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Note

Control of melanosis and spoilage during chilled storage of pacific white shrimp (*Penaeus vannamei*) using sulphite alternatives

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ABSTRACT

Effect of various additives *viz.,* sodium metabisuphite (SMS), combination of SMS, sodium citrate (SC) & ethylene diamine tetra acetic acid (EDTA); and pomegranate peel extract (PE) in controlling melanosis and quality deterioration in ice stored *Penaeus vannamei* was investigated. Shrimp was dip treated in: A) water without any additive (control), B) 1.25% SMS (w/v), C) 0.5% SMS, 0.5% SC and 200 ppm EDTA, D) 0.5% SC and 200 ppm EDTA and E) 0.5% PE for 5 min. The samples stored in ice were evaluated at 0, 12, 24, 36, 48 and 54 h for melanosis score, total volatile base nitrogen (TVB-N), pH and aerobic plate count (APC). Treatments E and D significantly delayed melanosis development compared to control (A) followed by B and C combination. At the end of 54 h, melanosis score reached 8; 5.5; 5; 4 and 5, respectively for A, B, C, D and E samples. The APC of E and D samples were relatively lower compared to other treatment and control samples. The APC at the end of 54 h of chilled storage was 2.2 $x10^5$; 1.23$x10^5$; 4.5$x10^5$; 2.3$x10^4$ and 2.2$x10^4$ cfu g^{-1}, respectively for A, B, C, D and E samples. TVB-N and pH of PE treated samples remained lower compared to other samples. The results indicated that pomegranate peel extract and chemicals like sodium citrate and EDTA can be used in combination with sodium metabisulphite to control development of melanosis in farmed shrimps during chilled storage.

Keywords: Biochemical quality, Melanosis, Metabisuphite, Shrimp

Melanosis is a severe quality defect occurring in crustaceans post-mortem as a result of a natural biomechanism in which the phenols are oxidised to quinones and melanin by polyphenol oxidase (PPO) enzyme. Its intensity varies among species as it is dependent on substrate and enzyme concentration (Benjakul *et al*., 2005) and is rapid in shrimp especially on the carapace, pleopods and telson, making it unacceptable to consumers. Market value of shrimp is primarily decided by its colour and appearance. Although harmless, the black spot on the carapace diminishes the market value of shrimp, leading to less profitability. Metabisulphites have been in use for many years to control melanosis development. However, metabisuphites are treated as allergens and European Union demands labeling on shrimp package where sulphite residue exceeds 10 ppm (Mark Edmonds, 2006). Stringent regulations on use of sulphites and increased awareness on food safety have necessitated the search for safe chemicals or natural additives for melanosis inhibition.

Shrimp spoils rapidly compared to fishes on account of its neutral pH, high content of moisture, free amino acids and nitrogenous compounds. Its wholesomeness under iced storage is affected by chemical changes, bacterial growth and melanosis. Studies have confirmed that natural extracts from plant sources can slow down black spot formation in shrimps during storage (Jang *et al*., 2003; Gokoglu and Yerlikaya, 2008; Nirmal and Benjakul, 2011). Chelating/reducing agents can also be used for inhibiting the enzymatic activity during melanois; however, scientific literature proving the role of those agents in farmed *Penaeus vannamei* is scarce. Ethylene diamine tetra acetic acid (EDTA) is a chelating agent, approved by the United States Food and Drug Administration (USFDA) as a food additive and is being used in various foods (Montero *et al.,* 2001). Teerawut and Pratumchart (2014) have observed improved physical and sensory properties of EDTA-soaked *P. vannamei* during iced storage. Sodium citrate is also an approved food additive and is commonly used as an acidulate/ flavouring agent in food. In this context, the present study was aimed to evaluate the efficacy of a blend of sodium citrate (SC), EDTA and sodium metabisulphite (SMS) and the individual effect of a natural antioxidant, pomegranate peel extract (PE) on controlling melanosis and quality changes in *P. vannamei* during short term iced storage.

Freshly harvested shrimp of size 30 g was procured from a shrimp farm in Srikakulam District of Andhra Pradesh and brought to the laboratory within

6 h of harvest in thermocol boxes under iced condition. Pomegranate peel was collected from a fruit juice vendor in Visakhapatnam city. All the chemical reagents were purchased from Merck Chemicals, India and Qualigens, India.

The shrimp was de-iced, washed with potable water and those specimens which exhibited melanosis above 20% were removed and the remaining quantity was divided into 5 lots. Samples were treated in, A) water without any additive (control) for 5 min, B) solution containing 1.25% SMS (w/v) for 1 min, C) solution containing 0.5% SMS, 0.5% SC and 200 ppm EDTA for 5 min, D) solution containing 0.5% SC and 200 ppm EDTA for 5 min and E) solution containing 0.5% (w/v) PE for 5 min. The chemicals and their concentrations were chosen based on their regulatory requirements for food applications. The shrimp after treatment were packed in polyethylene bags and stored in ice in 1:1 ratio in insulated boxes. The samples were evaluated at 0, 12, 24, 36, 48 and 54 h for melanosis score, pH, total volatile base nitrogen (TVB-N) and aerobic plate count (APC). The melted ice was removed but not replaced at any time during the storage period.

Progress of melanosis on the shrimp surface was evaluated by a panel of 5 members based on the scoring method reported by Nirmal and Benjakul (2009). Six numbers of shrimp from each treatment group was presented for evaluation. The panelists were directed to visually inspect the shrimp and give scores from 0-10, where 0 = no black spots, 2 = slight (up to 20% of shrimp surface affected), 4 = moderate (20-40% of shrimp surface affected), 6 = notable (40-60% of shrimp surface affected), 8 = severe (60-80% of shrimp surface affected) and 10 = extremely heavy (80-100% of shrimp surface affected). The scores were summed up and average was taken for evaluating the extent of melanosis.

pH of the samples was measured with a pH meter (LABMAN, LMPH-10) after mixing 5 g sample in 20 ml distilled water. Total volatile base-nitrogen (TVB-N) was estimated from trichloroacetic acid extract of the samples following Conway's microdiffusion method (Conway, 1950). APC was determined as per standard methods (BAM, 2011).

The progress of melanosis is illustrated in Fig. 1. Melanosis gradually increased significantly ($p<0.05$) in all samples during storage, but at varying degrees of advancement. In control samples (group A), melanosis increased rapidly after 12 h of treatment compared to its counterparts treated with melanosis inhibitors. Among the different inhibitors used, PE and the blend containing 0.5% sodium citrate and 200 ppm EDTA were more

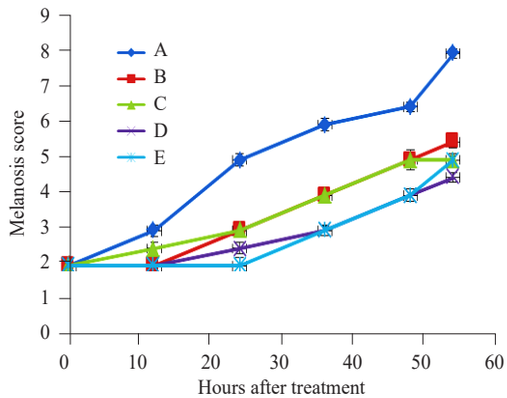

Fig. 1. Changes in melanosis in ice stored *P. vannamei* treated with melanosis inhibitors
A: control, B: SMS treated, C: SMS+SC+EDTA treated, D: SC+ EDTA treated and E: PG extract treated samples

effective in delaying melanosis development. Upto 24 h after treatment, melanosis in treated samples was only slight to moderate, affecting 20-40% of shrimp surface. At the end of 54 h, melanosis score reached 8, 5.5, 5, 4 and 5, respectively for A, B, C, D and E samples. Sulphites are the most widely used inhibitors for several years and it works by two mechanisms *viz.,* i) forming sulphaquinones by reacting with intermediate quinines in the melanosis process and ii) completely inactivating PPO enzyme by irreversible reactions (Ferrer *et al*., 1989). EDTA is an approved chelating agent for food applications; it can chelate the copper prosthetic group at the active site of PPO enzyme and can also inhibit digestive metalloproteases responsible for PPO activation (Montero *et al*., 2001). Being an acidifying agent, citrate can bring down the pH, retarding the optimum activity of PPO enzyme. It can also chelate copper ions during browning reactions. Gomez-Guillen *et al*. (2005) have investigated the synergistic effect of sulphite with citric acid and EDTA and obtained positive result for inhibiting melanosis in deep water pink shrimp. PE is reported to inhibit the PPO activity responsible for black spot formation in shrimp during refrigerated storage (Fang *et al*., 2013; Basiri *et al*., 2015). Our study further confirmed that PE can retard melanosis in shrimp. As Kubo and Kinst-Hori (1998) stated, phenolic compounds can inhibit melanosis by interacting with the active sites of the PPO or by chelating Cu^{2+} required for the enzyme activity.

During spoilage, a number of basic compounds are liberated as a result of proteolysis by bacterial enzymes. Hence, the muscle pH increases and its value is used as an index for quality deterioration. As evident from Fig. 2, PE could effectively control the deterioration in shrimp whereas in control samples pH was the highest, reaching 7.29 at the end of 54 h in ice. The pH of groups

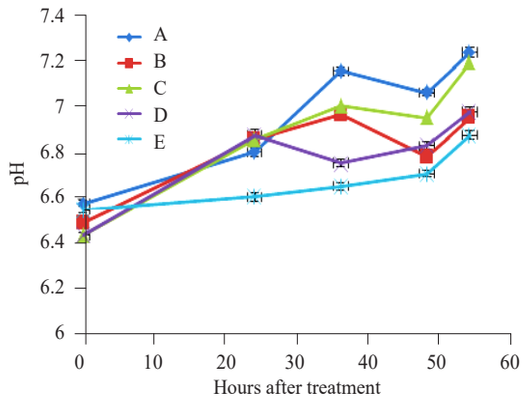

Fig. 2. Changes in pH of ice stored *P. vannamei* treated with melanosis inhibitors.
A: control, B: SMS treated, C: SMS+SC+EDTA treated, D: SC+EDTA treated and E: PE treated sample

B, C and D were not significantly different ($p>0.05$) till 24 h and afterwards, group C sample had significantly higher pH than groups D and B. The lower pH observed in PE treated samples could be due to its antibacterial effects. Similar results were observed by Yuan *et al*. (2016) in *P. vannamei* treated with a combination of chitosan and PE. Increase in pH was in parallel with TVB-N levels of the samples.

Progress of TVB-N generation during storage was examined and is shown in Fig. 3. Over the storage period, significant increase ($p<0.05$) in TVB-N was noticed where control group had the maximum TVB-N content followed by the sample treated with EDTA+SC combination. The results indicate that melanosis inhibitors are effective in controlling TVB-N formation at varying intensities. Groups treated with PE as well as combination of SMS+SC+EDTA showed better control over TVB-N generation compared to SMS alone and SC+EDTA combination which can be attributed to its antimicrobial effect. The lower TVB-N levels obtained by treated samples could be due to the inhibitory action on spoilage bacteria. Surendran *et al*. (1985) have reported decreased TVB-N formation in Na-EDTA dip treated shrimp during iced storage.

Fig. 4 shows the bacterial counts in shrimp during iced storage. Initial bacterial count of shrimp was 4.89 log cfu g^{-1}. An initial count of 4.19 and 4.45 log cfu g^{-1} were reported for *P. vannamei* by Mu *et al*. (2012) and Okpala *et al*. (2014), respectively. Gram-negative bacteria such as *Pseudomonas* spp., *Achromobacter* spp., *Flavobacterium* spp. as well as Gram-positive bacteria namely *Micrococcus* spp. were reported as aerobic spoilage bacteria mainly associated with fresh shrimps (Lu, 2009). Bacteria grew at a moderate rate in A, B and C groups, while a reduction from initial count was noticed in D and E groups. Bacterial proliferation was highest in control samples followed by the samples treated with SMS+SC+EDTA blend. The reduction or suppressed growth in D and E samples can be attributed to the antibacterial effect of the chemicals and phytochemicals used. Maximum acceptable limit for TPC in chilled/frozen shrimp meant for export (EIC, 1995) and for sale in domestic market (FSSAI, 2011) is 5,00,000 cfu g^{-1} (5.7 log cfu g^{-1}). None of the samples in the present study crossed this limit during the 54 h of storage in ice. At the end of 54 h, APC in control group reached 5.34 log cfu g^{-1} while count of PE treated shrimp remained 1 log lesser to control *i.e.* 4.34 log cfu g^{-1}, signifying its antibacterial properties. Reduction in total viable counts by treatment with PE has also been reported by Basiri *et al*. (2015) in ice stored *P. vannamei.*

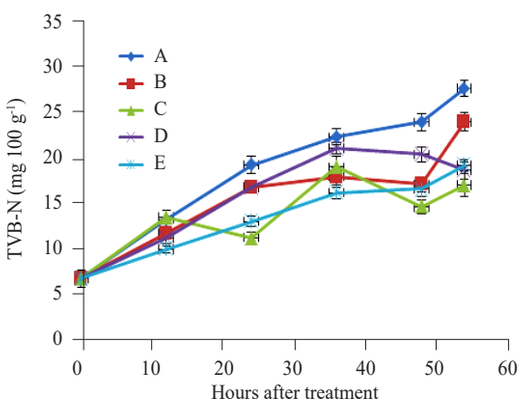

Fig. 3. Changes in TVB-N of ice stored *P. vannamei* treated with melanosis inhibitors.
A: control, B: SMS treated, C: SMS+SC+EDTA treated, D: SC+EDTA treated and E: PG extract treated samples

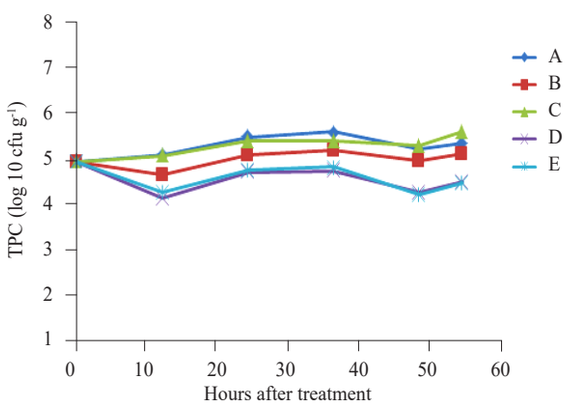

Fig. 4. Changes in TPC of ice stored *P. vannamei* treated with melanosis inhibitors.
A: control, B: SMS treated, C: SMS+SC+EDTA treated, D: SC+EDTA treated and E: PG extract treated samples

Phenolic compounds of PE might be accountable for its antibacterial effect which can denature the bacterial enzymes (Furneri *et al*., 2002) and also can bind with nutrients including vitamins, minerals and carbohydrates, making it unavailable to microorganisms (Shahidi and Naczk, 2004). The results of the present study also indicate antimicrobial effect of antimelanotic chemicals used. EDTA chelates Ca^{2+} and Mg^{2+} ions from the bacterial cell wall, leading to subsequent weakening/permeability of the cell wall, contributing to its antibacterial activity against most of the Gram-negative bacteria (Al-Bakri *et al*., 2009). A non-linear concentration dependent inhibition of spoilage bacteria, *P. aeruginosa* was established by Lambert *et al*. (2004). Sodium citrate also possesses bactericidal activity and Nagaoka *et al*. (2010) suggested two possible mechanisms to its antibacterial activity *viz*., direct disruption of cell membrane and disturbance of cation uptake,.

In the present study, changes in melanosis, biochemical and microbial changes of Pacific white shrimp treated with antimelanotic agents were monitored over 54 h of ice stored condition. The results obtained was promising, as the alternatives to metabisulphite used including pomegranate peel extract, sodium citrate and EDTA combinations had significantly controlled melanosis development. Additionally, the quality deterioration of *P. vannamei* as measured by pH, TVB-N and total plate count (TPC) was also delayed by PE and SC plus EDTA combinations compared to control. The study revealed that safe and approved chemicals like sodium citrate, EDTA or its combination with minimum concentration of metabisulphite can be used in place of metabisulphite alone to control black spot formation in shrimp during post-harvest handling. This approach can effectively manage the unscientific practice of soaking shrimp in highly concentrated metabisulphite solutions as a measure to arrest black spot formation.

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