to the previous experiment discussed earlier and also the antimicrobial effects of SMS, the commercially used antimelanosis agent and its potential pathological effects (Lopez-Caballero *et al.*, 2006; Encarnacion *et al.*, 2010; Bono *et al.*, 2012). Plant phenolics are reported with good potential of antibacterial and antioxidant activity (Banerjee, 2006).

Sensory score

The overall acceptability of white shrimp with and without beetroot extract treatment during ice storage is shown in Fig. 4b. The treatment with beetroot extract imparted a pinkish colour to the shrimp in case of treatment at 4% level (Fig. 1) and it did not affect the overall acceptability of shrimp. In the case of white shrimp treated at 1%BT, there was no observable pink colouration to shrimp after treatment. There was a significant decline in the overall acceptability score during ice storage in all the treatments. The acceptability of SMS treated shrimp was significantly ($p < 0.05$) higher than other samples from 5th day onwards till the end of storage. But the decrease in overall acceptability score was lower in the case of beetroot extract treatment at 1 and 4% levels than

the control. Increase in melanosis formation of shrimps during storage decreased the sensory acceptability based on appearance and colour.

The overall acceptability score of control and treatments at room temperature showed a declining trend throughout storage (Fig. 4d). The acceptability of control was significantly ($p < 0.05$) lower than that of treatments in which SMS treated sample had better acceptability at the end of storage period from 8th hour. Melanosis formation was more pronounced in beetroot extract treated sample than the SMS treated shrimp. Control was rejected by the sensory panel at 8th hour of storage while SMS and BT at 10th hour of storage due to off odour. The phenolic compounds from beetroot extract inhibit quinone formation by polyphenoloxidase and thus prevent melanosis. It also contains bio-active compounds and exhibit biological properties like antioxidant and chelating activities.

The post-mortem deterioration in shrimp quality by blackening or melanosis, impacts consumer acceptability and reduces market value, although it is harmless to the consumers. Application of beetroot extract at 4% level by dipping method effectively inhibited melanosis formation in white shrimp, comparable to commercial treatment with sodium metabisulfite for up to 7 days of iced storage. Similarly, beetroot extract treatment remained effective upto 6 h of room temperature storage in preventing melanosis formation, with no adverse effects on sensory attributes. Furthermore, the treatment improved biochemical and microbial quality compared to untreated control. Therefore, beetroot extract presents a promising natural alternative for inhibiting melanosis in shrimp.

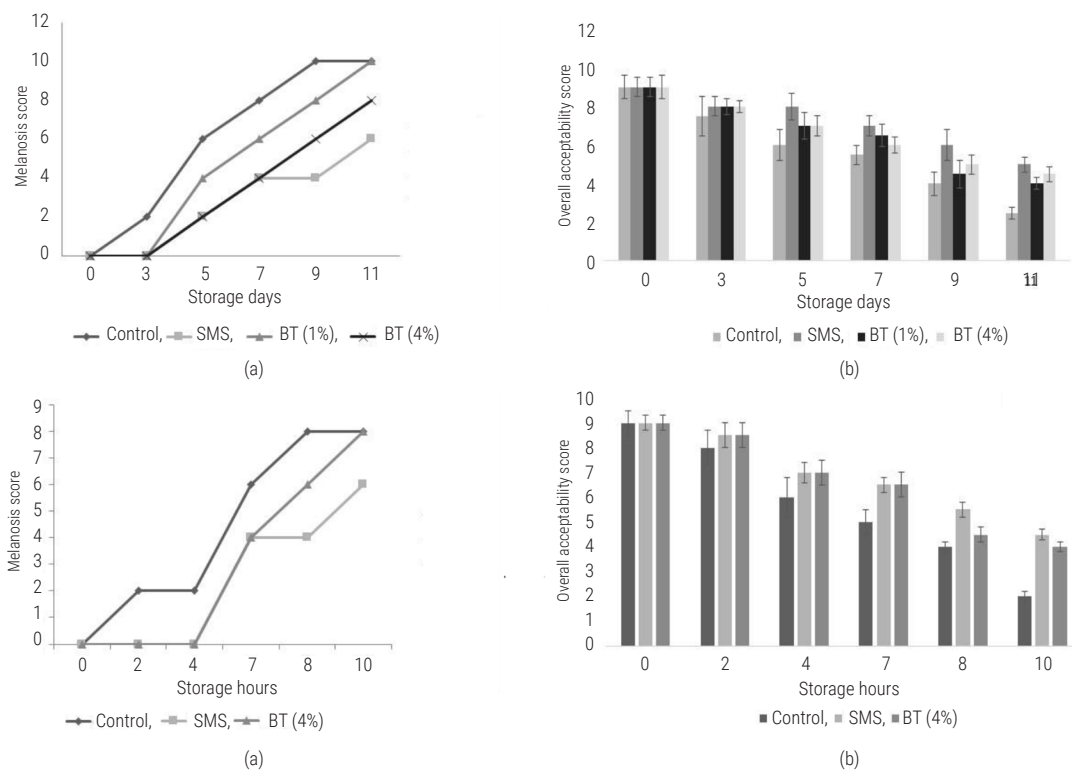


Fig. 4. Changes in melanosis score and overall acceptability score of control and treatment samples during iced storage (a and b) and room temperature storage (c and d)

Acknowledgements

This research work was carried out with the support of Indian Council of Agricultural Research, New Delhi, India. The authors sincerely thank the Director, ICAR-CIFT, Kochi for providing facilities and support to undertake this work.

References

- Akintola, S. L. and Bakare, S. B. 2012. Effects of ice storage on the biochemical composition of *Macrobrachium vollehovenii* (Herklots, 1857). *J. Fish Aquat. Sci.*, <https://doi.org/10.3923/jfas.2013.213.217>.
- Amerine, M. A., Pongborn, R. and Roesler, E. B. 1965. *Principles of sensory evaluation of food*. Academic Press, New York, USA, 602 p.
- AOAC 2005. *Official methods of analysis*, 17th edn. Association of Official Analytical Chemists, Washington, DC, USA.
- Banerjee, S. 2006. Inhibition of mackerel (*Scomber scombrus*) muscle lipoxigenase by green tea polyphenols. *Food Res. Int.*, 39(4): 486-491. <https://doi.org/10.1016/j.foodres.2005.10.002>.
- Baydar, H., Sagdic, O., Ozkan, G. and Karadogan, T. 2004. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Cont.*, 15: 169-172. [https://doi.org/10.1016/S0956-7135\(03\)00028-8](https://doi.org/10.1016/S0956-7135(03)00028-8).
- Benjakul, S., Visessanguan, W. and Tanaka, M. 2005. Properties of phenoloxidase isolated from the cephalothorax of kuruma prawn (*Penaeus japonicus*). *J. Food Biochem.*, 29: 470-485. <https://doi.org/10.1111/j.1745-4514.2005.00042.x>.
- Bono, G., Badalucco, C. V., Cusumano, S. and Palmegiano, G. B. 2012. Toward shrimp without chemical additives: A combined freezing-MAP approach. *LWT. Food Sci. Technol.*, 46(1): 274-279. <https://doi.org/10.1016/j.lwt.2011.09.020>.
- Canadanovic-Brunet, J. M., Savatovic, S. S., Cetkovic, G. S., Vulic, J. J., Djilas, S. M., Markov, S. L. and Cvetkovic, D. D. 2011. Antioxidant and antimicrobial activities of beetroot pomace extracts. *Czech J. Food Sci.*, 29: 575-585. <https://doi.org/10.17221/210/2010-CJFS>.
- Chanthachum, S. and Beuchat, L. R. 1997. Inhibitory effect of kiam (*Cotylelobium lanceotatum craih*) wood extract on Gram positive food-borne pathogens and spoilage micro-organisms. *Food Microbiol.*, 14: 603-608.
- Connell, J. J. 1995. Intrinsic quality. In: *Control of fish quality*. Fishing News Books/Blackwell Science, London, UK, pp 5-36.
- Conway, E. J. 1950. *Micro-diffusion analysis and volumetric error*, Crosby. Lockwood and Son Ltd, London, UK.
- DeWitt, B. J. 1998. An improved methodology for the estimation of sulfur dioxide in shrimp. *Resumen Int.*, 48: 3463-3464.
- Diaz-Tenorio, L. M., Garcia-Carreno, F. L. and Pacheco-Aguilar, R. 2007. Comparison of freezing and thawing treatments on muscle properties of white leg shrimp (*Litopenaeus vannamei*). *J. Food Biochem.*, 31: 563-576. <https://doi.org/10.1111/j.1745-4514.2007.00130.x>.
- EC, 2005. Commission Regulation (EC) No. 2074/2005 of 05 December 2005 on total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. *OJEC L338*, 36e39.
- Edmonds, M. 2006. Sodium metabisulfite alternatives. In: *Seafish technology implementation*. www.seafish.org.
- Encarnacion, A. B., Fagutao, F., Hirayama, J., Terayama, M., Hirono, I. and Ohshima, T. 2011. Edible mushroom (*Flammulina velutipes*) extract inhibits melanosis in kuruma shrimp (*Marsupenaeus japonicus*). *J. Food Sci.*, 76(1): C52-C58. <https://doi.org/10.1111/j.1750-3841.2010.01890.x>.
- Fang, X. B., Sun, H. Y., Huang, B. Y. and Yuan, G. F. 2013. Effect of pomegranate peel extract on the melanosis of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *J. Food Agric. Env.*, 11: 105-109.
- Ferrer, O. J., Koburger, J. A., Otwell, W. S., Gleeson, R. A., Simpson, B. K. and Marshall, M. R. 1989. Phenoloxidase from the cuticle of Florida spiny lobster (*Panulirus argus*): mode of activation and characterization. *J. Food Sci.*, 54: 63-67. <https://doi.org/10.1111/j.1365-2621.1989.tb08568.x>.
- Ginson, J., Panda, S. K., Bindu, J., Kamalakanth, C. K. and Srinivasa Gopal, T. K. 2015. Effect of high pressure treatment on microbiological quality of Indian white prawn (*Fenneropenaeus indicus*) during chilled storage. *Food Microbiol.*, 46: 596-603. <https://doi.org/10.1016/j.fm.2014.10.008>.
- Gokoglu, N. and Yerlikaya, P. 2008. Inhibition effects of grape seed extracts on melanosis formation in shrimp (*Parapenaeus longirostris*). *Int. J. Food Sci. Technol.*, 43: 1004-1008. <https://doi.org/10.1111/j.1365-2621.2007.01553.x>.
- ICMSF 2018. *Microorganisms in foods 7, Microbiological testing in food safety management*. International Commission on Microbiological Specifications for Foods. Kluwer Academic/Plenum Publishers, New York, USA.
- Jeyakumari, A., Narasimha Murthy, L., Visnuvinayagam, S. and Ravishankar, C. N. 2018. A comparative study on the quality changes of croaker (*Johnius dussumieri*) fish stored in slurry ice and flake ice. *J. Aquat. Food Prod. Technol.*, <https://doi.org/10.1080/10498850.2018.1449152>.
- Jeyakumari, A., Visnuvinayagam, S., Parvathy, U., Sarma, K. S., Rawat K. P., Khader, S. A., Narashimha Murthy, L. and Ravishankar, C. N. 2020. Effect of electron beam irradiation on the biochemical, microbiological and sensory quality of *Litopenaeus vannamei* during chilled storage. *J. Food Sci. Technol.*, <https://doi.org/10.1007/s13197-020-04250-7>.
- Kahkonen M. P., Hopia A. I., Vuorela H. J., Rauha J. -P, Pihlaja K., Kujala T. S. and Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.*, 47: 3954-3962. <https://doi.org/10.1021/jf990146l>.
- Kubo, I. and Kinst-Hori, I. 1998. Tyrosinase inhibitors from cumin. *J. Agric. Food Chem.*, 46: 5338-5341. <https://doi.org/10.1021/jf980226+>.
- Lopez-Caballero, M. E., Martinez-Alvarez, O., Gomez-Guillen, M. D. C. and Montero, P. 2006. Quality of Norway lobster (*Nephrops norvegicus*) treated with a 4-hexylresorcinol-based formulation. *Euro. Food Res. Technol.*, 222(3): 425-431. <https://doi.org/10.1007/s00217-005-0125-8>.
- Lopez-Caballero, M. E., Martínez-Alvarez, O., Gómez-Guillen, M. D. C. and Montero, P. 2007. Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formulations during chilled storage. *Int. J. Food Sci. Technol.*, 42: 1029-1038. <https://doi.org/10.1111/ifs.2007.42.issue-9>
- Lu, S. 2009. Effects of bactericides and modified atmosphere packaging on shelf life of Chinese shrimp (*Fenneropenaeus chinensis*). *LWT. Food Sci. Technol.*, 42: 286-291. <https://doi.org/10.1016/j.lwt.2008.03.004>.
- Manheem, K., Benjakul, S., Kijroongrojana, K. and Visessanguan, W. 2012. The effect of heating conditions on polyphenol oxidase, proteases and melanosis in pre-cooked Pacific white shrimp during refrigerated storage. *Food Chem.*, 131: 1370-1375.
- Maqsood, S., Benjakul, S. and Shahidi, F. 2013. Emerging role of phenolic compounds as natural food additives in fish and fish products. *Crit. Rev. Food Sci. Nutr.*, 53: 162-179. <https://doi.org/10.1080/10408398.2010.518775>.

- Martinez-Alvarez, O., Gomez-Guillen, M. C. and Montero, P. 2008. Chemical and microbial quality indexes of Norwegian lobsters (*Nephrops norvegicus*) dusted with sulphites. *Int. J. Food Sci. Technol.*, 43: 1099-1110. <https://doi.org/10.1111/j.1365-2621.2007.01576.x>.
- Mead, G. C. and Adams, B. W. 1977. A selective medium for the rapid isolation of pseudomonads associated with poultry meat spoilage. *Brit. Poult. Sci.*, 18(6): 661-670. <https://doi.org/10.1080/00071667708416418>.
- Montero, P., Lopez-Caballero, M. E. and Perez-Matreos, M. 2001. The effect of inhibitors and high pressure treatment to prevent melanosis and microbial growth on chilled prawns (*Penaeus japonicus*). *J. Food Sci.*, 66: 1201-1206. <https://doi.org/10.1111/j.1365-2621.2001.tb16105.x>.
- Nirmal, N. P. and Benjakul, S. 2009a. Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *Food Chem.*, 116: 323-331. <https://doi.org/10.1016/j.foodchem.2009.02.054>.
- Nirmal, N. P. and Benjakul, S. 2009b. Melanosis and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) treated with catechin during iced storage. *J. Agricult. Food Chem.*, 57: 3578-3586. <https://doi.org/10.1021/jf900051e>.
- Nirmal, N. P. and Benjakul, S. 2011a. Inhibition of melanosis formation in Pacific white shrimp by the extract of lead (*Leucaena leucocephala*) seed. *Food Chem.*, 128: 427-432. <https://doi.org/10.1016/j.foodchem.2011.03.048>.
- Nirmal, N. P., and Benjakul, S. 2011b. Use of tea extracts for inhibition of polyphenoloxidase and retardation of quality loss of Pacific white shrimp during iced storage. *LWT Food Sci. Technol.*, 44: 924-932. <https://doi.org/10.1016/j.lwt.2010.12.007>.
- Nirmal, N. P. and Benjakul, S. 2011c. Inhibition of melanosis formation in Pacific white shrimp by the extract of lead (*Leucaena leucocephala*) seed. *Food Chem.*, 128(2): 427-432. [http://refhub.elsevier.com/S0023-6438\(15\)30182-1/sref43](http://refhub.elsevier.com/S0023-6438(15)30182-1/sref43).
- Pedreno M. A. and Escribano J. 2001. Correlation between antiradical activity and stability of betanine from *Beta vulgaris* L roots under different pH, temperature and light conditions. *J. Sci. Food Agric.*, 81: 627-631. <https://doi.org/10.1002/jsfa.851>.
- Shahidi, F. 1994. Seafood proteins and preparation of protein concentrates. In: Shahidi, F. and Botta, J., (Eds.), *Seafoods: Chemistry, processing, technology and quality*. Blackie Academic and Professional, London, UK, pp 3-9.
- Sun, H. Y., Lv, H., Yuan, G. F. and Fang, X. B. 2014. Effect of grape seed extracts on the melanosis and quality of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *Food Sci. Technol. Res.*, 20: 671-677. <https://doi.org/10.3136/fstr.20.671>.
- Tarladgis B. G., Watts, B. M. and Younthan, M. T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.*, 37: 44-47.
- Viji, P., Madhusudana Rao, B., Jesmi, D. and Prasad, M. M. 2018. Control of melanosis and spoilage during chilled storage of Pacific white shrimp (*Penaeus vannamei*) using sulphite alternatives. *Indian J. Fish.*, 65(3): 135-139. <https://doi.org/10.21077/ijf.2018.65.3.78879-20>.
- Vinson, J. A., Hao, Y., Su, X. and Zubik, L. 1998. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.*, 46: 3630-3634. <https://doi.org/10.1021/jf980295o>.
- Yuan, G., Zhang, X., Tang, W. and Sun, H. 2016. Effect of chitosan coating combined with green tea extract on the melanosis and quality of Pacific white shrimp during storage. *J. Food*, 14(1): 35-40. <https://doi.org/10.1080/19476337.2015.1040459>.