TECHNICAL BULLETIN

Number: 08
Date Published: 2021/08/17

Issue	**Melanosis in Shrimp/Prawn**

Background

Prior to the current practices for shrimps/prawns culture, prawns and shrimps were only considered as incidental by-products of milkfish production resulting from wild fry entering the ponds. The beginning of the Philippine shrimp industry was the outcome of the low density monoculture study conducted by the Dagat-dagatan Experimental Station of the Bureau of Fisheries on giant tiger prawn (*Penaeus Monodon)* in 1943. More studies were conducted in the mid 1970's and the study results guided farmers in stocking more than 10,000 postlarvae per hectare using hatchery fry [1].

The commercial scale production of shrimps/prawns grown in brackish water and seawater contributed largely to the income from the Philippine aquaculture industry. For five consecutive years (2015-2019), the shrimp/prawn industry has an average production of 61,225.25 MT [2], with an average export volume of 7,803.80 MT making it one of the top five aquaculture commodities consumed domestically and exported internationally to countries including Japan, Korea and the United States [3].

With the growing market for shrimp products, the Bureau of Fisheries and Aquatic Resources (BFAR) approved the establishment of shrimp processing plants in Manila, Bohol, Butuan, and General Santos City. Some of the shrimp by-products developed and processed in these facilities are pasteurized bottled sauteed shrimp, fresh frozen black tiger or white shrimp (head-on or headless, and un-hulled or shelled), shrimp powder, frozen block shrimp, and frozen pre-cooked peeled shrimp [4].

Despite the availability of trained technicians for implementation of Good Manufacturing Practices (GMP), and other food safety standards and requirements to ensure high quality produce, meeting the global standards and establishing international market access is still a challenge to Philippine shrimp farmers and exporters. Shrimp/prawn is highly perishable and aesthetic issues such as melanosis, effects of post-harvest treatments and bacterial contamination are of great concern that degrades quality, as it relates to food safety and marketability.

Although there is no definite data on the rejection/refusal of Philippine shrimp exports, quality issues may potentially impact trade with international trade partners, such as the US and the European Union (EU).

When a country's production and export conditions for seafood products do not meet EU food safety and quality standards (e.g. melanosis affecting greater than 25% of sample unit), the importation of seafood from that country to the EU may be prohibited [5]. Meanwhile, in the US, grading standards are used in inspecting sample units of shrimps/prawn, noting quality physical defects, and assigning corresponding numerical values to determine their grade assignment. Aside from the compliance to food safety requirements, the grading standards also play an important role in obtaining a certification [6].

General Description

Melanosis, or blackspot is a common discoloration or darkening of specific parts of the shrimp/prawn due to increased concentration of melanin. The monophenol present is catalyzed by a system of enzymes, polyphenol oxidase (PPO), generally used to refer to tyrosinase, catechol oxidase, o-diphenol oxidase, monophenol oxidase, among other (according to the type of substrate they use) into quinone that when oxidized becomes complex brown polymers or dark pigments called melanin. These polymers occur primarily along the swimmerets, head, tail and nearby shell areas, before spreading further along shell edges and through the body [7].

The development rate of melanosis is significantly affected by the following factors [7]:

1. High pH of the muscle tissue;
2. Temperature;
3. Water activity;
4. Presence of oxygen;
5. Presence of copper;
6. High level of substrate (tyrosine existent in the body of the organisms;
7. The level of enzyme particularly PPO level in the blood, carapace, head and legs;
8. Species of crustacean;
9. Sex;
10. Season, particularly the molting period;

	11. Geographical origin of crustaceans; 12. Technological factors (postharvest practices); and 13. Mechanical stress during harvesting [8].
Quality Issues in Food	Based on the PNS/BAFPS 70:2008 – Quick frozen shrimps and prawns, the sample unit exhibiting discoloration with distinct blackening (melanosis) or green or yellow discoloration, singly or in combination of more than 10% of the surface area of individual shrimp/prawn which affects more than 25% of the sample unit is considered as defective [9]. The progression scale of melanosis in shrimp and the corresponding descriptions of each scale can be seen in Figure 1 and Table 1, respectively.
Adverse Health Effect in Human	Shrimps and prawns with melanosis do not pose food safety hazards to consumers. However, it can negatively affect quality perception resulting in diminishing market value that can greatly affect the income of farmers/fisherfolk. In addition, the use of sulfites as melanosis control agents is generally considered non-toxic, but are irritant and can cause health problems for some consumers, especially people suffering from asthma or deficiencies of sulfite oxidase [8].
Mitigation Measures	The existence of melanosis as a quality issue can be controlled and eliminated promptly at the deck where GMP plays an important role. To date, the following conventional and alternative methods are being practiced to delay the onset of black spot on shrimps and prawns: **Non-chemical Methods** [7] 1. *Freezing*. Traditionally, the preservation of fresh fishery or aquatic products is through freezing. It requires -18°C or below for long-term preservation of seafood quality, however, there were observed occurrences of black spots even after 2 days of freezing and a rubbery texture once the product was thawed. 2. *Pre-cooking*. At boiling temperature, pre-cooking shrimps/prawns for 2 minutes can deactivate PPO activity, thereby preventing the onset of melanosis, however, this technique is not always applicable as it reduces the products options.

3. *High Pressure Processing (HPP)*. This treatment is a non-thermal processing technology that has proved to extend shrimp shelf life by slowing down microbial growth and inhibiting melanosis. At 600 MPa, only 20% of the residual PPO activity remained [10], while using 900 MPa for 30 minutes at 45°C, the enzymes are completely controlled [7].

4. *Modified Atmosphere Packaging (MAP)*. Studies have revealed that using optimal MAP reduces bacterial growth, thus, increasing the product shelf life by 100% as oxygen and water activities are controlled. In result, absence of oxygen that triggers oxidation reactions delays enzymatic activities like melanosis.

5. *Food Irradiation*. The application of ionizing radiation to food improves the safety and extends the shelf life of food products as it eliminates microorganisms and insects [11]. Low dose can reduce melanose, but only before the onset of melanosis [7]. For raw, headless tropical shrimps (*Penaeus* spp.), 1.5-2.0 kGy has been found as the optimum dose, which also extends shelf-life [12].

   Furthermore, only the US FDA approved sources of radiation (*i.e.* gamma rays, X-rays and electron beams) are allowed and considered safe to be used [11]. In the Philippines, food irradiation services are available through the Department of Science and Technology - Philippine Nuclear Research Institute (DOST-PNRI) irradiation facilities [13]. A public-private project under DOST-PNRI is also in the pipeline, which aims to establish a fully commercial irradiation facility [14].

Chemical Methods

1. *Ascorbic acid and ascorbyl derivatives*. The PPO is active in basic medium and inactive in acid medium. By adding acidulants (citric acid, phosphoric acid, ascorbic acid and ascorbyl derivatives), pH in tissue muscles can be increased, therefore, deactivating PPO activity.

2. *Chelating agents*. The use of ethylenediamine tetraacetic acid or EDTA reduces copper available in the product that is an essential component of melanosis [7].

3. *Sulfites, especially sodium metabisulfite (SMS)*. Sulfites and its derivatives are the most widely used additives to prevent melanosis in crustaceans. However, improper handling of these

chemicals may become potential health hazards as they release sulfur dioxide upon contact with acid and water. This SO_2 gas reacts with respiratory tissue forming sulfurous acid, and inducing a pulmonary reaction causing hypoxemia [15].

To mitigate this potential hazard, different countries had established a regulatory limit for sulfite residue. The U.S. Food and Drug Administration (FDA) has established a regulatory limit of 100 ppm for sulfite residue on their domestic and imported shrimp while in European regulations the sulfite residue limit is at 150 ppm [16]. Based on PNS/BAFPS 70:2008 - Quick frozen shrimps or prawns, Philippines allows 100mg/kg or 100 ppm sulfite residue on the edible part of the raw shrimp or 30mg/kg in the edible part of the cooked product, singly or in combination, expressed as sulfur dioxide (SO_2) [9].

Alternatives for Inorganic Chemical Additives

Moreover, there is a growing demand for sulfite-free shrimp/prawn products in the market as more consumers are becoming conscious of its possible hazards. Several studies have already been conducted by researchers to develop safer, locally available and natural alternatives to currently used synthetic antimelanosic compounds in the food industry.

1. *Application of Edible Oyster Mushroom (Pleurotus ostreatus). Extract as Melanosis Control Agent* [17]. The mushroom extract was processed by mixing 500 grams of fresh oyster mushroom fruiting body trimmings with 1,000 mL of distilled water and boiled gently at 90°C for 30 minutes. The mixture was filtered using cheesecloth and by qualitative filter paper No. 1 subsequently. At 50°C, the filtrate was evaporated to obtain 500 mL of the hot water extract concentrate (1 g wet mushroom/mL of hot water extract) and then, packed in sterile plastic tubes and stored at -20°C until used.

   With the observed remarkable scavenging activity of crude water extract against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and inhibitory effect on PPO activity, the experiment proceeded with the use *P. vannamei* shrimp, immersed in a 1.0% crude extract of *P. ostreatus* for 60 minutes.

   The result showed the biochemical interventions of edible oyster mushroom extract inhibited the PPO activity thus, melanosis was effectively controlled and comparable with the commercial antimelanosic agents.

2. *Inhibitory Effect of Olive Phenolic Compounds Isolated from Olive Oil By-Product on Melanosis of Shrimps* [18]. The study used Atlantic Ditch Shrimp (*Palaemonetes varians*) that were purchased at different seasons and treated with dipping solutions made from two potent, natural antioxidants isolated from olive waste (hydroxytyrosol or HT and 3,4-dihydroxyphenylglycol or DHPG, and three novel HT-derivatives containing selenium and sulfur). The results showed a positive inhibitory effect of these antioxidants on tyrosinase activity, thus delaying the onset of melanosis on shrimp.

   In addition, U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have considered HT as functional food and safe for human consumption making it a safer alternative to inorganic chemicals like sulfites and their derivatives.

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References

[1] J. H. Primavera, "Prawn/shrimp culture industry in the Philippines," In A. W. Fast & L.J. Lester (Eds.), Marine Shrimp Culture: Principles and Practices (Development in Aquaculture and Fisheries Science Vol. 23, pp. 701-728). Amsterdam: Elsevaier, 1992. Available: http://hdl.handle.net/10862/352.
[2] Philippine Statistics Authority, "Volume and Value of Production by Species," *Fisheries Statistics of the Philippines*, 2015, 2016, 2017,

2018, 2019. [Online]. Available: https://psa.gov.ph/content/fisheries-statistics-philippines

[3] Bureau of Fisheries and Aquatic Resources, "Major Fishery Exports in Terms of Values," *Philippine Fisheries Profile*, 2015, 2016, 2017, 2018, 2019. [Online]. Available: https://www.bfar.da.gov.ph/publication. [Accessed July 12, 2021].

[4] Department of Trade and Industry and Board of Investments, "Processed Shrimp," [Online]. Retrieved from http://industry.gov.ph/industry/processed-shrimp/. [Accessed July 12, 2021].

[5] D. S. Dabadé, "Shrimp quality and safety management along the supply chain in Benin," Ph.D. dissertation, Wageningen University, Wageningen, NL, 2015.

[6] National Oceanic and Atmospheric Administration - U.S. Department of Commerce, "PART 5 – U.S. Grading Standards and Procedures for Grading," *Seafood Inspection Manual*, pp. 118-126, 2021. [Online]. Available: NOAA, https://media.fisheries.noaa.gov/dam-migration/part_5_grade_standards.pdf. [Accessed Aug. 02, 2021].

[7] A. A. Goncalves, A. R. M. De Oliveira, "Melanosis in crustaceans - importance and characteristics," *Melanosis in crustaceans: A Review,* pp. 792-798, 2016. Available: https://www.researchgate.net/publication/281591200_Melanosis_in_crustaceans_A_review#pf8.

[8] H. Lucien-Brun, "*Preventing melanosis in shrimp*," Global Aquaculture Advocate, 2005. Available: https://www.aquaculturealliance.org./advocate/preventing-melanosis-in-shrimp/.

[9] Bureau of Fisheries Standards, *PNS/BAFS 70:2008 Quick frozen shrimps or prawns,* 2008. [Online]. Available: http://www.bafs.da.gov.ph/bafs_admin/admin_page/pns_file/2021-02-24-PNS-BAFPS%2070-2008-Quick%20Frozen%20Shrimps% 20or%20Prawns.pdf. [Accessed July 12, 2021].

[10] J. Gomez-Estaca, P. Montero, F. Fernandez-Martin, M.M. Calvo, M.C. Gomez-Guillen, "*The effect of high pressure treatment on functional components of shrimp (Litopenaeus vannamei) cephalothorax*," Innovative Food Science & Emerging Technologies, vol. 34, pp. 154-160, 2016. Available: https://www.sciencedirect.com/science/article/abs/pii/S1466856416300054.

[11] US Food and Drug Administration, "*Food Irradiation: What You Need to Know,"* 2018. Available: https://www.fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know. [Accessed July 12, 2021].

[12] International Atomic Energy Agency. Irradiation of fish, shellfish and frog legs: A compilation of technical data for authorization and control. Vienna: IAEA, 2000.
[13] Department of Science and Technology - Philippine Nuclear Research Institute, *Irradiation Facilities,* 2015. [Online]. Available: https://www.pnri.dost.gov.ph/index.php/services/facilities/irradiation-services.
[14] BusinessMirror, "PNRI partners with private sector for commercial irradiation facility," *BusinessMirror*, Jun. 20, 2021. [Online]. Available: https://businessmirror.com.ph/2021/06/20/pnri-partners-with-private-sector-for-commercial-irradiation-facility/
[15] D. A. Atkinson, T. C. Sim, J. C. Grant, "Sodium metabisulfite and SO2 release: an under-recognized hazard among shrimp fishermen," Ann Allergy, Dec;71(6):563-6, PMID: 8267251, 1993. Available: https://pubmed.ncbi.nlm.nih.gov/8267251/.
[16] M. Khan, "Quality of United States and Bangladesh Shrimp Due to Growth and Post-harvest Practices," Ph. D. dissertation, The School of Renewable Natural Resources, 2018.
[17] M. G. Llanto, A. B. Encarnacion, "Application of Edible Oyster Mushroom, *Pleurotus ostreatus* Extract to Control Postharvest Melanosis in Shrimp, *Penaeus vannamei*," Philippine Journal of Science, vol. 147 No. 2, 2018. Available: https://philjournal sci.dost.gov.ph/images/pdf_upload/pjs2018/2ndQtr/application_of_edible_oyster_mushroom.pdf.
[18] A. Lama-Muñoz, A. Gomez-Carretero, F. Rubio-Senent, A. Bermudez-Oria, I. Maya, I. Fernandez-Bolaños, "*Inhibitory Effect of Olive Phenolic Compounds Isolated from Olive Oil By-Product on Melanosis of Shrimps,"* Antioxidants, vol. 10 no.5: 728, 2021.
[19] R. Thepnuan, "Melanosis progression scale of shrimp," Prince of Songkla University, pp. 10-11, 2007. Available: https://kb.psu.ac.th /psukb/bitstream/2553/1571/7/292762_ch1.pdf.

Annex

Figure 1. Melanosis Progression scale of shrimp [19]

Table 1. Progression scale used to describe of melanosis on shrimp [19]

Melanosis Scale	Description
0	Absent
2	Slight, noticeable on some shrimps
4	Slight, noticeable on some shrimps
6	Moderate, noticeable on some shrimps
8	Heavy, noticeable on some shrimps
10	Heavy, totally unacceptable