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Melanosis and quality attributes of chill stored farm raised whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract: Loss of market value of shrimp is mainly due to the formation of black spot called melanosis. A study was conducted for 14 days to determine the extent of melanosis and quality changes during that period of freshly harvested whiteleg shrimp (*Litopenaeus vannamei*) under chilled storage (2°C). Among quality parameters,total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBAR-S), were varied from 13.17 mg % to 44.50 mg % and 0.04to 2.57 mg malondehaldehyde/kg of fat respectively whereas melanosis score and metric chroma (C) exhibited significant increases during chilled storage ($P<0.05$). There was a slight increase in moisture, crude fat and pH from 73.96 % to 74.57 %, 1.05 % to 1.14 % and 6.52 to 7.60 respectively at 14th day of storage. Loss of protein from 22.51 % to 21.28 % may be due to decrease in available amino acids during chilled storage and total plate count (TPC) showed gradual increase of bacterial load up to $1.73*10^7$ log CFU/g at the end of chilled storage. The sensory analysis by panellists indicated, the acceptability of whiteleg shrimp was up to 6 days in chilled condition and formation of black spot is one of the major parameter for rejection by the panellists.

Keywords: *Litopenaeus vannamei,* Melanosis, Quality, Sensory characteristics

INTRODUCTION

The global capture and culture production of whiteleg shrimp (*Litopenaeus vannamei*) is 10924 tonnes and 3668681 tonnes respectively in 2014 (FAO Fish Stat., 2016). It is an important aquaculture species having high market value in all over the world and leading farm-raised species in the western hemisphere, representing more than 99 % of production. *L. vannamei* is mainly consumed in the North, Latin America, Europe and Asian countries. The market value of shrimp is generally based on the visual appearance of their body colour. The appearance of product and the resulting quality implications play a significant role in maintaining a high consumer acceptance. Shrimps are very perishable with short shelf life and susceptible to black spot formation called melanosis during post-mortem handling and storage. Melanosis starts in frozen and refrigerated crustaceans after a few hours of capture and it is brought on by the action of polyphenol oxidase, which oxidizes phenols to quinones. Polymerization of this quinones give rise a black pigment, which are not dangerous to human health, but affects the crustaceans appearance and loses its market value (Montero *et al*., 2001).

Apart from melanosis, quality of shrimp is affected due to several changes like lipid oxidation, protein denaturation during chilled storage (Imran *et al*., 2013). Colour, flavour, taste are also affected due to bacteriological activity during storage period (Sriket *et al*., 2007). pH is also one of the most important quality parameter during storage which is related with the growth of microbes in the sample. This is an important index for determining the quality of fish (Okeyo *et al*., 2009). The main reason of spoilage in fresh seafood is due to the activity of microorganisms which leads to undesirable flavours and odours. According to Qingzhu (2003), total viable count (TVC) is used as the acceptability index in standards, guidelines and specifications. Similarly, total volatile basic nitrogen (TVB-N) is an indicator of seafood freshness mainly due to spoilage bacteria (Huss, 1995).

Besides the protein and lipid fractions, deterioration in sensory quality, loss of nutritional value and changes in physico-chemical properties has been reported by Bennour *et al.*, 1991, Nunes *et al*., 1992. Riaz *et al.* (1990) has reported about the chemical changes like lipid oxidation, volatile basic nitrogen in shrimp quality during frozen storage. Similarly, the shelf life of shrimp at variable temperature of frozen conditions has been studied by Tsironi *et al.* (2009). They selected colour, texture, pH, microbial load, and T-VBN as the indices of quality for frozen shrimp. Specifically, the breakdown of fat and proteins are important stages of

decomposition observed in fishery products which are kept either chilled or in frozen condition (Essien,1995 and Fatima *et al*., 1988).

As *L. vannamei* is one of the most important export item among shrimps, unfavourable colour change associated with melanosis on its surface and quality changes during that period has been of great concern to food processors. Based on this background, the aim of present investigation was to study the extent of melanosis on whiteleg shrimp (*L. vannamei*) as well as to monitor the physico-chemical, microbial and sensory properties during melanosis in chilled storage.

MATERIALS AND METHODS

Shrimp collection and preparation: Whiteleg shrimp (*L. vannamei*) with the count of 30–35 shrimps/kg were purchased from a farm in Surat, India. The shrimp, freshly caught and completely free of additives, were kept in ice with a shrimp: ice ratio of 1:2 (w/w) and transported to the Department of Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Mumbai within 3 hours. Upon arrival, shrimp were stored in a low temperature freezer (at 2℃). Time zero was taken as the day of harvest. Physico-chemical, microbial, sensory and melanosis assessments were done in every alternative day up to14 days.

Physico-chemical analysis: Moisture, crude protein, pH, and ash contents were measured following standard method (AOAC, 2000). Differences in weight were recorded after drying the sample in hot air oven at 100 ± 5℃ overnight to determine the moisture content. The crude protein content was measured by using the micro -kjeldahl method. Ten-gram samples were homogenized with 50 ml distilled water in a homogenizer (Polytron system PT 2100, Kinematica, AG, Germany) for 30s and pH value of fish homogenate was measured by a digital pH meter (Eutech tutor pH/ ^{0}C meter, Eutech Instruments, Singapore) standardized earlier by buffers at pH 4 and 9. Ashing was done by incineration in a muffle furnace at 550 ± 50 ℃ until white ash was obtained. Ten percent trichloroacetic acid (TCA) extract was used to estimate total volatile basic nitrogen (TVB-N) by using Conway's micro-diffusion method (Conway, 1947) and expressed as mg %. Crude fat was measured by Soxhlet extraction with diethyl ether. Thiobarbituric acid reactive substances (TBARS) were determined by the titrimetric method of Tarladgis*et al*. (1960) using thiobarbituric acid standard in 90 % glacial acetic acid and expressed as mg malondehaldehyde/kg of fat.

Determination of colour attributes: The colour measurements were carried out using the Hunter *L*, *a*, and *b* scales, according to the method of Arfat and Benjakul, (2012) with slight modifications to evaluate the surface of shrimp samples. All colour determinations were carried out three times on different shrimp samples. The colorimeter (Hunter Lab scan XE, U.S.A.) employed was calibrated first with a black standard followed by a white standard to obtain the final setting, both placed centrally over the sample port. The dressed shrimp meat (~5 g) was placed in an optically clear glass cup, which was placed on the port and covered with an opaque cup cover. The opaque cup cover whilst accommodating the shrimp sample contained, it excluded any external light interference. In this colour system, the L* variable represents lightness (L*= 0 for black, L* = 100 for white). The a* scale represents the red/ green (+a* is red and –a* is green) and the b* scale represents the yellow/ blue (+b* is yellow and –b* is blue) (Hunterlab, 1996). The measurements were performed at room temperature. The parameters metric chroma ($C = (a^{*2} + b^{*2})^{0.5}$) was calculated using a* and b* values. Chroma is expressed as saturation or intensity and clarity of the colour. Metric chroma describes the saturation as well as measure of vividness of colour (Senapati *et al*., 2016).

Sensory evaluation: Organoleptic quality such as shell colour, meat colour, odour, taste, flavour, freshness and overall acceptability of whiteleg shrimp was

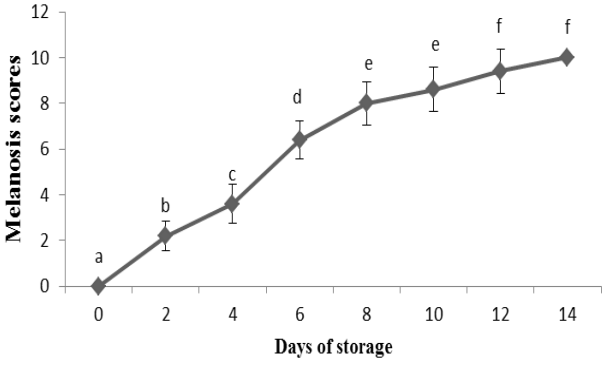

Fig. 1. *Changes in melanosis scores of whiteleg shrimp (L. vannamei) during chilled storage.*

Values are mean (SD), n = 3, Different small letters in the barsare significantly different at ($p < 0.05$)

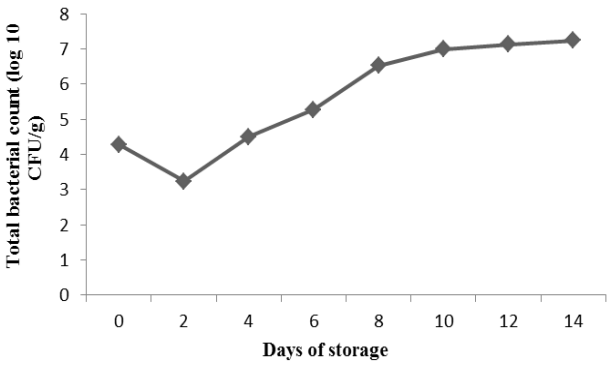

Fig. 2. *Change in total bacterial counts of whiteleg shrimp (L. vannamei) during chilled storage.*

Values are mean (SD), n = 3, Different small letters in the barsare significantly different at ($p < 0.05$)

Table 1. Changes in proximate composition of whiteleg shrimp (*L. vannamei*) during chilled storage.

Paramaters	Fresh	2nd Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
Moisture (%)	73.96^{ab}±0.16	74.65^{c}±0.32	73.58^{a}±0.39	73.66^{a}±0.19	74.33^{bc}±0.65	74.32^{bc}±0.59	74.30^{bc}±0.23	74.57^{bc}±0.22
Crude protein (%)	22.51^{bc}±0.13	22.04^{abc}±0.08	22.87^{c}±0.51	21.93^{ab}±0.65	21.38^{a}±0.85	21.81^{ab}±0.33	21.85^{ab}±0.35	21.28^{a}±0.55
Crude fat (%)	1.05^{a}±0.07	1.07^{a}±0.10	1.13^{a}±0.16	1.11^{a}±0.14	1.14^{a}±0.10	1.17^{a}±0.12	1.20^{a}±0.09	1.14^{a}±0.22
Ash (%)	1.96^{b}±0.10	1.99^{b}±0.09	1.90^{b}±0.05	1.80^{b}±0.07	1.54^{a}±0.09	1.53^{a}±0.21	1.49^{a}±0.27	1.38^{a}±0.07

Values are mean (SD), n = 3, Means on the same row with different superscripts are significantly different at ($p < 0.05$)

Table 2. Changes in TVB-N, TBAR-S and pH of whiteleg shrimp (*L. vannamei*) during chilled storage.

Paramaters	Fresh	2nd Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
TVB-N (mg %)	13.17 ±0.34[a]	14.10±0.64[a]	16.68±0.17[b]	18.51±0.31[c]	26.13±1.02[d]	30.53±0.47[e]	36.91±0.90[f]	44.50±0.43[g]
TBAR-S (mg malondehaldehyde /kg of fat	0.04±0.01[a]	0.15±0.05[b]	0.47±0.03[c]	0.93±0.01[d]	1.24±0.04[e]	1.40±0.01[f]	2.18±0.05[g]	2.57±0.04[h]
pH	6.52±0.08[a]	6.87±0.15[b]	7.08±0.17[bc]	7.18±0.03[cd]	7.40±0.10[de]	7.47±0.06[e]	7.40±0.30[de]	7.60±0.10[e]

Values are mean (SD), n = 3, Means on the same row with different superscripts are significantly different at ($p < 0.05$)

evaluated throughout the storage time. The shrimps were peeled, cooked for 5 minutes and given only for taste and other parameters were evaluated by unpeeled samples in another plate with random coding. Panellists were acquainted with shrimp consumption and had no allergies to shrimp. All panellists (n=10) were asked to judge and give the overall score on a 9 point Hedonic scale where 9 = like extremely; 7 = like moderately; 5 = neither like or nor dislike; 3 = dislike moderately; 1 = dislike extremely. (Nirmal *et al*. 2011)

Melanosis Assessment: Melanosis or blackening of whiteleg shrimp was evaluated through visual inspection by ten trained panellists using 10-point scoring test (Montero *et al*., 2001). Samples (10 shrimps) were selected randomly and evaluated for melanosis score, throughout the storage time. Panellists were asked to give the melanosis score (0–10), where 0 = absent; 2 = slight (up to 20 % of shrimps' surface affected); 4 = moderate (20–40 % of shrimps' surface affected); 6 = notable (40–60 % of shrimps' surface affected); 8 = severe (60–80 % of shrimps' surface affected); 10 = extremely heavy (80–100 % of shrimps' surface affected).

Microbiological analysis: Total plate count (TPC) analysis was carried out as per the spreadplate technique. Whole shrimp sample (10 g) placed in sterileplastic stomacher bag was homogenised (60 s) with 90 mL of thephysiological saline (0.85 %) using a BagMixer. A serial 10-fold dilution of thehomogenate was prepared and 0.1 mL aliquots were pipetted intosterile petri dishes. About 10-15 mL aliquot of molten autoclaved Plate Count Agar (PCA) (Hi-media, India) was poured into the petri dish and gently swirled 2-3 times. Plates were incubated for 48 hours at 37 ℃ and counted. The microbiological analysis was conducted in duplicates and the results were expressed as logarithm of colony forming units (log CFU/g) of shrimp muscle (Arashisara *et al*., 2004; Sallam, 2007).

Statistical analysis: Analysis of variance (one way - ANOVA) was performed to determine the differences between storage periods of *L. vannamei* followed by Duncan's multiple range tests (for Post hoc analyses) to compare the means ($p<0.05$) of parameters analysed during the experiment. The results are presented as means ± SD. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 16.0 for windows).

RESULTS AND DISCUSSION

Changes in proximate composition of whiteleg shrimp during chilled storage: Changes in proximate composition of *L. vannamei* are given in Table 1. The moisture, protein, fat and ash of fresh shrimp were 73.96 %, 22.51 %, 1.05 % and 1.96 % respectively.

The moisture content varied between 73.96 % and 74.57 %. On the 2nd day, the slight increase in moisture content (74.65 %) might be due to the sudden temperature difference in the low temperature freezer comparing with room temperature. Thereafter, there was a slight decrease in moisture content on 4th and 6th day of sampling. This decrease in moisture content might be due to sublimation of surface water of the meat in the refrigerator. However, no significant difference ($p>0.05$) was observed in the moisture content in later stages. It can be compared with the moisture differences reported by Huidobro *et al*. (2002) during iced-stored deep water pink shrimp which were processed on-board a ship. The ash content of species is an indication of mineral concentration in the organisms (Anon, 1995). The ash content was ranged from 1.96 % to 1.38 %. There was a slight reduction in crude protein content of whiteleg shrimp from 22.51 % to 21.28 %. This decrease might be related to the increased microbial growth resulted from higher water activity (aw) and enzymatic autolysis at low temperature (Kandeepan and Biswas, 2007). Another reason might be due to the loss of available amino acids during storage as reported by Fatima *et al*.(1988). Ko-

Table 3. Colour changes in muscles of whiteleg shrimp (*L. vannamei*) during chilled storage.

Parameters	Fresh	2nd Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
L*	31.51^{cd}±1.06	32.45^{d}±0.91	31.56^{cd}±0.89	32.59^{d}±1.88	41.14^{e}±1.02	30.29^{bc}±0.78	29.12^{ab}±1.13	27.61^{a}±1.13
a*	-0.45^{c}±0.12	-0.53^{bc}±0.08	-0.54^{bc}±0.11	-0.79^{ab}±0.10	-0.66^{abc}±0.13	-0.80^{ab}±0.09	-0.92^{a}±0.26	-0.85^{a}±0.22
b*	2.71^{a}±0.55	3.15^{a}±0.60	3.38^{ab}±0.46	4.48^{b}±0.66	5.92^{c}±0.82	8.12^{d}±0.91	8.48^{d}±0.68	8.89^{d}±0.80
C	2.75^{a}±0.55	3.19^{a}±0.60	3.42^{ab}±0.46	4.55^{b}±0.65	5.96^{c}±0.80	8.16^{d}±0.91	8.54^{d}±0.65	8.93^{d}±0.78

L*: Lightness, a*: Redness, b*: Yellowness, C: Metric chroma, Values are mean (SD), n = 3, Means on the same row with different superscripts are significantly different at ($p < 0.05$).

Table 4. Sensory evaluation of whiteleg shrimp (*L. vannamei*) during chilled storage.

Sensory parameters	Fresh	2nd Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
Shell colour	9.10^{f}±0.21	8.95^{f}±0.16	7.90^{e}±0.57	5.20^{d}±0.42	3.50^{c}±0.53	1.70^{b}±0.48	1.40^{ab}±0.52	1.10^{a}±0.32
Meat colour	9.10^{f}±0.32	8.75^{f}±0.26	7.90^{e}±0.66	6.45^{d}±0.76	4.40^{c}±0.52	3.00^{b}±1.05	1.40^{a}±0.52	1.40^{a}±0.52
Odour	9.10^{f}±0.21	8.70^{f}±0.26	8.00^{e}±0.41	6.70^{d}±0.48	3.50^{c}±0.53	3.30^{bc}±0.48	2.90^{b}±0.57	2.00^{a}±0.67
Taste	9.15^{f}±0.24	9.10^{f}±0.39	8.40^{e}±0.39	6.70^{d}±0.54	4.60^{c}±0.52	4.30^{c}±0.82	3.20^{b}±0.42	2.70^{a}±0.67
Flavour	9.10^{f}±0.21	8.90^{f}±0.46	8.40^{e}±0.47	6.45^{d}±0.50	3.30^{c}±0.48	3.30^{c}±0.53	2.60^{b}±0.52	1.50^{a}±0.53
Freshness	9.05^{e}±0.16	8.80^{de}±0.35	8.45^{d}±0.55	6.50^{c}±0.53	3.20^{b}±0.42	3.00^{b}±0.82	1.50^{a}±0.53	1.30^{a}±0.48
Overall acceptability	9.15^{f}±0.24	8.95^{f}±0.28	7.80^{e}±0.42	6.60^{d}±0.39	3.60^{c}±0.52	3.25^{c}±0.54	2.40^{b}±0.46	1.85^{a}±0.24

Values are mean (SD), n = 3, Means on the same row with different superscripts are significantly different at ($p < 0.05$)

lodjiejska *et al*. (1987) observed a remarkable rate of denaturation and autolysis of fish protein while working on biochemical changes in fish muscle during low temperature storage. The present study showed the slight increase of crude fat during the whole storage. However, the values were not statistically significant ($p>0.05$) and ranged between 1.05 % and 1.20 %.

Changes in TVB-N, TBAR-S and pH: Changes in TVB-N value of whiteleg shrimp during chilled storage up to 14 days are shown in Table 2. The TVB-N value generally indicates conversion of proteins and non-proteins to ammonical nitrogen and amines, usually having high pH. In fresh *L. vannamei*, TVB-N content was 13.17mg %, which was below the maximum limit of acceptance of 30 mg / 100 g suggested by Food safety and standards authority of India (FSSAI). Within the first two days of storage no differences in TVB-N content of whiteleg shrimp ($p>0.05$) and 4th day onwards there was a significant increase ($p < 0.05$) in TVB-N values. According to Gopakumar (2002), TVB-N value recommended for good quality fish is less than 35 - 40 mg N /100 g. In the present study, after 10th day the values exceeded the acceptable limit.

The pH is an important index for determining the quality of fish (Okeyo *et al*. 2009). Changes in pH of whiteleg shrimp during chilled storage up to 14 days are depicted in Table 2. Fresh whiteleg shrimp had a pH of 6.52. As the storage time increased, the pH increased significantly ($P<0.05$) and reached at 7.60 by 14th day. This increase might be caused by the growth of spoilage bacteria leading to the accumulation of alkaline components (e.g., ammonia and trimethylamine) (Chaijan *et al*., 2005).

Fat oxidation in the fish muscle in terms of thiobarbituric acid (TBA) value during the course of chilled storage of whiteleg shrimp are presented in Table 2. TBA value is an index of oxidative rancidity in the fish muscle (Gopakumar, 2002). The present study shows significant increment ($p<0.05$) of TBA value due to the formation of fatty acid hydro-peroxides during the course of storage and the value increased from 0.04 (0th day) to 2.57 mg malondehaldehyde /kg of fat (14th day). For a seafood product to be acceptable for consumption, a recommended TBA value is less than 2 (Gopakumar, 2002). The *L. vannamei* exceeded the acceptable limit on 12th day of storage in this study.

Instrumental colour analysis: Colour is one of the essential parameters which determine the quality attributes of food (Du and Sun, 2011). Instrumental colour measurements of whiteleg shrimp are presented in the Table 3. This instrumental analysis is normally used as supportive information to determine the degree of quality of whiteleg shrimp in melanosis and sensory analysis. Present study assessed the colour attributes with respect to metric chroma under chilled storage. During chilled storage, up to day 6th , the lightness remained unchanged ($P> 0.05$) and would increase up to a peak on 8th day (41.14), later stage there was a decline till the end of storage ($P<0.05$). This result seems to be in agreement with the previous observations of Okpala *et al*. (2014) who reported increases in metric chroma of untreated Pacific white shrimp during iced storage. There was no significant difference observed in a* value of shrimp during chilled storage ($P>0.05$). Concerning yellowness (b*) value and particularly during the initial 4 days of storage, there was no significant difference observed ($P>0.05$) whereas after 4th day, the b* significantly increased up to a peak (8.93) by day 14 ($P<0.05$). The significant increase in yellowness (b*) was observed by Bak *et al*.(1999) for the untreated

shrimp with iced storage, where a colour shift was observed from somewhat red to a more yellow appearance. This might have occurred due to fair breakdown of astaxanthin, which might attribute to high pressure exerted by presence of oxygen.

Melanosis and sensory analysis: Melanosis and sensory characteristics of the white leg shrimp during chilled storage are shown in Figure 1 and Table 4 respectively. Shell colour, meat colour, odour, taste, flavour, freshness and overall acceptability of whiteleg shrimp were evaluated during the storage period. As storage time increased, the sensory scores given by panellists on 9 point hedonic scale for all sensory parameters were decreased significantly ($P<0.05$). Similarly, the study of melanosis scores by visual inspection were exhibited significant increase ($P<0.05$) after 1st day of storage. The higher scores for melanosis were in agreement with the low colour values of shell and meat after 6th days of storage. At this point, the shrimp surface was severely affected by black spot (60 %-80 %) and the product was disliked by the panellists. This result can be related to the Alvarez *et al*. (2005), where the black spot increased after 1 day in untreated tiger prawns (*Marsupenaeus japonicus*) during chilled storage.

Microbiological analysis: Figure 2 shows the total bacterial counts of whiteleg shrimp during chilled storage of 14 days. The initial count was $1.89*10^4$ log CFU/g and decreased to $1.73*10^3$ log CFU/g on 2nd day and later stage it was increased to a value of $1.73*10^7$ log CFU/g at the end of chilled storage. But actually, microbial load was enlarged after 6th day of storage and this result is equal to Lopez-Caballero *et al*., 2006. The decrease of microflora in earlier stage of storage might be due to the effect of low temperature (Zeng *et al*., 2005) and the later increase might be due to the tolerance to cold conditions of those microorganisms up to a certain limit in shrimp (Nirmal *et al*., 2009). This finding is similar to Panchavarnam *et al*. (2003) in which the initial counts increased to a value of 5.2 *l0^7 after 14 days of storage of whole rohu in ice.

Conclusion

The present study concluded that the degrading changes in biochemical and microbial composition had increased with period which had a direct effect on shelf life and market value of *L. vannamei*. The melanosis had started from 6th day onwards. But TVB-N as a quality index, shrimp is acceptable till 10th day. By correlating the results of colour, sensory scores and melanosis index, it was indicated that *L. vannamei* can be stored till 6 days in chilled storage without any black discoloration. However, the information created by this experiment provides the baseline for preservative treatments applied to whiteleg shrimp to prevent melanosis.

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