

Effectiveness of green tea extract as a natural inhibitor of melanosis in pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

Melanosis, an enzymatic browning process catalysed by polyphenol oxidase, significantly reduces the commercial value of shrimp post-harvest. Freshly harvested shrimp were treated with 2% and 3% concentrations of either SMBS or green tea extract and stored at room temperature and <4 °C. Visual assessments were conducted every 3 hours over an 18-hour period to record melanosis progression. Results demonstrated that both treatments significantly reduced black spot development compared to the untreated control, with SMBS showing superior inhibition, especially under room temperature conditions and at higher concentrations. However, green tea extract also provided substantial protection against melanosis, particularly when combined with cold storage, highlighting its potential as a natural alternative to synthetic preservatives. The study confirmed a dose-dependent effect, as 3% concentrations of both treatments yielded fewer melanised shrimp pieces than 2% concentrations. Furthermore, refrigeration markedly slowed melanosis progression across all groups. These findings align with previous research emphasizing the antioxidant and polyphenol-rich profile of green tea as a promising natural preservative. The study concludes that while SMBS remains highly effective, green tea extract can serve as a safer, natural inhibitor of melanosis in shrimp, thereby extending shelf life and maintaining product quality during storage. This research contributes to sustainable seafood preservation strategies, prioritizing consumer safety and environmental responsibility.

Keywords: Shrimp preservation, natural preservatives, green tea extract, sodium metabisulfite (SMBS)

Introduction

The food industry with the fastest rate of growth in the world is aquaculture, which has enormous growth potential. It plays a critical role in global food production and more than half of the aquatic foods consumed by humans are produced through farming (FAO.org). According to the report of FAO 2022, in 2020 marine species represented 39 percent of total aquatic animal production. Today the shrimp is one of the major species grown in aquaculture worldwide. Main cultural species in freshwater are carp, tilapia, giant freshwater prawn, catfish, and others (Water quality and management, 2019, Science Direct) [32]. And marine water culture represented by shrimp, Atlantic salmon, seaweed, lobster, oysters and others (FAO 2022). And major species cultivated in brackish water environment are white leg shrimp, tiger shrimp, seabass, pearl spot and others (www.agrifarming.in).

White leg shrimp (*Litopenaeus vannamei*) are native to the eastern Pacific Ocean, extending from the Mexican state of Sonora to northern Peru. In 1973, Florida initiated aquaculture production of white leg shrimp, sourcing the initial breeding stock from Panamanian shrimp used in hatcheries for larval production. The majority of nations, from Mexico to Peru, established sizable production regions in the 70s and 80s. Ecuador rise to prominence as a global leader in shrimp production (FAO).

This species was introduced to aquaculture in Asia at the start of the millennium to replace *Penaeus monodon*. China, Vietnam, and India lead the world in *Litopenaeus vannamei* production.

A total of 5.4 million tons of shrimp were produced globally in 2023. Which is expected to be at 5.7 million tons in 2024 and 6.1 million tons in the next year (Fletcher R, The Fish Site, 2024) [35].

The top five global shrimp producers in 2023 were India, Ecuador, China, Vietnam, and Indonesia, together contributing approximately 74% of the total output. The Pacific white shrimp (*Litopenaeus vannamei*) continues to be the most produced species globally (Jory D, Global Seafood Alliance, 2023) [36].

A marginal increase of 0.33% was observed in Vannamei shrimp exports during 2023–2024, with figures moving from 623,432 MT to 625,475 MT. The US accounted for 53.52% of all Vannamei shrimp exports, with China (15.78%), the EU (9.01%), Southeast Asia (5.71%), Japan (3.41%), the Middle East (2.90%), and other nations (9.57%) (MPEDA, 2024).

Gujarat's shrimp farming industry is experiencing rapid growth, particularly in Surat, Valsad, Navsari, and Bharuch. According to survey data, there may be up to 376,000 hectares of land accessible for the production of brackish water shrimp, which now occupies about 7,000 hectares (Jahnvi tandel, 2018) [27].



Fig 1: *Litopenaeus vannamei*

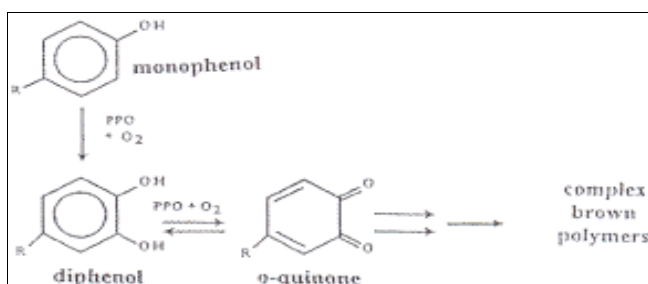
Melanosis, also known as black spot, is a serious problem in shrimp after harvesting, caused by a natural biomechanism where the polyphenol oxidase (PPO) enzyme oxidizes phenols to melanin (Benjakul *et al.*, 2005) [5]. It starts from the carapace, pleopods and telson. The color and appearance of shrimp influence its commercial value (Viji *et al.*, 2018) [29, 30].



Fig 2: Black spot in shrimp

Melanosis incidence varies by species; moulting cycles, harvesting practices, and handling techniques, as well as capture, rough handling of the catch, and other traumatic events, appear to set off the crustacean defence mechanism, which in turn leads to an increasing formation of black spots (Bartolo & Birk, 1998; McEvily *et al.*, 1991) [4, 22].

Fig. 03 shows how blackspot develops. In the presence of oxygen, a naturally occurring enzyme called PPO starts a reaction that turns colourless monophenols into diphenols, which in turn produce brightly coloured quinones, which form complex brown polymers (Garcia-Carreno *et al.*, 2008) [12].



Source: Ding *et al.* 2002

Fig 3: Enzymatic oxidation induced by PPO

First used on shrimp in the 1950s to prevent melanosis (Fieger, 1951) [10], they are being used today all over the world. Sulphites' mode of action in stopping enzymatic browning. When using catechol as a substrate, the sulphite ion in position 4 of the o-quinone nucleophilically attacks the substrate to produce 4-sulfocatechol, which is then followed by the addition of a hydrogen ion to stop browning. As a result, this process has reduced the quinone (Kim *et al.*, 2000) [19, 20].

However, Sodium metabisulfite is considered an allergen, and the European Union requires that shrimp packages with

a sulphite residue of more than 10 parts per million be labelled (Mark Edmonds, 2006) [11].

Green tea polyphenols are natural antioxidants (Tanizawa *et al.*, 1984) [28] and are considered to be responsible for the anticarcinogenic and antimutagenic properties of green tea.

Methodology

Extraction of Green Tea (*Camellia sinensis*)

To prepare the green tea extract, dried green tea (*Camellia sinensis*) leaves were first ground into a fine powder to maximize surface area for subsequent extraction using a high-speed blender. The powdered leaves were then passed through a stainless-steel sieve with an 80-mesh size to ensure uniform particle size and remove any coarse material. Distilled water was heated to a temperature range of 80–90 °C, a range known to be effective for extracting bioactive compounds while minimizing degradation. The sieved green tea powder was added to the hot water at a concentration of 25 g/L. The mixture was stirred continuously for a period of 15 to 30 minutes to enhance the extraction of polyphenols and other beneficial compounds. After stirring, the resulting mixture was filtered through Whatman No. 1 filter paper to remove solid residues and obtain a clear extract. The prepared extract was then collected and stored for further use in shrimp treatment and melanosis inhibition studies.

Treatment with extract and SMBS

Freshly harvested shrimp were used to evaluate the effectiveness of green tea extract and sodium metabisulfite (SMBS) in controlling black spot formation. The extract and SMBS were dissolved in water chilled to <4 °C at two different concentrations: 2%, and 3%. A control group, treated solely with chilled water (at <4 °C) without any additives, was also maintained to serve as a baseline for melanosis development. The shrimp were immersed in the prepared solutions to undergo

A chill kill treatment, a process used to quickly lower body temperature and reduce enzymatic activity that contributes to melanosis. After treatment, the shrimp samples were divided and stored under two different conditions: one set at refrigeration temperature <4 °C and another at room temperature. The samples were stored for a maximum of 18 hours and visual observations were made every 3 hours to monitor the appearance and progression of black spots on the shrimp. The effectiveness of each concentration, as well as the impact of different storage temperatures, was evaluated based on melanosis development. All observations were carefully documented to determine which treatment best inhibited black spot formation and maintained the visual and physical quality of the shrimp during short term storage.

Result

The study incorporated a control group (without treatment) to establish the natural course of melanosis progression under both high and low-temperature conditions. Recorded results underwent analysis to assess the effectiveness of each extract, at each given dose, within both experimental temperatures.

Sodium metabisulfite (SMBS) is a frequently utilized chemical preservative, recognized for its capability to inhibit melanosis in shrimp. It works by acting as an

antioxidant, which reduces melanin activity and consequently prevents the formation of black spots. Green tea extract is recognized for its high concentration of catechins and polyphenols. These compounds are recognized for their diverse health-promoting attributes, including significant antioxidant and anti-viral effects.

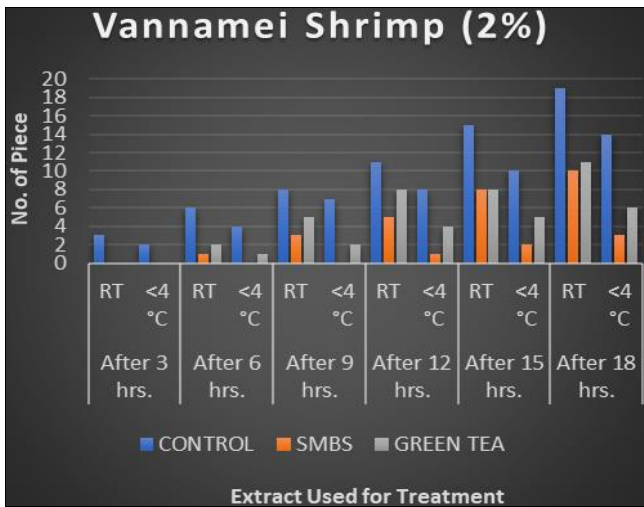


Fig 4: Vannamei shrimp treated with 2% dose

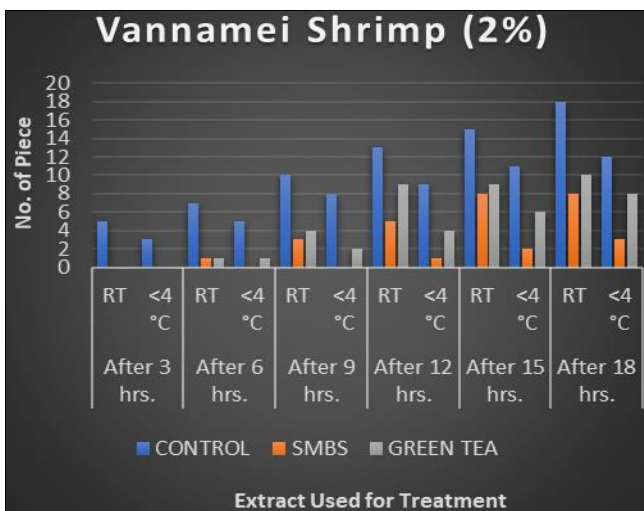


Fig 5: Vannamei shrimp treated with 2% dose

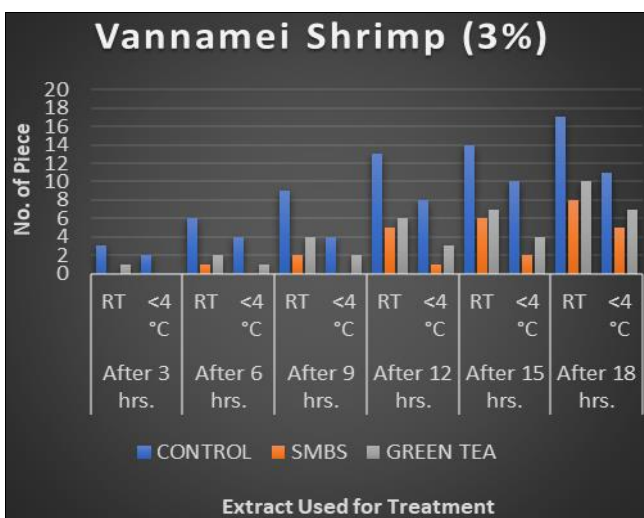


Fig 6: Vannamei shrimp treated with 3% dose

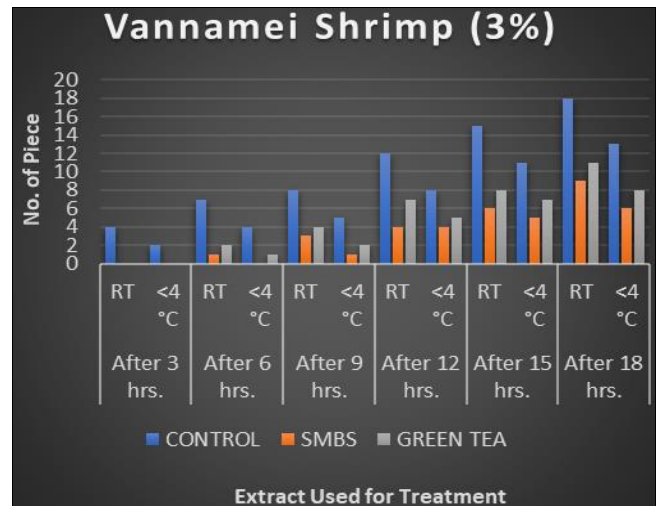


Fig 7: Vannamei shrimp treated with 3% dose

The comparative performance of various treatments, an untreated control, Sodium metabisulfite (SMBS) and Green tea extract. In suppressing melanosis (black spot formation) on vannamei shrimp. The study recorded the number of affected shrimp pieces over 18 hours under two distinct storage conditions: room temperature (RT) and <4°C temperature. Data is presented for both 2% and 3% concentrations of the active treatments.

Across both the 2% and 3% treatment concentrations, the control group consistently served as a standard for natural melanosis development. It shows a steadily increasing count of melanised shrimp pieces throughout the observation period, particularly under room temperature conditions, where the black spots phenomenon accelerated rapidly.

In contrast to the control, both the SMBS and green tea applications gave substantial protection against melanosis. Every 3-hour time period and for both concentrations, these treated groups exhibited a significantly lower incidence of black spots. All treatments, including the control, showed considerably slower melanosis progression at <4°C temperatures.

When directly comparing the two active treatments, SMBS frequently emerged as the more potent agent in preventing melanosis, especially evident at later stages of the storage period and in conditions where melanosis was more prevalent (i.e., at room temperature). Nevertheless, green tea extracts also demonstrated commendable anti-melanosis activity, offering considerable protection, particularly when combined with cold storage. Furthermore, the data suggests a dose-dependent improvement in efficacy: the 3% concentration of both SMBS and green tea generally resulted in marginally fewer melanised pieces compared to their 2% counterparts at equivalent time points and temperatures, indicating an incremental benefit with higher concentrations.

Discussion

A. Firdous *et al.* (2020) underscore the significant potential of green tea extract as an effective natural preservative for Indian white prawns during chilled storage. Green tea extract exhibits robust antioxidant properties, stemming from its rich phenolic content, which is crucial for neutralizing free radicals. Critically, they effectively inhibit polyphenol oxidase activity, a key enzyme responsible for undesirable melanosis, thus preventing black spot

formation. Furthermore, these natural treatments notably reduce biochemical spoilage indicators like TVB-N, FFA, and PV, while simultaneously curbing bacterial proliferation. This comprehensive action preserves the prawn's sensory appeal, extends its marketable shelf life, and presents a viable, natural alternative to synthetic preservatives for minimizing post-harvest losses. In the present study, we found that green tea extract shows potential as a natural preservative for vannamei shrimp during chilled storage due to its strong antioxidant properties and high phenolic content. It effectively inhibits polyphenol oxidase (PPO) activity, preventing black spot formation (melanosis). Additionally, it offers a natural alternative to synthetic preservatives.

Conclusion

The study concludes that both sodium metabisulfite (SMBS) and green tea extracts offer substantial protection against melanosis (black spot formation) in vannamei shrimp. While the control group showed a rapid increase in melanosis, especially at room temperature, both treated groups consistently exhibited a significantly lower incidence of black spots. Melanosis progression was considerably slower at temperatures 4°C across all treatments, including the control. Green tea extracts demonstrated commendable anti-melanosis activity, especially in 4°C temperature. The data further indicates a dose-dependent improvement in efficacy, with 3% concentrations of both SMBS and green tea generally leading to fewer melanised pieces compared to 2% concentration.

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