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Research Article

Quality Assessment of Vannamei Shrimp from Indonesian Waters

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Abstract: The special feature that indicates quality degradation in shrimp is the existence of blackspot. The spreading rate of blackspot on shrimp will grow in line with the storage time, thus it can be used as a parameter on quality assessment of shrimp deterioration.. This study aims to determine the quality of shrimp during chilling storage in organoleptic, histology, and enzymatic. The procedure of this study includes sampling, observations, organoleptic test, histological analysis, observing polyphenoloxidase and cathepsin activity. The results showed that pre-rigor phase on shrimp occurs shortly after the death (storage 0 d to 2 d), rigor mortis occurs on 3 d to 11 d of storage, post-rigor occurs on day 12 d to 17 d of storage, and deterioration (decay) occurs on the 18 d of storage until 23 d. Early appearance of blackspot appeared in cephalothorax and continues over time. Muscle fibers in pre- rigor phase has not been damaged. Muscle fibers still looks compact and solid. Muscle fibers in rigor mortis phase begins to experience shrinkage. Muscle fibers in post rigor phase had been cut into pieces. Cathepsin was most active on rigor mortis phase.

Keywords: Blackspot, cathepsin, histology, polyphenoloxidase, quality deterioration

1. INTRODUCTION

Vannamei shrimp [*Litopenaeus vannamei* (Boone, 1931)] is one of cultivated shrimps that is in great demand. The production of the shrimp increased 529×10^6 kg in 2012 to 608×10^6 kg in 2013 [1]. Shrimps are commodities that prone to deteriorate. The deterioration of shrimps is faster than fish, because the shrimp body surface area is smaller. The special feature that shows shrimp deterioration is the blackspot. The spread of blackspot starts from the cephalothrax and will spread to other parts of the body along with the length of storage time. The spread of blackspot can be used as a parameter on quality assessment of shrimp deterioration. Quality degradation process can be caused by autolytic process (enzymatic and chemical), oxidation process, bacteriological process, and dehydration process. Quality degradation process can be seen physically (organoleptic and histology), chemically/ biochemically (pH value and total volatile base (TVB)), and enzymatically (polyphenol oxidase and proteolytic). The shrimps also deteriorate as a result of bacteriological activity, which can be measured by total plate count (TPC) method [2]. Imran et al. (2013) reported that losses of the quality in white shrimps can be determined through TVB, color parameter, pH, TVC, and sensory index. The correlation matrix of the dependent variabtles showed that color parameters (L*,b*), pH, and sensory index were positively correlated ($P < 0.05$) with H, TVC, and TVB-N, respectively [3].

The existence of blackspot correlates to the deterioration of shrimp quality. The wider the spread of blackspots shows the lower the quality. This can be observed by using a stereoscope. The damage on shrimp's body tissues can be observed

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microscopically through histology observation. Pornrat et al. [4] observed changes in the texture and structure of prawns [*Macrobrachium rosenbergii* (De Man, 1879)] at a temperature of 5°C during 14 d of storage and the results of this observation indicates a change in the microscopic structure of myofibrils during the storage time. Zamorano et al. [5] observed the spread of melanosis on pink shrimp [*Parapenaeus longirostris* (Lucas, 1846)] at 4°C during the storage period and the result indicates that the highest spreading rate of blackspot occur in shrimp carapace. Information regarding quality degradation of vannamei shrimp (*L. vannamei*) in organoleptic, blackspot, histology, and enzymatic studies are still few, thus this research is needed to facilitate the shrimp handling process. This study aims to determine shrimp quality organoleptically, histologically, and enzymatically during chilling temperature

2. MATERIALS AND METHODS

2.1 Sample preparation

Vannamei shrimps were obtained from suppliers in Muara Karang, Jakarta, Indonesia and transported in a live condition with wet system transporting method that uses water as a medium in a closed container. Shrimp were killed with a shock chilling temperature (4°C). The dead shrimp were placed into trays, covered by plastic, and stored in refrigerator at 4°C.

2.2 Observation

Vannamei shrimps (*L. vannamei*) were stored at 4 °C for 23 d. Sensory analysis were conducted twice. The first, observations on shrimp were done to determine the quality degradation phase (pre-rigor, rigor mortis, and post-rigor). Number of panelis are five people. The sensory analysis for shrimp quality according SNI 2346:2015: guidelines for sensory testing of fishery products.[6] The second, organoleptic test was also conducted every 6 h for 84 h at chilling temperatures to observe blackspot. Observation was made on the eyes, cephalothorax, abdomen, and pereiopod. Subsequent observations were histology on shrimp abdomen and shrimp shells which were cut longitudinally, and measurement of polyphenoloxidase and cathepsin activity at each phase of quality degradation, i.e. pre rigor, rigor mortis, and post rigor . The analysis includes blackspot observation, histological analysis with BNF method [6], polyphenoloxidase activity [8], cathepsin activity [9], and measurement of protein concentration [10].

2.3 Blackspot Observation

Blackspot on shrimp were observed every 6 h using a digital stereoscope (SDA-1) that was connected to the computer. The observation result was presented in a photo of shrimp per segment and then analyzed descriptively.

3. RESULTS AND DISCUSSION

Quality degradation of shrimp includes four phases, namely pre-rigor, rigor mortis, post-rigor, and deterioration (decay). Stages of shrimp quality degradation was determined through organoleptic observations. The description of organoleptic

Table 1. Organoleptic quality of *L. vannamei*.

	Sightings	**Smell**	**Texture**
Pre rigor	Intact, the original colors such as shrimp, clear luminous, inter-segment sturdiness.	Fresh smell very specific kind.	Very elastic, compact and solid.
Rigor mortis	Intact, the original colors such as shrimp, yellowish slightly reduced (dull), inter-segment is less robust, appears blackspot.	The smell of fresh to neutral.	Less elastic, compact and solid.
Post rigor	intact, the color changes to pink shrimp, clarity is lost, inter-segment is less robust, blackspot lots	Neutral until the resulting ammonia odor.	No elastic, compact and solid.
Deterioration	a dull red color, blackspot aplenty, easily removable skin from the meat.	The smell of ammonia strong that rotten.	Soft.

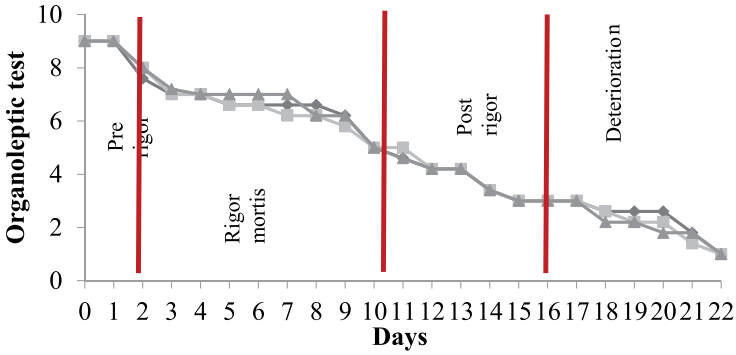

Fig. 1. Shrimp quality decline organoleptically: appearance; smell; texture.

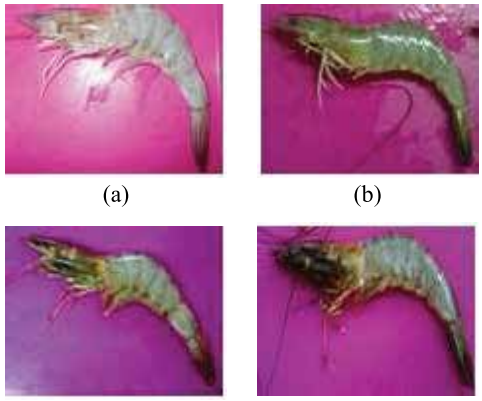

Fig. 2. Shrimp on each phase of deterioration of quality, description: (a) pre rigor, (b) rigor mortis, (c) post rigor, (d) deterioration.

observations is presented in Table 1.

Quality degradation of white shrimp was determined by observation of phase organoleptic. Organoleptic value of white shrimp has decreased in line with the long storage time. Determination of quality degradation of white shrimp was conducted for 23 d. The observed parameters were appearance, smell, and texture. The shrimp in the phase of rigor mortis has organoleptic grades 7 to grades 5 which is the safe limit of shrimp condition. Rigor mortis occurs on 3 d to 11 d. (Fig. 1) The appearance of shrimp in this phase remains the same as the shrimp in the phase of pre-rigor with a slight difference on the clarity of the body. This is due to increasing storage time. The freshness of the shrimp in this phase has started to decrease when compared to the phase of pre-rigor. It can be seen in the results of organoleptic parameters, namely the odor (Table 1). At this phase, the shrimp becomes stiff because actin and myosin in the muscle are not contracting, thus the texture is less elastic. Morkore [11] during rigor mortis, a decline in pH occurs due to the accumulation of lactic acid that activates proteolytic enzymes, for example cathepsin. This phase is characterized by exhaustion of ATP, therefore the contraction between actin and myosin is interrupted. Pornrat et al. [4] states that on 7 d to 9 d during storage, shrimp has less elastic texture than the texture of the shrimp at the beginning of storage. Blackspot at this phase begins to appear at the cephalothorax, pereiopod, pleopod, telson, and abdomen.

The shrimp in the phase of post rigor has organoleptic value ranging from 5 to 3. The phase of post rigor occurs on 12 d until 17 d (Fig. 1). The meat in this phase already started to experience softening as compared to the phase of rigor mortis due to bacterial activity. Blackspot at this phase changes shrimp body color to black. More blackspot was observed in cephalothorax. This phase is the beginning of deterioration. The alkaline pH is suitable for the growth of bacteria [12]. White shrimp in this phase is not acceptable to the consumer [4]. Furthermore, shrimp undergoes decay condition, thus not suitable for consumption. The shrimp that is in the phase of deterioration has organoleptic value from 3 to 1. Appearance of shrimp at different phases of quality degradation is presented in Fig. 2.

3.1 Quality Degradation of Shrimp: Blackspot

Observation of blackspot was performed every 6 h for 84 h at chilling temperatures. Observation was made on the eyes, cephalothorax, abdomen, and pereiopod using a digital stereoscope (SDA-1) that was connected to the computer. These parts represent observations for deployment blackspot. Quality degradation can be observed from the eyes of the shrimp. Shrimp's eye at 0 h was still fresh. Contradictive for 48 h and 66 h, eyes of the shrimp became concave due to the storage time. This condition indicates that the shrimp was not fresh. Observations deployment blackspot on the hour all 0's if it is associated with the results of organoleptic included in the phase of pre-rigor. Initial appearance of blackspot took place after 48 h, if it is associated

with the emergence of blackspot organoleptic results occur in the transition phase of pre-rigor into rigor mortis. Blackspot appeared at the center of the cephalothorax where the digestive organ located. Blackspot continued to appear after 54 h and 72 h, thus the entire surface of cephalotorax became black.

Shrimp abdomen on segment 1 and segment 6 had a slow growth of blackspot compared to cephalothorax and pereiopod. This is due to exposure to blackspot to enter into a longer abdomen due to abdominal shrimp meat. Black dots on the shrimp abdomen is the trigger for the growth of blackspot. Black dot can expand and grow over time storage and becomes blackspot. However, not all of the black dot can be blackspot. Blackspot point which was still small in 0 h grew larger after 84 h.

Blackspot on pereiopod spread as fast as in cephalothorax because pereiopod lies below cephalothorax. Pereiopod was still white translucent on 0 h and was not covered by the blackspot. Early emersion of blackspot occurred at 48 h, and spread faster on 66 h and 84 h. Observations deployment blackspot in accordance with Nirmal and Benjakul [13] which states that during storage at 4°C increase in the value of melanosis.

According to Montero et al. [14], shrimps which were stored in chilling temperatures have melanosis reaction starting at cephalothorax to tail with warious growth rate among different species. Early appearance of blackspot located on the body of the shrimp that has no flesh, namely cephalothorax, pereiopod, tail, and pleopod. Blackspot initially grows on the cephalothorax, tail, pereiopod, and pleopod, then continued in the shrimp's body from the first segment to the sixth vertebra.

Melanosis or blackspot is usually found on exoceleton or carapace, telson, tail and abdominal cuticle [14]. According to Zamorano et al. [5] blackspot is a natural process in the post-mortem which is derived from phenol polymerase and dissolved into a black pigment. Melanosis is caused by a biochemical mechanism of the enzyme polyphenoloxidase (PPO) that leads to phenol oxidation to quinone. Quinone is highly reactive and undergoes non-enzymatic oxidation (e.g. caused by oxygen) which will cause dark colored pigment having a high molecular weight, thus blackspot appears on the surface of shrimp shells [15].

3.2 Quality Degradation of Shrimp: Histology

There are two muscle fibers in shrimp, i.e. longitudinal and transverse muscle (Fig. 3). Muscle fibers in the phase of pre rigor is compact (Fig. 3). Image magnification on muscle fibers shows the muscle fibers fused with a septum that has not been damaged. This is consistent with the results of organoleptic which suggested that texture of the shrimp was very elastic, compact and solid. According to Pornrat et al. [4], after 2 d of storage at 5 ºC, no change in muscle tissue of the shrimp was observed. Muscle tissue is compact and integrates tightly. Early deterioration occurred on the shells, which was indicated by blackspot formation. Infection early emergence blackspot is the carapace that followed the release of particles blackspot towards muscle tissue. Blackspot is happening in the muscle tissue, including the heaviest infection. Quality deterioration shrimp shells when the phase of pre-rigor (Fig. 2) shows that the carapace is still compact. This is consistent with observations deployment blackspot on the hour-0 which indicates the undeveloped blackspot. Hematoxylin worked as a dye base means that this substance basophilic coloring elements on the network becomes purple-blue. Eosin acidic and coloring components acidophilic be pink. Cells are acidophilic so it can absorb the dye eosin. Inter muscle fibers in the phase of rigor mortis begins to experience shrinkage so that begins to form an empty space. Miomer which began to malfunction during this phase due to the shrinkage of the muscle fibers, causing started their empty white space. Shrinkage of the muscle fibers caused by stiffness in the shrimp meat as a result of not contra actin and myosin [11].

3.3 Quality Deteoration Shrimp: Enzymatic

Another cause of the decline in the quality of the shrimp meat freshness that is the proteolytic enzyme activity after dead shrimp. The enzyme hydrolyze of protein into smaller component, *i.e,* peptides and amino acids. One of these enzymes are enzymes

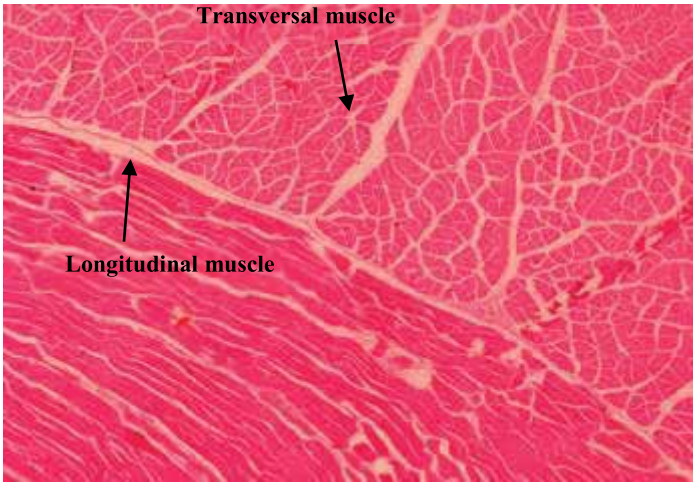

Fig. 3. Muscle fibers shrimp.

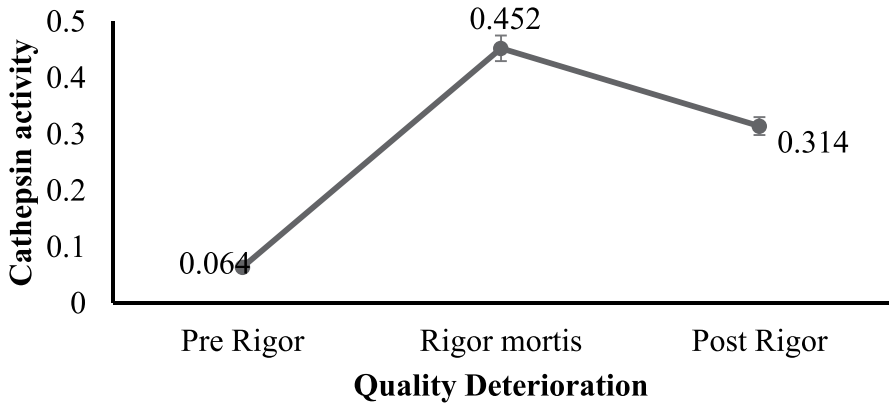

Fig. 4. The results of the measurement of enzyme activity during the decline of the quality of shrimp cathepsin.

that degrade proteins cathepsin miofibril [16]. The results of the measurement of enzyme activity cathepsin the shrimp meat is presented in Fig. 4 . Fig. 4 shows the value of the enzyme activity of shrimp meat on a phase of pre-rigor (0.064 U mL^{-1}), rigor mortis (0.452 U mL^{-1}), and post-rigor (0.314 U mL^{-1}). Cathepsin actively working at optimum pH in the range of acidic pH [17]. According to Qiu et al. [18], an enzyme cathepsin B on crustacean optimum at pH 6.0 and 45 ºC. When the shrimp die, the shrimp will fall from pH neutral range (pH 7) to a pH range of pH 6.5 and then ride back [19]. Decrease in pH causes lysosomal membrane on the muscle fibers become damaged and activate the cathepsin. Cathepsin belong to the proteolytic enzymes or proteins into peptone, amino acids, and polypeptides [20]. Cathepsin and other hydrolisis enzyme in shrimps contained in sub-cellular organelles called lysosomes. Lysosomes of cells found in two places, the muscle fibers and cell membranes [21]. The process of protein breakdown occurs due to decreased pH in the muscle tissue due to the formation of lactic acid.

Cathepsin B enzyme most abundant in shrimp meat [22]. The enzyme is also found in Pandalus borealis (Makarov, 1935) is in the lymph nodes and the enzyme was detected when the pH is acidic [23]. According to Stephens et al. [24], in *L*. vannamei cathepsin B enzyme low content of the skin (carapace) and high in the gills, while according to Ren et al. [25], there are many cathepsin L in hepatopancreas of Fenneropenaeus chinensis. Cathepsin D is not found in the shrimp, but there

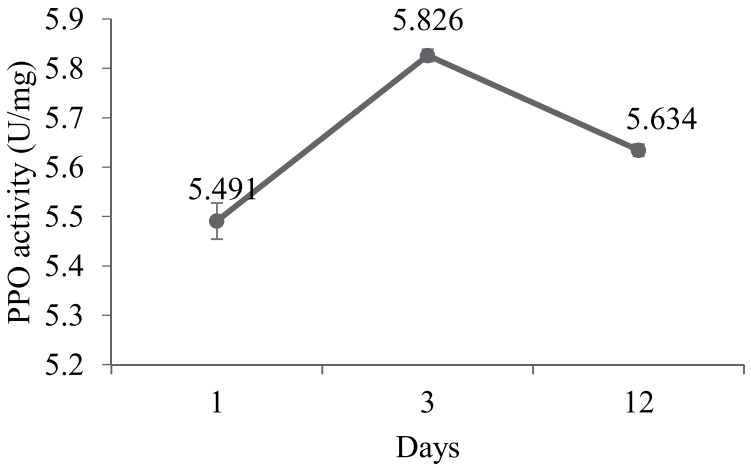

Fig. 5. Polyphenol oxidase enzyme activity vaname shrimp during 12 d of storage.

are in other crustaceans, such as lobsters [26].

3.4 Enzyme Activity Polyphenoloxsidase (PPO) Shrimp Vaname

Polyphenoloxidase (PPO) is an enzyme that catalyzes the two basic reactions or catalyze hydroxylation to the position of group O which is adjacent to the other hydroxyl. PPO enzyme using a substrate such as phenol and oxygen. The reaction that occurs in the PPO enzyme is oxidation of diphenol to o-benzoquinone which is oxidized to melanin (brown). Changes to the brown color occurs in a non-enzymatic [20].

Results of testing the activity of the enzyme extract from shrimp shells are presented in Fig. 5. The specific activity of the enzyme PPO Testing is conducted every phase of deterioration of quality of shrimp that is in phase pre-rigor, rigor mortis, and post-rigor. The specific activity of the enzyme PPO vaname shrimp stored at a temperature of ± 4 ºC increased activity in pre-rigor phase and the phase of rigor mortis, but decreased slightly in phase post-rigor.

PPO enzyme activity obtained at vaname shrimp has a high specific activity or the optimum phase of rigor mortis. PPO optimum enzyme activity can be caused by various environmental factors such as pH factor, i.e., the pH value of the phase of rigor mortis of pH 6.8, which means approaching pH 7. PPO enzyme activity will be increased due to the pH value of 7 on the substrate. Suhandana [27] mentions the PPO enzyme achieve optimum activity at pH 7. The enzyme activity at lower pH values and higher will result in lower enzyme activity. Phase post-rigor PPO enzyme activity decreased, this was due to the phase postrigor have alkaline pH value is obtained at pH 7.37 so that the PPO enzyme activity decreased. Phase postrigor, activity of PPO enzyme was decreased. This is presumably because the longer storage on deployment blackspot shrimp and more in line with the decrease concentration of tyrosine substrate, so that specific activity of the enzyme PPO was decreased.

4. CONCLUSIONS

Vanamei shrimp stored at chilling temperature (i.e., 4 °C) retain good quality for 11 days. This conclusion is supported by organoleptic analysis, cathepsin enzyme activity, polyphnenoloxidase activity, tissue structure, and blackspot development.

5. ACKNOWLEDGEMENTS

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