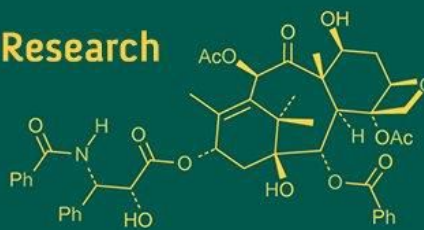


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Mechanistic insights into polyphenol oxidase activity and black spot onset in Aquacultured white leg shrimp (*Litopenaeus vannamei*)

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Abstract

The black spot formation in *Litopenaeus vannamei* kept in chilled storage (2 °C) was examined for 14 days. The crustacean enzyme polyphenol oxidase (PPO) was partially purified through 70% ammonium sulfate precipitation, followed by dialysis. The PPO activities in various parts of *L. vannamei* were assessed using 3,4-dihydroxy phenylalanine (L-DOPA) as the substrate. The melanosis scores considerably ($p < 0.05$) increased during storage, while the PPO activity showed a negative correlation with metric chroma (C). Except for the cephalothorax, correlation matrices revealed that specific PPO activity in various regions was negatively correlated with lightness (L *) and positively correlated with redness (a *) and yellowness (b *). During chilled storage, the carapace exhibited the highest enzyme activity (98.99 U/mg of protein), followed by the cephalothorax (45.98 U/mg of protein). The study found that the black spot formation began in the shrimp's head and progressively spread to other parts, starting on the sixth day under chilled conditions. Organoleptic evaluations indicated that the shrimp maintained acceptable quality for up to six days.

Keywords: Black spot, polyphenol-oxidase, commencement, crustacean, shrimp

1. Introduction

Shrimp forms one of the flagship seafood export commodities from India, which is largely consumed around the globe (Singh *et al.* 2021) [19]. Though it is a highly nutritious and easily digestible protein source, the enzymatic composition of shrimp makes them susceptible to fast spoilage after post-mortem handling and storage via several biochemical reactions (Gonçalves and de Oliveira 2016) [8]. Melanosis, also called black spot in shrimps, forms a major post-harvest loss in marine exports, which causes huge consumer rejection due to quality deterioration (unsightly visual appearance) and huge economic loss to the producer countries. It begins immediately after a few hours of harvest, especially in the head shell segments of crustaceans, which are in chilled and frozen states, and is caused by an enzyme called polyphenol oxidase (Hong *et al.* 2024) [9]. Considering the nutritional significance of fish in developing countries like India, it is imperative that such post-harvest losses at all levels cause significant income loss across the fisheries value chain and hence need to be minimized (Mandal *et al.* 2024) [11].

Enzymes are highly selective biological molecules that act as catalysts that significantly increase the rate and specificity of metabolic reactions. Phenol oxidase (PPO), classified under the oxidoreductase group (EC 1.14.18.1), is a tetrameric enzyme that incorporates four copper atoms per molecule. It features binding sites for two aromatic compounds as well as oxygen (Lv *et al.* 2021) [10]. PPO catalyzes the hydroxylation of monophenol molecules, where the benzene ring has a single hydroxyl group, converting them into *o*-diphenols (phenol molecules with two hydroxyl groups). It can also further catalyze the oxidation of *o*-diphenols to form *o*-quinones. The rapid polymerization of these *o*-quinones (non-enzymatic reaction) with a variety of compounds in the presence of oxygen produces black or brown, insoluble, and high molecular weight pigment (polyphenols) called melanin under the shell of the crustaceans, which causes melanosis. The amino acid tyrosine, which is naturally present in shrimp, contains a single phenolic ring, and can be oxidized by PPOs to produce *o*-quinone. Therefore, PPO is also known as tyrosinase (Fan 2023) [5].

Polyphenol oxidase, found in the haemolymph and cuticle of living shellfish in its inactive pro-enzyme form, becomes activated by proteolytic enzymes during post-mortem storage (Gokoglu *et al.* 2022) [7]. In live crustaceans, this enzyme is essential for primary immune response, cuticle sclerotization, and wound healing (Gonçalves and de Oliveira 2016) [8]. In chilled prawns and shrimps, melanosis typically begins at the head and gradually spreads toward the tail. The rate at which this occurs varies among species, likely due to differences in substrate availability, enzyme concentration, and activities in each species.

The biochemical properties of PPO from the cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) were examined by Nirmal and Benjakul (2012) [16]. Meanwhile, the characterization and tissue distribution of polyphenol oxidase in deep-water pink shrimp (*Parapenaeus longirostris*) were investigated by Zamorano *et al.* (2009) [21]. However, there is no established data in the literature about the spreading pattern of melanosis in white leg shrimp (*Litopenaeus vannamei*), a crucial aquaculture species that holds significant market value globally and represents 70% of total shrimp production.

Surface melanosis has been of great concern to researchers as well as shrimp processors due to its unfavourable color changes, which reduce the market value of the shrimp. Therefore, the current investigation aimed to study the PPO activity in different parts of white leg shrimp (*L. vannamei*) with color changes, melanosis assessment, and organoleptic properties under chilled storage. Additionally, the pattern of melanosis spread could be valuable in explaining its development and formulating effective strategies to control it.

2. Materials and Methods

2.1 Reagents

L-β-(3, 4-dihydroxyphenyl) alanine (L-DOPA), Triton X 100, polyvinyl Pyrrolidone (PVP), sodium dihydrogen phosphate, disodium hydrogen phosphate, ammonium sulphate, and purified sea sand were purchased from Sigma-Aldrich (St. Louis, MO, USA). Albumin Bovine fraction V powder was taken by Merck (Darmstadt, Germany).

2.2 Shrimp collection

Freshly harvested, additive-free shrimps (*L. vannamei*) were sourced from a private farm in Surat, India, with a count of 30-35 shrimp per kilogram. The shrimp were packed with ice at a ratio of 1:2 (w/w) and transported to the laboratory ICAR-CIFE, Mumbai within 3-4 hours. Upon arrival, whole shrimps were packed in 200-gauge HDPE pouches and stored in a low-temperature freezer (at 2 °C) under-saturated relative humidity for subsequent analysis.

2.3 Sampling of individual tissues

For a sampling of individual tissues of shrimp, seven organs (cephalothorax, carapace, abdomen exoskeleton, pereopod, pleopod, uropod, and telson) were selected as shown in Plate 1. The development of blackspots in those organs was evaluated for every alternative day till the shrimp became unaccepted by the panellists.

2.4 Enzyme (PPO) extraction from different body parts of shrimp

Enzyme extraction from shrimp was done according to the methodology stated by Choudhury and Chakrabarti (2001)

[2] with some minor modifications. The cephalothorax, carapace, abdomen exoskeleton, pleopod, pereopod, uropod and telson of about ten shrimps were taken separately in a mortar and ground with purified sea sand in chilled condition and extracted with two parts its weight of cold 10 mM sodium phosphate buffer (pH 7.0) containing 0.5% triton and 0.5% polyvinyl pyrrolidone (PVP) with pH 4.0. The homogenate was strained through cloth. The obtained suspension or homogenate was stirred for 30 minutes at 4 °C, then centrifuged at 8000 g for 30 minutes at 4 °C using a refrigerated centrifuge (Eltek centrifuge, Model: MP 400R, Elektrocraft Pvt. Ltd. India). The supernatant was filtered through muslin cloth and called a crude PPO soluble fraction.

2.5 Partial purification of the enzyme PPO

Partial purification of PPO obtained from different body parts of shrimp was done by the precipitation method using solid ammonium sulfate followed by dialysis based on the modified method of Manheem *et al.* (2012) [12]. Saturated ammonium sulfate @ 70% was added to the supernatant and allowed to stand for 30 min at 4 °C. The precipitate was collected at 12,000 g for 30 minutes at 4 °C by centrifugation using a refrigerated centrifuge. The resulting pellet was dialyzed against 15 volumes of the same buffer in a dialysis bag (MW cut off < 12-14 kDa) at 4 °C, with the buffer being replaced three times over 24 hours. The pellet was dissolved in a small volume of 0.05 M sodium phosphate buffer (pH 7.2), and insoluble components were eliminated by centrifugation at 3000 g for 30 minutes at 4 °C. The resulting supernatant was then collected and used as the “purified PPO extracts”.

2.6 Enzyme Assay of PPO

The enzyme activity of PPO in different body parts of shrimp was assessed using L-DOPA as the substrate based on the modified method of Basiri *et al.* (2015) [1]. The assay mixture included 100 µl of crude PPO extract, 600 µl of 15 mM L-DOPA in deionized water, 400 µl of 0.05 M phosphate buffer (pH 6.0), and 100 µl of deionized water. The reaction mixture was incubated for 3 min at 45 °C to initiate the reaction, which was then allowed to proceed for an additional 3 min at the same temperature. The formation of dopachrome was monitored every 30 seconds by measuring the absorbance at 475 nm (A_{475}) using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). One unit of PPO activity was defined as a 0.001 increase in absorbance per minute. Enzyme and substrate blanks were prepared by omitting the enzyme and substrate, respectively, from the reaction mixture with deionized water. All assays were conducted in triplicate, and enzyme activity was reported as units per mg of protein.

2.7 Determination of protein

The Biuret method (Fang *et al.* 2022) [6] was followed to determine the protein content in all organs used for the PPO assay. The protein concentrations were calculated by interpolating the values on a calibration standard curve with bovine serum albumin (BSA), measured at 540 nm.

2.8 Measurement of colour

The colour parameters were analyzed using the colorimeter (Hunter Lab scan XE, U.S.A.) from different parts (cephalothorax, carapace, abdomen exoskeleton, pereopod,

pleopod, uropod, and telson) of shrimp at room temperature using the method followed by Senapati *et al.* (2017) [19]. The analysis was carried out in triplicates with prior proper calibrations using standard coloured (black and white) tiles.

2.9 Organoleptic evaluation

Throughout the storage period, the organoleptic quality of the shrimp, including its shell colour, meat colour, odor, taste, flavour, freshness, and general acceptability, was assessed. The shrimp were peeled, cooked for about a minute to reach a core temperature of 60 °C, and then served solely for taste and flavor. Unpeeled samples in a separate tray with random coding were used to assess other parameters. The panellists had no shrimp allergies and were familiar with eating shrimp. On a nine-point hedonic scale sheet, each panellist (n = 10) was asked to rate and provide an overall score, with 09 representing "like extremely," 07 representing "like moderately," 05 representing "neither like nor dislike," 03 representing "dislike moderately," and 01 representing "dislike extremely". (Nirmal and Benjakul, 2011) [15].

2.10 Melanosis Assessment

10 trained panellists used a 10-point scoring test (Montero *et al.* 2001) [13] to visually inspect white leg shrimp for melanosis or blackening. Ten shrimp samples were chosen randomly, and their melanosis scores were assessed throughout storage. Panellists were asked to rate their level of melanosis on a scale of 0 to 10, with 0 denoting absence, 2 denoting slightness (affecting up to 20% of the shrimps' surface), 4 denoting moderateness (20-40%), 6 denoting notableness (affecting 40-60%), 8 denoting severity (affecting 60-80% of the shrimps' surface), and 10 denoting extreme weight (affecting 80-100% of the shrimps' surface).

2.11 Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to assess the variation throughout the storage periods of white-leg shrimp. Then Post hoc analyses were performed using Duncan's multiple range test to compare the means ($p < 0.05$) of the parameters evaluated for PPO-specific activity. The results are presented as means $\pm$ SE. Additionally, correlation matrices were performed between the specific activity of PPO and the CIE color values to explore their relationship. All statistical analyses were done using SPSS, version 16.0 for Windows.

3. Results and Discussion

3.1 Specific activity of PPO in crude extract from different organs during chilled storage

The PPO-specific activities in crude extract from different organs of the shrimp throughout the storage are depicted in Figure 1. From the results, the crude extracts from different parts of the shrimp showed high activity up to the 6th day of storage. The carapace exhibited the highest activity compared to all other samples, possibly due to interference in the assay from turbidity in the extract. However, this concern was resolved by the absence of PPO activity in the partially purified PPO extract, which was obtained through solid ammonium sulfate precipitation at a 70% concentration, followed by dialysis. This precipitation technique has been used by some researchers for PPO purification from different crustaceans (Zhang *et al.* 2021) [22]. After dialysis, the carapace extract showed the highest

PPO activity, followed by the cephalothorax, abdomen exoskeleton, telson, uropod, and pereopod. The pleopod extract displayed very low activity (Table 1).

Typically, the reaction between PPO and phenolic substrates during enzyme extraction can result in enzyme precipitation and loss of activity (Manheem *et al.* 2012) [12]. In many studies, protective agents were not incorporated into the extraction buffer to prevent this loss, so longer extraction times could promote enzyme-substrate interactions, lead to a decrease in PPO activity. In the present experiment, the inclusion of polyvinyl pyrrolidone (a phenol scavenger) in the extraction buffer likely prevented this negative effect.

Zamorano *et al.* (2009) [21] examined the distribution of PPO in the tissues and organs of various crustaceans, including tiger shrimp, crayfish, two species of deep-sea crab, snow crab, and swimming crab using crude extracts. Their findings indicated that diphenol oxidase activity was exclusively present in the haemolymph and gills or the haemolymph of two species-tiger shrimp (*Penaeus japonicus* and crayfish *Cambarus clarkii*), respectively. PPO's monophenol oxidase (tyrosinase) activity was detected in the gills. PPO activity was measured in crude extracts of various imperial tiger prawn tissues by Montero *et al.* (2001) [13]. The carapace had the highest activity of all the organs, followed by the telson, while the abdomen and exoskeleton showed the lowest activity. However, PPO was only discovered from the carapace, when they conducted additional research using ammonium sulfate precipitation.

3.2 Specific activity of PPO in dialyzed extract from different shrimp organs

PPO-specific activity in dialyzed extract collected from different shrimp organs during chilled storage is given in Table 1. Melanosis begins with the enzymatic action of PPO, and its mechanism has already been discussed. In this study, we aimed to investigate the role of different organs in the commencement of melanosis during the chilled storage of whole shrimp (Plate 2). In the fresh sample, PPO activity was detected only in the carapace and cephalothorax. The blackening in the entire shrimp began on the second day of storage, starting in the ventral region of the head (cephalothorax and carapace). Then it extended up to the dorsal area and little to the carapace, 1st segment of the abdomen exoskeleton, and telson (on 4th day). Then it continued to pereopod and a little to pleopod and uropod (on the 6th day). The highest activity was detected on the 6th day, i.e., in the carapace (98.99 units/mg of protein). Then the black spot dispersed to the exoskeleton of the first three abdominal segments (28.08 units/mg of protein) on the 8th day. On the 10th and 12th days of storage, telson got the highest PPO activity among all (8.31 and 25.37 units/mg of protein). This might be due to the spreading of melanosis towards the tail region. But on the last day (on the 14th day), the black spot expanded towards the cephalothorax and showed high activity (45.98 units/mg of protein) after the carapace during the whole storage period. Specific PPO activity 95.7 (u/mg) was found by Nirmal and Benjakul (2012) [16] using ammonium sulphate fractions from the cephalothorax of pacific white shrimp (*L. vannamei*).

During the storage period, the PPO activity in the cephalothorax increased significantly ($p < 0.05$). This enzyme activation could be attributed to the action of metal-dependent serine proteases (Derardja *et al.* 2019) [3]. Zamorano *et al.* (2009) [21] found elevated enzyme activity

in carapace extracts and noted significant melanosis in the cephalothorax and head after only 1 day of storage at 4 °C when they assessed PPO activity in partially purified extracts from various parts of deep-water pink shrimp (*Parapenaeus longistris*). Nevertheless, there was a reduction of PPO activity on the 14th day of storage in all organs except the cephalothorax. This reduction could be related to PPO degradation by proteases, especially thiol proteases (Derardja *et al.* 2019) [3].

There was no noticeable melanosis in the pleopod or pereopod by visual inspection. Still, we got the higher PPO activity in pereopod on the 8th day and pleopod on the 10th day. Similarly, Zamorano *et al.* (2009) [21] reported that melanosis was not observed in the carapace even after 7th day of storage at 4 °C. This indicates that the formation of black spots in different parts of the shrimp is influenced by multiple factors, not just PPO levels. These include the presence of proteases that activate proPPO, the concentration of PPO substrates, the moulting stage, and the occurrence of non-enzymatic melanin formation (Zamorano *et al.* 2009) [21]. Typically, the formation of black spots started spreading to different parts of the shrimp from the 6th day onward. The melanosis scores given by the panellists were also higher after the 6th day of storage.

The zymogens (proPPOs) that are synthesized from nearly all arthropod PPOs are activated by proteolysis by the enzyme serine protease, which is present in the haemolymph of living animals (Derardja *et al.* 2019) [3]. In response to an invading microbial threat, a series of serine proteases are activated in succession, ultimately triggering a terminal protease that acts as a pro-phenol oxidase activating protease/enzyme/factor (PAP/PPAE/PPAF) within the cascade. Therefore, the activation of proPPO is expected to occur first in the cephalothorax, particularly in the gill region (Zamorano *et al.* 2009) [21], which was observed in our experiment. Hepatopancreas extracts can activate cuticle proPPO. This finding aligns with our results, where melanosis is initiated more quickly in the cephalothorax and head, regions where the hepatopancreas is located (Sae-Leaw and Benjakul 2019).

3.3 Colour analysis by instrumental method

Color is a key characteristic that defines the quality attributes of food (Du and Sun 2011). The relationship between the instrumental colors of white leg shrimp and the specific activity of PPO is represented in Table 2. When determining the color spread during melanosis, this instrumental analysis is typically utilized as supporting data.

Correlation matrices concluded that, aside from cephalothorax, specific PPO activity in various parts had a positive correlation with redness (a^*) and yellowness (b^*), but a negative correlation with lightness (L^*). Cephalothorax showed negative relationship with L^* ($r = -0.941^{**}$), a^* ($r = -0.367$) and b^* ($r = -0.579^{**}$) values. Other than carapace and pereopod, L^* was significant in cephalothorax, pleopod, uropod, and telson at 0.01 levels and in abdomen exoskeleton at 0.05 levels during the end of chilled storage. However, yellowness was significant in cephalothorax, pleopod, abdomen exoskeleton, uropod, and telson at 0.01 levels during the entire storage period.

Changes in color intensity of different parts are depicted in Figure 4. Metric chroma C^* quantifies color vividness and describes color saturation (Okpala *et al.* 2014) [17]. C^* increased significantly ($p < 0.05$) in all parts of shrimp except cephalothorax and pereopod during storage. But in the case of cephalothorax, there was a slight increase or decrease of chroma, and a significant reduction was observed at the 12th (1.40) and 14th (2.14) days of storage, which can be correlated with high PPO activity during that period. C^* value increased up to the 10th day (10.21) and decreased (1.66) at a later stage in the pereopod, which again correlated with less PPO activity during that period. The lightness (L^*) of all organs was significantly decreased during the whole storage (data not shown). However, there was a negative relationship established between metric chroma and the specific activity of PPO.

3.4 Melanosis and organoleptic evaluation

When the shrimp were kept in chilled conditions of storage, their melanosis and organoleptic characteristics (shell color, meat color, odor, taste, flavor, freshness, and overall acceptability) were assessed and depicted in Figure 2 and Figure 3, respectively. Organoleptic scores by the panellists for every parameter significantly ($p < 0.05$) declined with increasing storage time, which was evaluated on a 9-point hedonic scale. Similarly, after the first day of storage, there was a significant increase ($p < 0.05$) in the melanosis scores determined by visual inspection. After six days of storage, the low colour values of the meat and shell were consistent with the higher melanosis scores (Figure 4). By this time, 60-80% of the shrimp's surface was covered in black spots, which was disliked by the panellists. This result may be related to the findings of Senapati *et al.* (2017) [19], who found that in untreated shrimp kept in cold storage, the black spot progressed after one day.

Table 1: Specific activity of PPO in dialysed extract from different organs of white LEG Shrimp (*L. vannamei*) During Chilled Storage (U/mg of protein)

Organs	Fresh	2 nd Day	4 th Day	6 th Day	8 th Day	10 th Day	12 th Day	14 th Day
Cephalothorax	0.06±0.00 ^a	0.55±0.00 ^a	1.99±0.18 ^{ab}	2.49±0.18 ^{ab}	2.96±0.35 ^{abc}	4.02±0.56 ^{bc}	5.86±0.42 ^c	45.98±2.65 ^d
Carapace	1.79±0.90 ^a	1.97±0.99 ^a	32.91±0.00 ^d	98.99±0.00 ^e	17.47±2.18 ^c	6.38±2.13 ^b	7.31±0.43 ^b	8.32±0.42 ^b
Pereopod	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	6.90±3.45 ^b	12.49±3.12 ^c	2.75±0.79 ^{ab}	1.64±0.41 ^{ab}	2.79±0.40 ^{ab}
Pleopod	0.00±0.00 ^a	0.00±0.00 ^a	1.55±0.00 ^{ab}	2.83±0.00 ^{bc}	3.46±0.43 ^{cd}	4.90±0.98 ^d	3.53±0.71 ^{cd}	3.91±1.03 ^{cd}
Abdomen Exoskeleton	0.00±0.00 ^a	0.00±0.00 ^a	7.63±1.91 ^{bc}	11.70±2.34 ^c	28.08±2.16 ^d	4.98±0.38 ^b	7.67±1.01 ^{bc}	9.63±1.02 ^c
Uropod	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	5.82±0.89 ^{bc}	10.21±2.55 ^{cd}	4.87±0.49 ^b	13.65±2.73 ^d	6.88±1.46 ^{bc}
Telson	0.00±0.00 ^a	0.00±0.00 ^a	4.72±0.00 ^{ab}	13.12±0.00 ^{cd}	6.77±0.00 ^b	8.31±0.00 ^{bc}	25.37±3.17 ^e	15.73±4.54 ^d

Values are mean (SE), n = 3

Means on the same row with different superscripts are significantly different at ($p < 0.05$)

Table 2: Corelation between different organs and CIE Colour values (L*, a*, b*) of white LEG shrimp (*L. vannamei*) during chilled storage

Cephalothorax

	Specific activity of PPO	L*	a*	b*
Specific activity of PPO	1			
L*	-0.941**	1		
a*	-0.367	0.478*	1	
b*	-0.579**	0.640**	0.800**	1

* Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Carapace

	Specific activity of PPO	L*	a*	b*
Specific activity of PPO	1			
L*	-0.121	1		
a*	0.041	-0.442*	1	
b*	0.272	-0.860**	0.507*	1

* Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Pereopod

	Specific activity of PPO	L*	a*	b*
Specific activity of PPO	1			
L*	-0.402	1		
a*	0.165	-0.891**	1	
b*	0.348	-0.049	-0.033	1

* Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Pleopod

	Specific activity of PPO	L*	a*	b*
Specific activity of PPO	1			
L*	-0.660**	1		
a*	0.680**	-0.617**	1	
b*	0.833**	-0.677**	0.792**	1

* Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Abdomen Exoskeleton

	Specific activity of PPO	L*	a*	b*
Specific activity of PPO	1			
L*	-0.465*	1		
a*	0.308	-0.835**	1	
b*	0.531**	-0.420*	0.335	1

* Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Uropod

	Specific activity of PPO	L*	a*	b*
Specific activity of PPO	1			
L*	-0.763**	1		
a*	0.613**	-0.835**	1	
b*	0.620**	-0.804**	0.532**	1

* Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Telson

	Specific activity of PPO	L*	a*	b*
Specific activity of PPO	1			
L*	-0.833**	1		
a*	0.677**	-0.903**	1	
b*	0.907**	-0.905**	0.813**	1

* Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

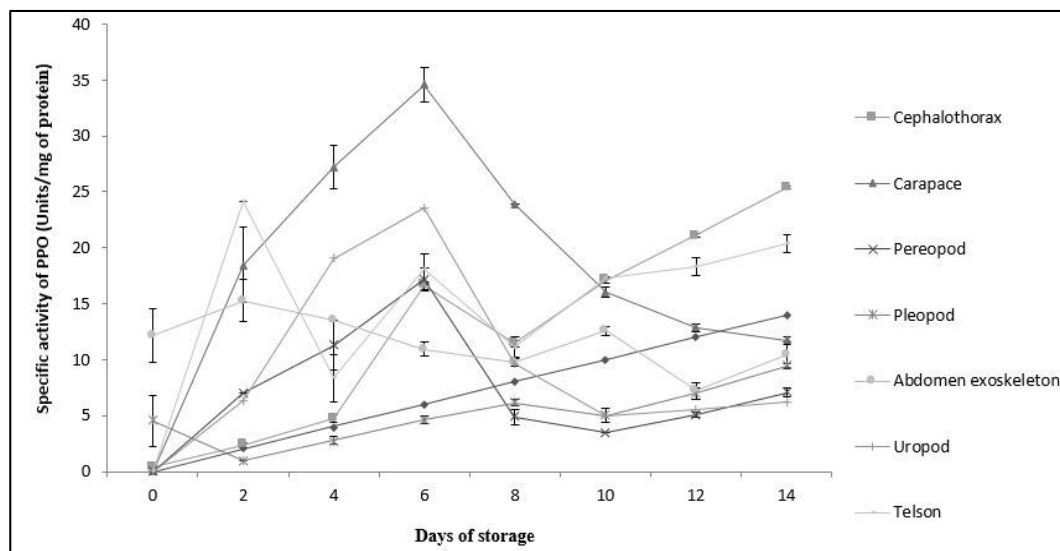


Fig 1: Specific activity of PPO in crude extract from different organs of white leg shrimp (*L. vannamei*) during chilled storage

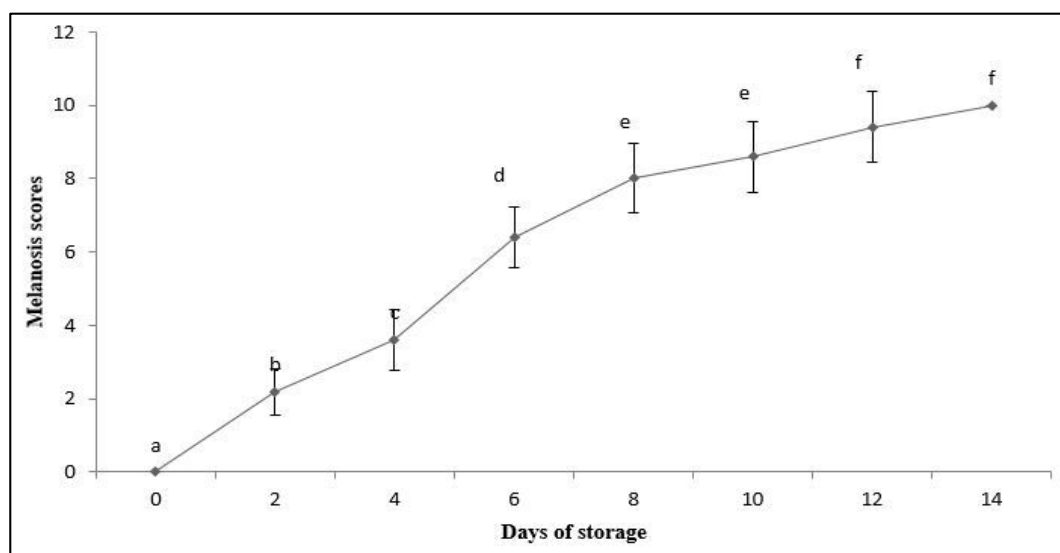


Fig 2: Changes in melanosis scores of white LEG Shrimp (*L. vannamei*) During Chilled Storage

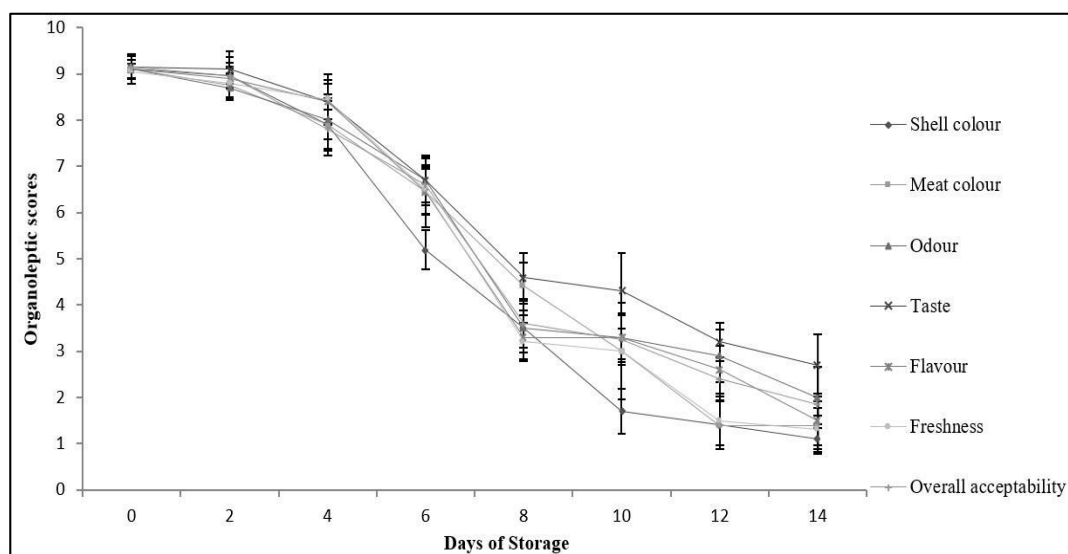
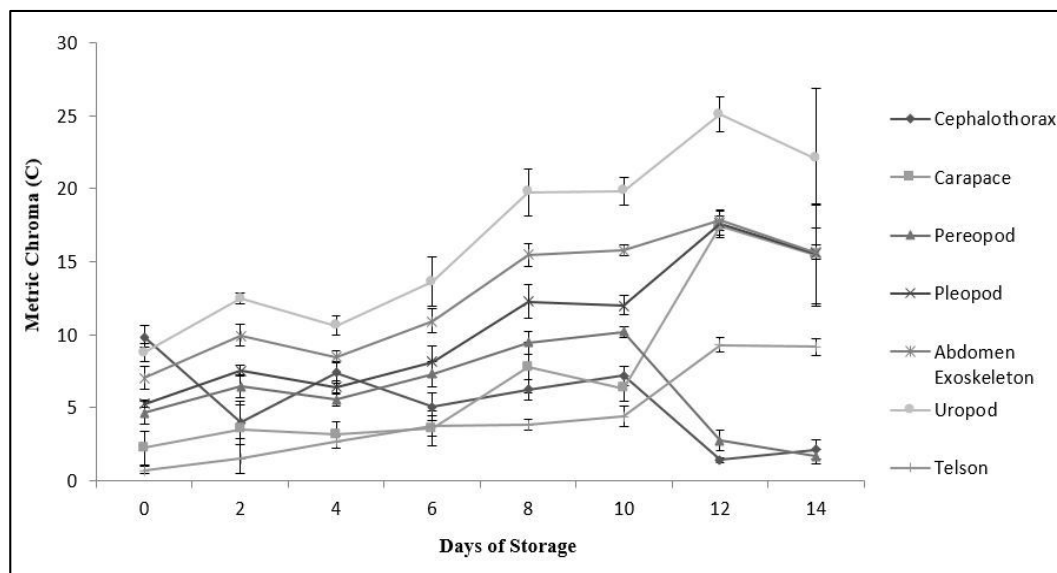


Fig 3: Changes in organoleptic scores of white LEG Shrimp (*L. vannamei*) During Chilled Storage



Values are mean (SD), n = 3

Fig 4: Changes of colour intensity in different parts of white leg shrimp (*L. vannamei*) during chilled storage

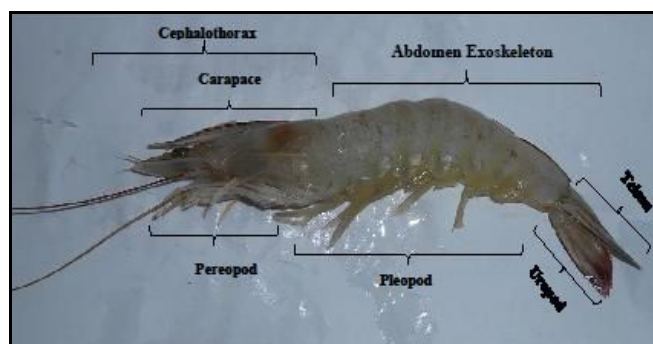


Plate 1: Sampling Parts of White Leg Shrimp (*L. vannamei*)

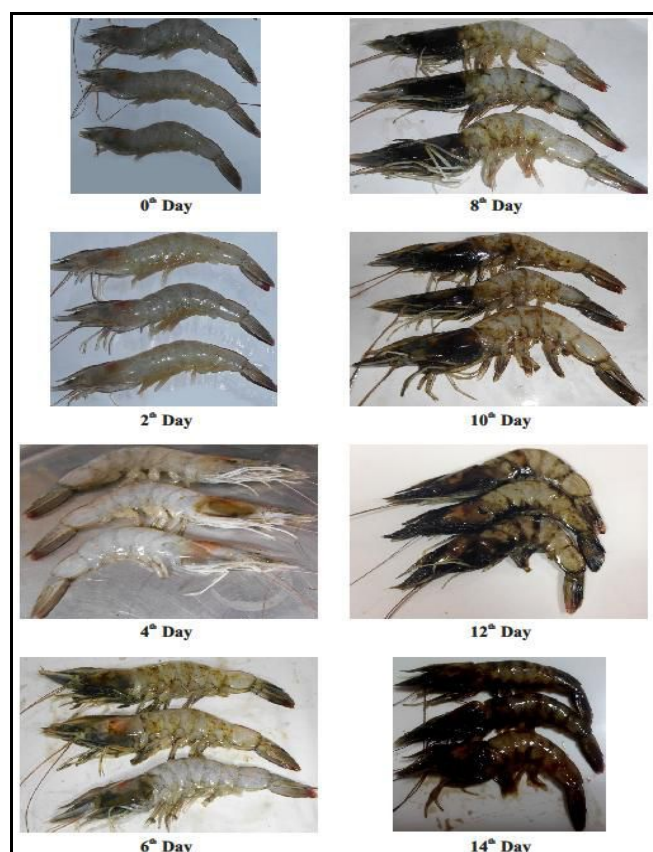


Plate 2: Melanosis in white leg shrimp (*Litopenaeus vannamei*)

4. Conclusions

The dispersal or spreading pattern of melanosis between the cephalothorax and the abdomen of white leg shrimp was determined. Using 70% ammonium sulfate fractions, the exoskeleton sections between the head and the abdomen showed the highest specific activity. Melanosis progressed from the head and spread from the 6th day onwards into various other parts of the shrimp. Statistical correlation concluded that there was a negative relationship established between metric chroma and the specific activity of PPO. The shrimps were rejected by the panellists after 6th days of storage as reflected in melanosis scores. This study could provide valuable insights for the shrimp industry in developing an effective plan to prevent the black spot in white leg shrimp.

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