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Quality of farmed shrimp (*Penaeus monodon,* Fabricius, 1798 and *Macrobrachium rosenbergii,* deman, 1879) as affected by melanosis inhibiting compounds

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Abstract

A problem associated with shrimp is blackspot or melanosis and to prevent blackspot, sulfites have been used for years but come with additional health risks. Newer 4-hexylresorcinol formulations exist that allow sulfite-free shrimp, but effects on the quality of *Penaeus monodon* Fabricius, 1798, and *Macrobrachium rosenbergii,* de Man, 1879, beyond melanosis prevention are unknown. Adverse effects on the quality of the shrimp from melanosis prevention treatments would negatively offset the benefits of a sulfite-free product. In order to determine if prevention compounds (sulfite powder or 4-hexylresorcinol based compounds, Everfresh® and Xyrex® Prawnfresh™) affect quality, proximate composition and total plate count of bacteria were determined for cultured (*P. monodon* and *M. rosenbergii*) shrimp in Bangladesh. The results showed no effect of melanosis prevention compound on proximate composition ($p > 0.05$), but for each parameter, species were significantly different ($p < 0.001$). For total plate count, treatment was not significantly different ($p = 0.09$), but species were significantly different in total plate count ($p < 0.001$). *M. rosenbergii* had a higher total plate count. Results of this study indicated that proximate composition and bacterial levels are not affected by 4-hexylresorcinol melanosis treatments. Melanosis prevention is necessary to reduce loss due to unacceptance, and 4-hexylresorcinoltreated shrimp can provide the industry with a sulfite-free alternative.

1. Introduction

Shrimp is one of the most consumed and traded seafood, and in Bangladesh, most shrimp are aquacultured. In 2018, black tiger shrimp (*Penaeus monodon,* Fabricius, 1798), and giant freshwater prawn (*Macrobrachium rosenbergii,* de Man, 1879) contributed 50% and 41%, respectively to the total 122 tonnes of farm shrimp production (Department of Fisheries in Bangladesh (DOF), 2018). Quality degradation in shrimp is a big issue related to food safety. Problems related to quality are found in both cultured and captured shrimp. Common problems of both wild and cultured shrimp are aesthetic issues such as blackspot, effects of post-harvest treatments, and bacterial contamination.

Melanosis, or blackspot, is a common visual defect in shrimp that affects marketability. Polyphenol oxidase enzymes, an endogenous enzyme complex with tyrosinase as the main active enzyme cause the shell's darkening (Huang *et al*., 2010; Andrade *et al*., 2015). Sodium sulfites ($NaHSO_3$) or sodium meta-bisulfites ($Na_2S_2O_5$) are the most widely used inorganic chemicals effective for crustacean melanosis control (López-Caballero *et al*., 2006; Nirmal and Benjakul, 2009; Miget, 2010; Bono *et al*., 2012). Sulfites are very effective in preventing melanosis, but they trigger asthma attacks and are known allergens (Collins-Williams, 1983). As more conscious consumers request sulfite-free shrimp, several products based off of 4-hexylresorcinol exist that are approved by the European Union (EU) and United States (US) for melanosis prevention (European Commission, 2003). These compounds (e.g., Everfresh® (Everfresh) and Xyrex® Prawnfresh™ (Prawnfresh)) use 4-hexylresorcinol to bind the melanosis-causing enzymes. While proven effective at preventing or delaying black spot development in shrimp, any negative effects on the quality of the shrimp from melanosis prevention

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treatments would negate the sulfite-free aspect (López-Caballero *et al*., 2006).

Shrimp flesh is considered a good source of protein, minerals and highly unsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acid (Felix *et al*., 2002; Yanar and Celik, 2005). The biochemical composition of shrimp can be influenced by season, origin and handling (Yanar and Celik, 2005; Puga-López *et al*., 2013). Information regarding the effect of melanosis prevention treatments with the use of sulfite, Everfresh and Prawnfresh on the nutritive composition of *P. monodon* Fabricius, 1798, and *M. rosenbergii*, de Man, 1879, is currently unknown. Previous reports have shown that sulfite and Everfresh affected bacteria levels (Martinez-Alvarez *et al*., 2005), but a comparative study on the effect of sulfite, Everfresh and Prawnfresh on the bacterial count in *P. monodon* and *M. rosenbergii* is not available. Therefore, this study aimed to determine the changes in basic chemical composition and bacterial quantity of shrimp due to the application of 4-hexylresorcinol melanosis prevention treatments.

2. Materials and methods

2.1 Sources of shrimp

Cultured giant freshwater prawns (*M. rosenbergii*) and black tiger shrimp (*P. monodon*) from Bangladesh were used for the experiments. All shrimp were free of post-harvest dips and purchased directly from the harvesters during winter 2017. Three separate replicates of each species of shrimp were treated and analyzed using approximately 2.3 kg of shrimp per replicate. The experiments were performed in the Department of Fisheries Technology Lab, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh.

2.2 Treatments

Once in the lab, shrimp were divided randomly into one of four treatments (Sulfite as the positive control, two 4-hexylresorcinol formulations Everfresh and Prawnfresh and control which was untreated as a negative control). All treatment dips (concentration, salinity, temperature, and time duration of the dip) were done following manufacturer recommendations, and all saltwater was a mix of deionized (DI) water and Instant Ocean. For Sulfite, 136.08 g of sulfite (sodium metabisulfite, NF/Food and Photographic grade, Esseco, USA) were mixed with 11.3 L of ambient saltwater (19° C) at 5 ppt. Shrimp were dipped for 1 min. For Everfresh, 24 g of Everfresh (Andenex-Chemie Engelhard + Partner GMBH, Humburg) were mixed with 11.3 L of ambient saltwater (19°C) at 2.2 ppt. Shrimp were dipped for 2 mins. For Prawnfresh, 12 mL of Prawnfresh (Prawnfresh +, Xyrex, United Kingdom) were mixed with 11.3 L of cold (5°C) saltwater at 30 ppt. Shrimp were dipped for 10 mins. For the Control, shrimp were rinsed well with ambient DI water (21°C).

2.3 Proximate analysis

For proximate composition analysis (moisture, ash, protein, and lipid), the head and shell were removed from the shrimp, and the flesh was ground to obtain uniformity immediately after treatment. Moisture and ash analysis started immediately. Samples for protein and lipid analysis were transferred to a microcentrifuge tube, stored in a freezer at -20°C, and testing was completed within 7 days of treatment. For each batch of shrimp, three replicates samples were run, and the average was calculated.

The moisture content was analyzed by the Pearson (1976) method. Shrimp samples were weighed using an electric balance (AL54 analytical balance, Mettler Toledo, Switzerland) and dried in a hot air oven (Precision™, Thermofisher Scientific, USA) at 105°C for 24 hrs. Plates were placed in desiccators until the final weight of the shrimp was taken.

For ash determination (AOAC, 1990), shrimp samples were placed in a crucible of known weight (AL54 analytical balance, Mettler Toledo, Switzerland) and placed into a muffle furnace (Lindberg, Thermo Scientific, USA). The temperature was maintained at 550°C for 6 hrs, and the final weight was taken.

The total lipid content of shrimp was estimated by the Folch method (1957). Modified Lowry (Lowry, 1951) protein method was used for the estimation of protein. A Modified Lowry protein assay kit (Thermo Scientific, number 23240, USA) was used. Estimation of total protein was done in two steps: chloroform-methanol precipitation and modified Lowry protein assay. Samples and standards were analyzed on a spectrophotometer (T70+ UV/VIS spectrophotometer, PG instrument Ltd., United Kingdom) at 750 nm.

2.4 Microbiological analysis

For the testing of total plate count (TPC) of bacteria, shrimp was stored on ice at 5°C, and testing was started within 12-18 hrs. Microbiological analysis was performed according to the standard procedure for the enumeration and identification of microorganisms (Maturin and Peeler, 2001). The shrimp head and shell were removed, and then 25 g homogenised sample was used for each replicate. A sample was transferred to a stomacher bag aseptically and mixed with 225 mL phosphate buffer saline (PBS) by stomacher (Easy Mix lab blender, AES Laboratoire, USA) for 60 s. Serial

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dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) were prepared. Then, 1 mL of each dilution was pipetted onto an appropriately marked Petri dish, in duplicate. Plate count agar (15 mL) was added to each plate. Sample dilutions and agar medium were mixed uniformly by rotation of plates. After solidification, Petri dishes were inverted and incubated for 48 hrs at 35°C.

2.5 Statistical analysis

A two-way ANOVA was used to test for significant differences between species and treatment and interactions for each proximate composition parameter and total plate count. Tukey's HSD post hoc was used to identify differences in significant ANOVAs. All statistical analysis was run in Sigma Plot Systat Software, V. 14; $p = 0.05$ for all statistical tests. All results are reported in mean±SD.

3. Results

For each of the treatments, the pH of the dip was 6.9 for Sulfite and Everfresh and 5.4 for the Prawnfresh. The Control had a pH of 7.6.

3.1 Proximate analysis

To test if melanosis prevention treatments had an effect on quality, moisture, ash, lipid, and protein were measured. Melanosis treatment did not affect moisture, ash, protein or lipid contents ($p > 0.05$). However, the results of each parameter were significantly different ($p < 0.001$) when compared between the two species. Moisture content ranged from 79.92±1.01% (*P. monodon)* to 79.50±1.16% (*M. rosenbergii*) (Figure 1a). *P. monodon* had a higher moisture level than *M. rosenbergii* but was not significantly ($p > 0.05$) different. For ash content, *M. rosenbergii's* (1.72±0.11%) was higher ($p < 0.001$) than *P. monodon* (1.38±0.30%) (Figure 1b). For lipid content, *M. rosenbergii* (1.73±0.06%) was significantly higher ($p < 0.026$) than *P. monodon* (1.23±0.08%) (Figure 1c). For protein content, *P. monodon* (782.34±66.72 µg.mL^{-1}) had the higher protein levels (Figure 1d) and was significantly higher than *M. rosenbergii* (669.35±65.87 µg.mL^{-1}) ($p < 0.001$).

3.2 Microbiological analysis

To determine if melanosis prevention treatment affected microbial levels, total plate count (TPC) was measured for each treatment or control group in both species of shrimp. Two microbiological sample replicates were done for each shrimp species. The treatment did not affect TPC ($p = 0.09$), although Sulfite and Everfresh always had the lowest TPC in each shrimp species (Figure 2). There was a significant difference in TPC ($p < 0.001$) with *M. rosenbergii* having a higher TPC (5.34 $\log_{10}$ CFU.g^{-1}) than *P. monodon* (5.14 $\log_{10}$ CFU.g^{-1}). The TPC of the two species were within the acceptable limit.

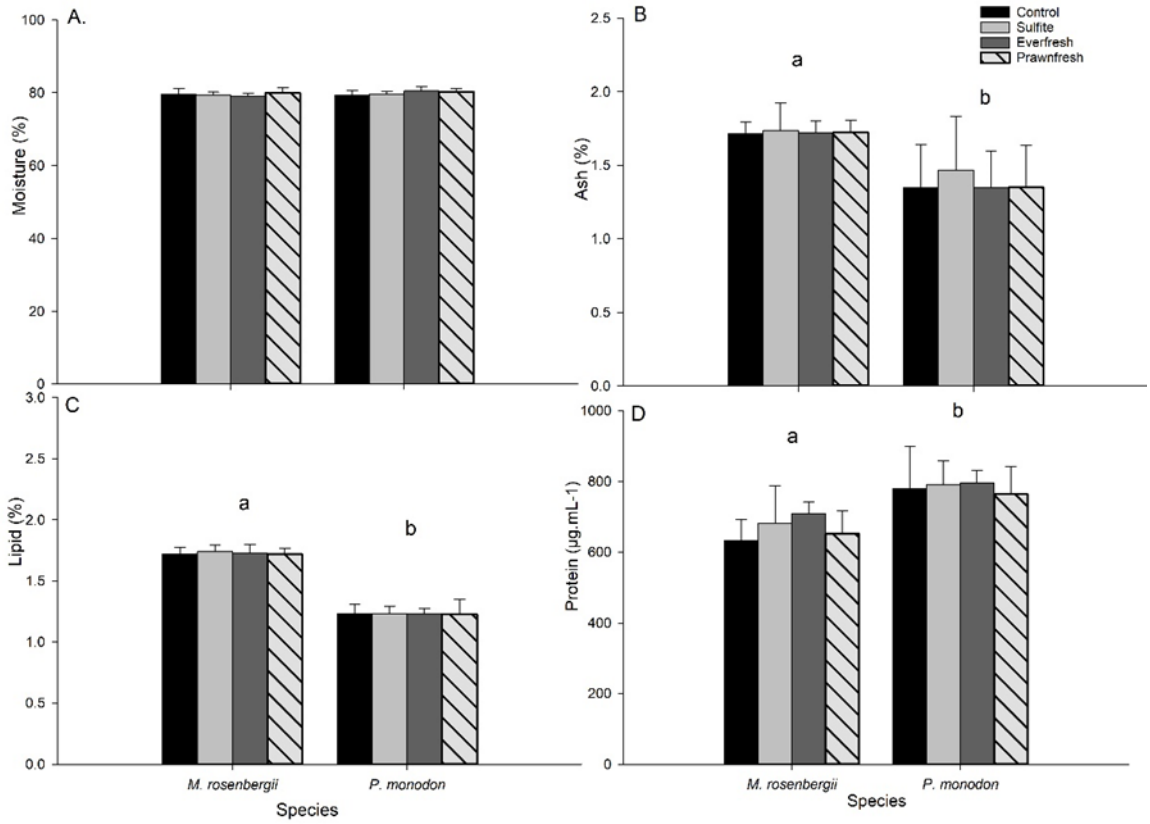

Figure 1. Proximate analysis for the two shrimp species including A. Moisture, B. Ash, C. Lipid and D. Protein. Error bars represent SD. Different letters indicate statistically significant differences by species ($p < 0.001$).

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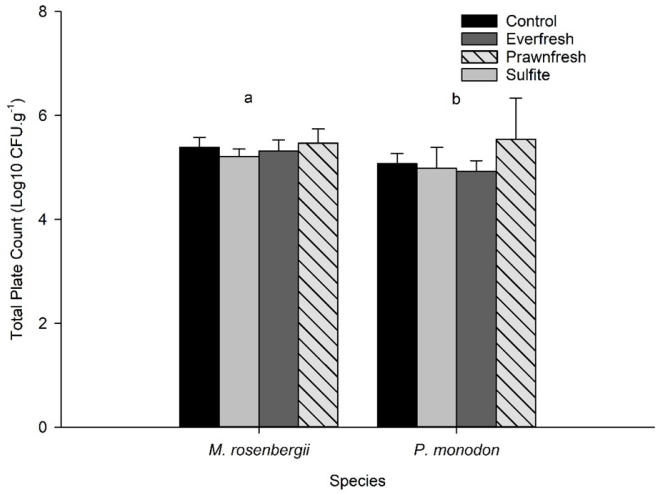

Figure 2. Total Plate Count of the two shrimp species. Error bars represent SD. Different letters indicate statistically significant differences by species ($p < 0.001$).

4. Discussion

Overall, there was no effect of melanosis prevention treatment on the proximate composition or TPC of the shrimp, which means 4-hexylresorcinol formulations did not affect the proximate composition or bacterial levels compared to traditional sulfites.

Post-harvest treatments can alter quality parameters. Cadun *et al*. (2008) marinated deep water pink shrimp with rosemary extracts to determine the shelf life by physical, chemical, microbiological, instrumental and sensory analysis but like the present study, found no effect of rosemary extract on shrimp composition. However, in the present study, the proximate compositions were different among species. In addition to known variations between species, season, habitat, diet, water temperature, maturity, spawning cycle and water temperature can cause variations within species (Razia, 2010; Venugopal and Gopakumar, 2017). One study reported that the proximate composition of wild-harvested white shrimp (*Litopenaeus vannamei,* Boone, 1931) is comparatively better than the farmed shrimp because of a wide variety of food in an aquatic environment (Puga-López *et al*., 2013). However, in this case, some factors were unknown, like the moulting time, types of feed for farmed shrimp, water condition or habitat, and those can influence the biochemical composition.

Previously, 0.05% 4-hexylresorcinoldid not inhibit the growth of microorganisms (European Commission, 2003). Additionally, 4-hexylresorcinol combined with organic acid did not prevent the growth of lactic acid bacteria in Norway lobster, *Nephrops norwegicus,* Linnaeus, 1758 (López-Caballero *et al*., 2006). In the present research (1.25% sulfite dip), results were similar in shrimp that were treated with 2% sodium sulfite; the 2% solution did not significantly affect the microbial load of the shrimp (Attala, 2012). The current study is the first report on the effect of Prawnfresh on the total plate count and although there was no significant effect of treatment on TPC, Prawnfresh-treated shrimp had higher bacterial counts than untreated or Sulfite and Everfresh-treated shrimp. The high TPC might be due to the longer (10 mins) dipping treatment recommended by the manufacturer.

According to the International Commission on Microbiological Specifications for Foods (1986), shrimp is unacceptable if the bacterial load exceeds 6-7 $\log_{10}$ CFU.g^{-1}. In the current research, the average range of bacterial load was 5.14 $\log_{10}$ CFU.g^{-1} for *P. monodon* to 5.34 $\log_{10}$ CFU.g^{-1} for *M. rosenbergii*. The TPC count of the two species was different, which is natural as fish and shellfish carry natural microflora. Water quality can also influence microbial quantity and quality (Ray and Bhunia, 2007). Previously in Bangladesh, the bacterial loads of *P. monodon* from four sources were different, ranging from 2.4×10^2 CFU.mL^{-1} to 4.8×10^5CFU.mL^{-1} (Yousuf *et al*., 2008). Differences in water source and quality, habitat structure, feeding habits, and density between the culture system and natural system may affect the gut microorganism of aquatic organisms (Prieur *et al*., 1990). Different environments and different seasons could result in different bacterial quantities.

Results of the current study indicate that in terms of proximate composition and total bacteria count, 4-hexylresorcinolformulations do not have an effect compared to traditional sulfites or untreated shrimp. Melanosis prevention is vital to reduce loss due to consumers' unacceptance of the product and low sale price. The results of this study will be helpful to consumers and shrimp harvesters seeking sulfite-free shrimp.

As 4-hexylresorcinol formulations do not have the same negative impacts associated with consumer health as sulfites, these could be a better choice for preventing melanosis in shrimp of Bangladesh. Processing plants tested raw shrimp for sulfite destined for export to maintain monitoring procedures under HACCP (Mazid and Mostafa, 2009) but shrimp destined for the local market are not tested for sulfite residue. Other research found that both *P. monodon* and *M. rosenbergii* collected from the retail market contain sulfite residue (Khan, 2018).

As small amounts of sulfite can be harmful to hypersensitive asthmatics patients, and no monitoring of local shrimp for sulfite residue exists, Bangladeshi people face a possibly severe health risk. To reduce this

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risk, the use of 4-hexylresorcinolformulations in shrimp can be a better option than sulfite.

5. Conclusion

There was no significant effect of melanosis treatment on the proximate composition and bacterial count of shrimp. However, based on species there was a significant difference in proximate composition and bacterial counts. Overall, there was no effect of melanosis prevention treatment, and sulfite, Everfresh or Prawnfresh could be used to prevent melanosis without affecting proximate composition. This provides the shrimp industry with sulfite-free alternatives for preventing melanosis without affecting quality.

Conflict of interest

The authors declare no conflict of interest.

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