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# Black Spot Inhibition and Microbial Inactivation on Shrimps Using Ultrasound Combined with Perasan

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**Abstract:** In the food industry, eliminating harmful microorganisms and inactivating enzymes are important for food quality and also for public health. That is the reason why heat treatment is the most utilized method for stabilizing foods because of its capacity to destroy microorganisms and also to inactivate enzymes. However, since, heat can alter the organoleptic properties of foods and diminish the contents or bioavailability of some nutrients, there is a growing interest in searching non-thermal method for food preservation. Ultrasonication combined sanitizers can accelerate the rate of sterilization and enzymatic inactivation. The advantages of ultrasound over heat treatment include: minimization of flavor loss and significant energy saving. In our research, we approach to a new way by using ultrasonic combined with perasan to inhibitate polyphenol oxidase activity. Microbial sanitation is also a benefit from this process. Main purposes of this research are to prevent black spot (melanosis) and sanitize microorganism on shrimp without effecting to texture (firmness) and apprearance (L) of finished product. Our results show that optimal parameters of ultrasonic combined PAA are as follows: amplitude (120 μ), chemical concentration (40 ppm), temperature (12°C) and time (9 min).

**Key words:** Ultrasound, perasan, black spot, microorganism, texture, appearance

## INTRODUCTION

Browning or melanosis in aquatic foods postharvest occurs primarily in crustaceans. These highly prized and economically valuable products are extremely vulnerable to enzymatic browning. Melanosis is usually more severe in lobsters if the head is retained during storage postharvest. If the head is removed, care should be taken to thoroughly wash the tail in order to eliminate proteases that activate latent polyphenol oxidases and promote browning. Although, the products of melanosis are not harmful and do not influence flavour or aroma, consumers will not select these products, since, their brown discolouration connotes spoilage. Severe melanosis on these products can cause tremendous economic losses due to the high value commanded by these aquatic products in the marketplace. There are many containers of imported aquatic products entering the United States, worth millions of dollars, those are reduced markedly or lost completely owing to the severity of melanosis. Unfortunately, a majority of these products originate in developing countries which lack both the scientific and technical resources and the processing infrastructure required in order to prevent the occurrence of these devastating losses. Limited susceptibility of a number of crustacean species to melanosis on the other hand, presents the processor with the problem of deciding how to treat the product in order to prevent melanosis.

Shrimps are highly perishable and quality deterioration is usually dominated by microbial activity. This deterioration is highly temperature dependent and can be reduced by low storage temperature. Shrimp is a very perishable product and postmortem changes occur rapidly, compared with fish. The high content of free amino acids and other soluble non-nitrogenous substances which partly contribute to desirable, delicate sweet taste of shrimp can also, serve as easily digestible nutrients for microbial growth. The production of protein breakdown products such as ammonia indole, methanethiol, putrescine, trimethylamine and other off-odor compounds are caused by the growth of spoilage bacteria. Apart from spoilage bacteria, we also concern to pathogenic ones. Some common microorganism normaly inpsected in seafood exported including: TPC, Coliform, Enterobacteriae, E. *Coli*, *Staphylococcus aureus, Salmonella, Vibrio cholerae, Vibrio parahaemolyticus, Clostrid perfringens, Listeria monocytogene* eu.min$^{-1}$.mL$^{-1}$m.

Perasan or peracetic acid (also known as Peroxyacetic Acid or PAA) is an organic compound. It is a colorless liquid with a characteristic pungent odor reminiscent of household vinegar. All commercially available PAA products contain an equilibr eu.min$^{-1}$.mL$^{-1}$m of PAA, hydrogen peroxide, acetic acid and water. Its formula is $CH_3CO_3H$. Peracetic acid is an ideal antimicrobial agent due to its high oxidizing potential. It is highly effective

against a broad range of microorganisms. In addition, PAA breaks down in food to safe and environmentally friendly residues (acetic acid and hydrogen peroxide) and therefore can be used in non-rinse applications. PAA is one of the most environmentally friendly antimicrobial agents, since, resulting wastewater can be land-applied for irrigation and is generally eligible for NPDES permits to discharge to natural waterways.

There were several researches mentioned to melanosis prevention by ultrasound. Marong *et al.* (2007) used supercritical $CO_2$ to extract of cinnamomum zeylanicum. Nirmal and Benjakul (2011) monitored the effect of catechin and ferulic acid on melanosis and quality of pacific white shrimp subjected to prior freeze-thawing during refrigerated storage. Wen-Zong *et al.* (2010) studied the effect and mechanisms of ultrasonic treatment on polyphenol oxidase activity. Jang and Moon (2011) examined the inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. Zhang *et al.* (2011) investigatesd the inactivation of polyphenol oxidase from pacific white shrimp by dense phase carbon dioxide. Nirmal and Benjakul (2011) examined the inhibitory effect of mimosine on Polyphenol Oxidase (PPO) from the cephalothorax of pacific white shrimp (*Litopenaeus vannamei*) was studied. Surasani and Patange (2012 verified melanosis inhibition and $SO_2$ residual levels in farmed tiger shrimp (*Penaeus monodon*) following different sulfite-based treatments. Rithmanee and Intipunya (2012) investigated the physicochemical quality and inactivation of Polyphenol Oxidase (PPO), Peroxidase (POD) in dried longan with high power ultrasonic pretreatment. Fang *et al.* (2013) evaluated the effect of Pomegranate Peel Extract (PPE) at different concentrations (0, 7.5 and 15 g/L) on the melanosis formation and quality of Pacific white shrimp (*Litopenaeus vannamei*). Cheng *et al.* (2013) studied the inactivation kinetics of polyphenol oxidase in mushroom (*Agaricus bisporus*) during thermal and thermosonic treatments in 55-75°C temperature range. Sae-Leaw *et al.* (2018) proved a retardation of melanosis and quality loss of pre-cooked pacific white shrimp using epigallocatechin gallate with the aid of ultrasound.

There were also, various studies mentioned to the microbial inhibition by untrasound. Qian *et al.* (1997) studied the effect of ultrasonic frequency upon enhanced killing of P. *Aeruginosa* biofilms. Bettner *et al.* (1998) evaluated the effect of ultrasonic cleaning on microorganisms. Joyce *et al.* (2002) investigated the development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power and sonication time on cultured Bacillus species. Hoover *et al.* (2004) examined the destruction of bacterial spores by phenomenally high efficiency non-contact ultrasonic transducers. Herceg *et al.* (2013) determine the effect of high intensity ultrasound (amplitude, temperature and treatment time) on the inactivation of food spoilage bacteria.

Purpose of our research is to demonstrate the effectiveness of ultrasonication combined perasan to prevent melanosis or black spot on shrimp tail while maintaining their quality parameters.

## MATERIALS AND METHODS

### Raw material, chemical and equipment

**Raw material:** Black tiger shrimp (*Paneaus monodon*), white leg shrimp (*Paneaus vannamei*), giant prawn (*Macrobrach rosenbergii* eu.min$^{-1}$.mL$^{-1}$m) were harvested from Mekong delta, Vietnam. They must be cultivated following Global GAP without using antibiotic to ensure food safety. After harvesting, they must be kept in ice chest (<4°C) and conveyed to laboratory within 2 h for experiments. Proteolysis and biochemical changes of muscle can be taken place to some degrees during iced storage.

**Chemical:** Perasan (PAA) supplied from Van Dai Phat Ltd. Perasan MP-2 is an FDA-approved antimicrobial specifically formulated for use in ice and water that directly contacts meat, poultry and seafood as well as further processed fruits and vegetables. Perasan MP-2 contains 15% peracetic acid, 6% hydrogen peroxide and acetic acid.

**Equipment:** Lab utensils and equipments included ultrasonication cleaner, penetrator, handheld colorimeter (L, a, b), centrifugator incubator, colony counter, etc.

### Researching procedure

**Effect of different washing methods:** Shrimps are chosen in this experiment including black tiger shrimp, white leg shrimp and giant prawn. Testing parameters include melanosis: PPO activity, melanosis score, microorganism: Coliform, quality: color, texture.

**Demonstration:**

- Before treatment
- Control: washing in fresh water at temperature (4°C), time (5 min)
- Ultrasonic alone: washing in ultrasonic cleaner at amplitude (30 μm), temperature (4°C), time (5 min)

- PAA alone: washing in bath at PAA concentration (50 ppm), temperature (4°C), time (5 min)
- Combined ultrasonic and PAA treatment: washing in ultrasonic cleaner at amplitude (30 μm), PAA concentration (50 ppm), temperature (4°C), time (5 min)

In respect of melanosis score evaluation, samples are trayed in normal room temperature in 15 min and then evaluated through visual inspection by ten trained panelists using 10-point scoring test.

**Effect of different parameters of ultrasonic combined PAA in melanosis inhibition (PPO activity, melanosis score) on different shrimps:** In respect of black spot evaluation, treated samples are trayed in normal room temperature in 15 minutes and then evaluated through visual inspection by ten trained panelists using 10-point scoring test (Table 1 and 2).

**Effect of different parameters of ultrasonic combined PAA in microbial sanitation (coliform) on different shrimps:** Table 3 is discussed in effect of different parameters of ultrasonic combined PAA in microbial sanitation (coliform) on different shrimps.

**Effect of different parameters of ultrasonic combined PAA in firmness (texture, kg/ $cm^2$), color apperance (L) on different shrimps:** Table 4 is discussed in effect of different parameters of ultrasonic combined PAA in firmness (texture, kg/$cm^2$), color apperance (L) on different shrimps.

**Effectiveness of optimal parameters of ultrasonic combined PAA on different quality and hygiene criteria of different shrimps:** Table 5 is discussed in effectiveness of optimal parameters of ultrasonic combined PAA on different quality and hygiene criteria of different shrimps.

Table 1: Effect of different washing methods on different shrimps (melanosis: PPO activity, melanosis score; microorganism: coliform, quality: color, texture)

| | | Testing parameters | | | | |
|---|---|---|---|---|---|---|
| Washing methods | Shrimps | PPO activity (eu.$min^{-1}$/mL) | Melanosis score (1-10) | Coliform (CFU/g) | Color (L value) | Texture (kg/$cm^2$) |
| Before treatment | B.T. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Control | B.T. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Ultrasonic alone | B.T. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| PAA alone | B.T. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Combined ultrasonic and PAA | B.T. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |

Table 2: Effect of different parameters of ultrasonic combined PAA in melanosis inhibition (PPO activity, melanosis score) on different shrimps

| Criteria | Different shrimps | Experimental parameters | | | | |
|---|---|---|---|---|---|---|
| Amplitude (30 μm) chemical concentration (vary) temperature (4°C) time (3 min) | | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm |
| PPO activity (eu.$min^{-1}$.$mL^{-1}$) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Black spot (score) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (vary) chemical concentration (40 ppm) temperature (4°C) time (3 min) | | 30 μm | 60 μm | 90 μm | 120 μm | 150 μm |
| PPO activity (eu.$min^{-1}$.$mL^{-1}$) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Black spot (score) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (120 μm) chemical concentration (30 ppm) temperature (vary) time (3 min) | | 4°C | 8°C | 12°C | 16°C | 20°C |
| PPO activity (eu.$min^{-1}$.$mL^{-1}$) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |

Table 2: Continue

| Criteria | Different shrimps | Experimental parameters | | | | |
|---|---|---|---|---|---|---|
| | G. Prawn | | | | | |
| Black spot (score) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (12°C) time (vary) | | 3 min | 6 min | 9 min | 12 min | 15 min |
| PPO activity (eu.min$^{-1}$.mL$^{-1}$) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Black spot (score) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |

Table 3: Effect of different parameters of ultrasonic combined PAA in microbial sanitation (coliform, CFU/g) on different shrimps

| Criteria | Different shrimps | Experimental parameters | | | | |
|---|---|---|---|---|---|---|
| Amplitude (30 μm) chemical concentration (vary) temperature (4°C) time (3 min) | | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm |
| Coliform (CFU/g) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (vary) chemical concentration (40 ppm) temperature (4°C) time (3 min) | | 30 μm | 60 μm | 90 μm | 120 μm | 150 μm |
| Coliform (CFU/g) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (120 μm) Chemical concentration (40 ppm) Temperature (vary) Time (3 min) | | 4°C | 8°C | 12°C | 16°C | 20°C |
| Coliform (CFU/g) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (120 μm)Chemical concentration (40 ppm)Temperature (12°C) Time (vary) | | 3 min | 6 min | 9 min | 12 min | 15 min |
| Coliform (CFU/g) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |

Table 4: Effect of different parameters of ultrasonic combined PAA in firmness (texture, kg/cm), color apperance (L) on different shrimps

| Criteria | Different shrimps | Experimental parameters | | | | |
|---|---|---|---|---|---|---|
| Amplitude (30 μm) chemical concentration (vary) Temperature (4°C) time (3 min) | | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Color (L) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (vary) chemical concentration (40 ppm) Temperature (4°C) time (3 min) | | 30 μm | 60 μm | 90 μm | 120 μm | 150 μm |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Color (L) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (vary) time (3 min) | | 4°C | 8°C | 12°C | 16°C | 20°C |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Color (L value) | BT. Shrimp | | | | | |
| | W.L. Shrimp | | | | | |
| | G. Prawn | | | | | |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (12°C) time (vary) | 3 min | 6 min | 9 min | 12 min | 15 min | |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | | | | | |

Table 4: Continue

| Criteria | Different shrimps | ----------Experimental parameters---------- |
|---|---|---|
| Color (L value) | W.L. Shrimp | |
| | G. Prawn | |
| | BT. Shrimp | |
| | W.L. Shrimp | |
| | G. Prawn | |

Table 5: Effectiveness of optimal parameters of ultrasonic combined PAA on different quality and hygiene criteria of different shrimps

| | Black tiger shrimp | | White leg shrimp | | Giant prawn | |
|---|---|---|---|---|---|---|
| Criteria | Before treatment | After treatment | Before treatment | After treatment | Before treatment | After treatment |
| Quality PPO ($eu.min^{-1}.mL^{-1}$) | | | | | | |
| Black spot score (1-10) | | | | | | |
| Texture ($kg/cm^2$) | | | | | | |
| Color (L) | | | | | | |
| Hygiene | | | | | | |
| Coliform (CFU/g) | | | | | | |
| E. *Coli* (CFU/g) | | | | | | |
| Salmonella (CFU/g) | | | | | | |

Table 6: Melanosis apperance after being frozen and thawed

| | ----------Melanosis score on shrimps after being frozen and thawed ---------- | | | |
|---|---|---|---|---|
| Way 1 | 0 month | 1 month | 2 months | 3 months |
| Black tiger shrimp | | | | |
| White leg shrimp | | | | |
| Giant prawn | | | | |
| Way 2 | 0 month | 1 month | 2 months | 3 months |
| Black tiger shrimp | | | | |
| White leg shrimp | | | | |
| Giant prawn | | | | |
| Way 3 | 0 month | 1 month | 2 months | 3 months |
| Black tiger shrimp | | | | |
| White leg shrimp | | | | |
| Giant prawn | | | | |

**Optimal parameters of ultrasonic combined PAA:** amplitude (120 μ), chemical concentration (40 ppm), temperature (12°C) and time (9 min).

**Melanosis apperance (melanosis score) after thawing** Freeze samples at -18°C in 1 month then thaw products under three different ways:

**Way 1:** Thaw the non-treated samples (non-ultrasonication) in cooling water 4°C without ultrasonication.

**Way 2:** Thaw the pre-treated samples (ultrasonication) in cooling water 4°C without ultrasonication.

**Way 3:** Thaw the pre-treated samples (ultrasonication) in cooling water 4°C with ultrasonication.

Then evaluate melanosis formation by numbering (score). After evaluating, re-freezing-thawing-evaluating for more 2 cycles (one cycle each month). In respect of black spot evaluation, thawed samples are trayed in normal room temperature in 15 min and then evaluated through visual inspection by ten trained panelists using 10-point scoring test.

**Physico-chemical analysis:**

- Determine the firmness by penetrometer
- Determine lightness (L*) by CIELAB instrument
- Determine PPO activity (Nirmal and Benjakul, 2011) melanosis, microbiological analysis (3M protocol)

**Statistical analysis:** The experiments were run in triplicate with three different lots of samples. Data were subjected to Analysis of Variance (ANOVA) and mean comparison was carried out using Duncan's Multiple Range Test (DMRT) statistical analysis was performed by the startgraphics (Table 6).

## RESULTS AND DISCUSSION

**Effect of different washing methods:** Shrimps are chosen in this experiment including black tiger shrimp, white leg shrimp and giant prawn. Testing parameters include melanosis: PPO activity, melanosis score, microorganism: Coliform, quality: color, texture. Our results were illustrated in Table 7. We highly valued the effect of combination between ultrasonic and PAA as a significant synergistic effect.

Table 7: Effect of different washing methods on different shrimps (melanosis: PPO activity, melanosis score; microorganism: coliform, quality: color, texture)

| | Testing parameters | | | | |
|---|---|---|---|---|---|
| Washing methods/Shrimps | PPO activity (eu.min$^{-1}$.mL$^{-1}$) | Melanosis score (1-10) | Coliform (CFU/g) | Color (L value) | Texture (kg/cm$^2$) |
| **Before treatment** | | | | | |
| B.T. Shrimp | 637 | 0 | 2.1×102 | 26.42 | 0.93 |
| W.L. Shrimp | 445 | 0 | 1.8×102 | 30.49 | 0.84 |
| G. Prawn | 520 | 0 | 2.4×102 | 28.24 | 1.05 |
| **Control** | | | | | |
| B.T. Shrimp | 637 | 10 | 1.9×102 | 26.42 | 0.93 |
| W.L. Shrimp | 445 | 10 | 1.6×102 | 30.49 | 0.84 |
| G. Prawn | 520 | 10 | 2.1×102 | 28.24 | 1.05 |
| **Ultrasonic alone** | | | | | |
| B.T. Shrimp | 280 | 2 | 0.2×101 | 32.97 | 0.92 |
| W.L. Shrimp | 195 | 2 | 0.1×101 | 38.47 | 0.83 |
| G. Prawn | 238 | 2 | 0.2×101 | 36.25 | 1.04 |
| **PAA alone** | | | | | |
| B.T. Shrimp | 524 | 3 | 0.3×101 | 27.54 | 0.93 |
| W.L. Shrimp | 385 | 3 | 0.1×101 | 32.66 | 0.84 |
| G. Prawn | 429 | 3 | 0.2×101 | 29.34 | 1.05 |
| **Combined ultrasonic and PAA** | | | | | |
| B.T. Shrimp | 211 | 0 | 0 | 38.41 | 0.92 |
| W.L. Shrimp | 127 | 1 | 0 | 41.26 | 0.83 |
| G. Prawn | 184 | 0 | 0 | 39.19 | 1.04 |

[a-d]Each value is the mean of three samples (n = 3)

Table 8: Effect of different parameters of ultrasonic combined PAA in melanosis inhibition (PPO activity, melanosis score) on different shrimps

| | Different shrimps | Experimental parameters | | | | |
|---|---|---|---|---|---|---|
| Amplitude (30 μm) chemical concentration (vary) temperature (4°C) time (3 min) | | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm |
| PPO activity (eu.min$^{-1}$.mL$^{-1}$) | BT. Shrimp | 429$^a$ | 374$^b$ | 304$^c$ | 211$^d$ | 209$^d$ |
| | W.L. Shrimp | 294$^a$ | 252$^b$ | 199$^c$ | 127$^d$ | 125$^d$ |
| | G. Prawn | 311$^a$ | 294$^b$ | 226$^c$ | 184$^d$ | 181$^d$ |
| Black spot (score) | BT. Shrimp | 8$^a$ | 5$^b$ | 4$^c$ | 0$^d$ | 0$^d$ |
| | W.L. Shrimp | 8$^a$ | 6$^b$ | 3$^c$ | 0$^d$ | 0$^d$ |
| | G. Prawn | 8$^a$ | 5$^b$ | 3$^c$ | 0$^d$ | 0$^d$ |
| Amplitude (vary) chemical concentration (40 ppm) temperature (4°C) time (3 min) | | 30 μm | 60 μm | 90 μm | 120 μm | 150 μm |
| PPO activity (eu.min$^{-1}$.mL$^{-1}$) | BT. Shrimp | 388$^a$ | 289$^b$ | 234$^c$ | 195$^d$ | 192$^d$ |
| | W.L. Shrimp | 207$^a$ | 185$^b$ | 164$^c$ | 110$^d$ | 109$^d$ |
| | G. Prawn | 293$^a$ | 234$^b$ | 202$^c$ | 162$^d$ | 160$^d$ |
| Black spot (score) | BT. Shrimp | 6$^a$ | 5$^b$ | 2$^c$ | 0d | 0d |
| | W.L. Shrimp | 6$^a$ | 5$^b$ | 1$^c$ | 0$^d$ | 0$^d$ |
| | G. Prawn | 6$^a$ | 4$^b$ | 1$^c$ | 0$^d$ | 0$^d$ |
| Amplitude (120 mm) chemical concentration (40 ppm) temperature (vary) time (3 min) | | 4°C | 8°C | 12°C | 16°C | 20°C |
| PPO activity (eu.min$^{-1}$.mL$^{-1}$) | BT. Shrimp | 270$^a$ | 224$^b$ | 190$^c$ | 189$^c$ | 187$^c$ |
| | W.L. Shrimp | 193$^a$ | 182$^b$ | 174$^c$ | 173$^c$ | 172$^c$ |
| | G. Prawn | 225$^a$ | 201$^b$ | 185$^c$ | 185$^c$ | 184$^c$ |
| Black spot (score) | BT. Shrimp | 3$^a$ | 2$^b$ | 0$^c$ | 0$^c$ | 0$^c$ |
| | W.L. Shrimp | 3$^a$ | 1$^b$ | 0$^c$ | 0$^c$ | 0$^c$ |
| | G. Prawn | 3$^a$ | 1$^b$ | 0$^c$ | 0$^c$ | 0$^c$ |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (12°C) time (vary) | | 3 min | 6 min | 9 min | 12 min | 15 min |
| PPO activity (eu.min$^{-1}$.mL$^{-1}$) | BT. Shrimp | 218$^a$ | 195$^b$ | 153$^c$ | 152$^c$ | 150$^c$ |
| | W.L. Shrimp | 175$^a$ | 149$^b$ | 102$^c$ | 102$^c$ | 100$^c$ |
| | G. Prawn | 197$^a$ | 178$^b$ | 121$^c$ | 120$^c$ | 120$^c$ |
| Black spot (score) | BT. Shrimp | 2$^a$ | 1$^b$ | 0$^c$ | 0$^c$ | 0$^c$ |
| | W.L. Shrimp | 2$^a$ | 1$^b$ | 0$^c$ | 0$^c$ | 0$^c$ |
| | G. Prawn | 1$^a$ | 0$^b$ | 0$^b$ | 0$^b$ | 0$^b$ |

*Each value is the mean of three samples (n = 3). The same characters (denoted above), the difference between them was not significant ($\alpha$ = 5%)

**Effect of different parameters of ultrasonic combined PAA in melanosis inhibition (PPO activity, melanosis score) on different shrimps:** We measured the effect of different parameters of ultrasonic combined PAA in melanosis inhibition (PPO activity, melanosis score) on different shrimps. Our results were depicted in Table 8.

Table 9: Effect of different parameters of ultrasonic combined PAA in microbial sanitation (coliform, CFU/g) on different shrimps

| | Different shrimps | Experimental parameters | | | | |
|---|---|---|---|---|---|---|
| Amplitude (30 μm) chemical concentration (vary) temperature (4°C) time (3 min) | | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm |
| Coliform (CFU/g) | BT. Shrimp | $0.2\times101^{a}$ | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ |
| | W.L. Shrimp | $0.2\times101^{a}$ | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ |
| | G. Prawn | $0.2\times101^{a}$ | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ |
| Amplitude (vary) chemical concentration (40 ppm) temperature (4°C) time (3 min) | | 30 μm | 60 μm | 90 μm | 120 μm | 150 μm |
| Coliform (CFU/g) | BT. Shrimp | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ |
| | W.L. Shrimp | $0c.1\times101^{a}$ | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ |
| | G. Prawn | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (vary) time (3 min) | | 4°C | 8°C | 12°C | 16°C | 20°C |
| Coliform (CFU/g) | BT. Shrimp | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ | $0^{b}$ |
| | W.L. Shrimp | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ | $0^{b}$ |
| | G. Prawn | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ | $0^{b}$ |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (12°C) time (vary) | | 3 min | 6 min | 9 min | 12 min | 15 min |
| Coliform (CFU/g) | BT. Shrimp | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ | $0^{b}$ |
| | W.L. Shrimp | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0b^{b}$ | $0^{b}$ |
| | G. Prawn | $0.1\times101^{a}$ | $0.1\times101^{a}$ | $0^{b}$ | $0^{b}$ | $0^{b}$ |

Table 10: Effect of different parameters of ultrasonic combined PAA in firmness (texture, kg/cm$^2$), color apperance (L value) on different shrimps

| | Different shrimps | Experimental parameters | | | | |
|---|---|---|---|---|---|---|
| Amplitude (30 μm) chemical concentration (vary) temperature (4°C) time (3 min) | | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | $0.93^{a}$ | $0.93^{a}$ | $0.93^{a}$ | $0.91^{b}$ | $0.90^{b}$ |
| | W.L. Shrimp | $0.84^{a}$ | $0.84^{a}$ | $0.84^{a}$ | $0.80^{a}$ | $0.80^{a}$ |
| | G. Prawn | $1.05^{a}$ | $1.05^{a}$ | $1.05^{a}$ | 1.01b | $1.01^{b}$ |
| Color (L) | BT. Shrimp | $32.97^{d}$ | $33.11^{c}$ | $34.29^{b}$ | $35.86^{a}$ | $35.90^{a}$ |
| | W.L. Shrimp | $38.47^{d}$ | $39.75^{c}$ | $40.29^{b}$ | $41.74^{a}$ | $41.78^{a}$ |
| | G. Prawn | $36.25^{d}$ | $38.01^{c}$ | $39.39^{b}$ | $42.94^{a}$ | $43.00^{a}$ |
| Amplitude (vary) chemical concentration (40 ppm) temperature (4°C) time (3 min) | | 30 μm | 60 μm | 90 μm | 120 μm | 150 μm |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | $0.91^{a}$ | $0.89^{ab}$ | $0.88^{b}$ | $0.82^{c}$ | $0.82^{c}$ |
| | W.L. Shrimp | $0.80^{a}$ | $0.80^{a}$ | $0.76^{b}$ | $0.71^{c}$ | $0.70^{c}$ |
| | G. Prawn | $1.01^{a}$ | 0.93b | $0.91^{c}$ | $0.84^{d}$ | $0.83^{d}$ |
| Color (L) | BT. Shrimp | $33.74^{d}$ | $34.95^{c}$ | $36.19^{b}$ | $38.55^{a}$ | $38.58^{a}$ |
| | W.L. Shrimp | $40.04^{d}$ | $41.34^{c}$ | $43.08^{b}$ | $44.27^{a}$ | $44.30^{a}$ |
| | G. Prawn | $39.17^{d}$ | $40.85^{c}$ | $42.38^{b}$ | $43.69^{a}$ | $43.70^{a}$ |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (vary) time (3 min) | | 4°C | 8°C | 12°C | 16°C | 20°C |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | $0.89^{a}$ | $0.89^{a}$ | $0.88^{a}$ | $0.81^{b}$ | $0.69^{c}$ |
| | W.L. Shrimp | $0.80^{a}$ | $0.80^{a}$ | $0.79^{a}$ | $0.70^{b}$ | $0.67^{c}$ |
| | G. Prawn | $0.93^{a}$ | $0.93^{a}$ | $0.92^{a}$ | $0.85^{b}$ | $0.80^{c}$ |
| Color (L) | BT. Shrimp | $36.33^{c}$ | $38.01^{b}$ | $39.18^{a}$ | $39.20^{a}$ | $39.21^{a}$ |
| | W.L. Shrimp | $43.21^{c}$ | $46.18^{b}$ | $47.49^{a}$ | $47.60^{a}$ | $47.63^{a}$ |
| | G. Prawn | $42.45^{c}$ | $44.10^{b}$ | $45.92^{a}$ | $45.95^{a}$ | $46.00^{a}$ |
| Amplitude (120 μm) chemical concentration (40 ppm) temperature (12°C) time (vary) | | 3 min | 6 min | 9 min | 12 min | 15 min |
| Firmness (texture, kg/cm$^2$) | BT. Shrimp | $0.89^{a}$ | $0.89^{a}$ | $0.82^{b}$ | $0.73^{c}$ | $0.67^{d}$ |
| | W.L. Shrimp | $0.80^{a}$ | $0.80^{a}$ | $0.75^{b}$ | $0.66^{c}$ | $0.59^{d}$ |
| | G. Prawn | $0.93^{a}$ | $0.93^{a}$ | $0.84^{b}$ | $0.72^{c}$ | $0.61^{d}$ |
| Color (L) | BT. Shrimp | $39.10^{c}$ | $42.11^{b}$ | $45.39^{a}$ | $45.41^{a}$ | $45.43^{a}$ |
| | W.L. Shrimp | $47.23^{c}$ | $49.80^{b}$ | $51.05^{a}$ | $51.08^{a}$ | $51.13^{a}$ |
| | G. Prawn | $45.37^{c}$ | $47.03^{b}$ | $49.34^{a}$ | $49.40^{a}$ | $49.43^{a}$ |

[a-d]Each value is the mean of three samples (n = 3). The same characters (denoted above), the difference between them was not significant ($\alpha$ = 5%)

**Effect of different parameters of ultrasonic combined PAA in microbial sanitation (coliform) on different shrimps:** We measured the effect of different parameters of ultrasonic combined PAA in microbial sanitation (coliform, CFU/g) on different shrimps. Our results were depicted in Table 9.

**Effect of different parameters of ultrasonic combined PAA in firmness (texture, kg/cm$^2$), color apperance (L) on different shrimps:** We measured the effect of different parameters of ultrasonic combined PAA in firmness (texture, kg/cm$^2$), color apperance (L) on different shrimps (Table 10).

Table 11: Effectiveness of optimal parameters of ultrasonic combined PAA on different quality and hygiene criteria of different shrimps

| | | Black tiger shrimp | | White leg shrimp | | Giant prawn | |
|---|---|---|---|---|---|---|---|
| Criteria | | Before treatment | After treatment | Before treatment | After treatment | Before treatment | After treatment |
| Quality | PPO (eu.min$^{-1}$.mL$^{-1}$) | 637$^{a}$ | 153$^{a}$ | 445$^{a}$ | 102$^{b}$ | 520$^{a}$ | 121$^{b}$ |
| | Black spot score | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| | Texture (kg/cm$^{2}$) | 0.93$^{a}$ | 0.82$^{b}$ | 0.84$^{a}$ | 0.75$^{b}$ | 1.05$^{a}$ | 0.84$^{b}$ |
| | Color (L) | 26.42$^{b}$ | 45.39$^{a}$ | 30.49$^{b}$ | 51.05$^{a}$ | 28.24$^{b}$ | 49.34$^{a}$ |
| Hygiene | Coliform (CFU/g) | 2.1×102$^{a}$ | 0$^{b}$ | 1.8×102$^{a}$ | 0$^{b}$ | 2.4×102$^{a}$ | 0$^{b}$ |
| | E. *Coli* (CFU/g) | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| | Salmonella (CFU/g) | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |

Table 12: Melanosis apperance after being frozen and thawed

| Criteria | Melanosis score on shrimps after being frozen and thawed | | | |
|---|---|---|---|---|
| **Way 1** | 0 month | 1 month | 2 months | 3 months |
| Black tiger shrimp | 0$^{d}$ | 5$^{c}$ | 6$^{b}$ | 9$^{a}$ |
| White leg shrimp | 0$^{d}$ | 4$^{c}$ | 5$^{b}$ | 8$^{a}$ |
| Giant prawn | 0$^{d}$ | 4$^{c}$ | 6$^{b}$ | 7$^{a}$ |
| **Way 2** | 0 month | 1 month | 2 months | 3 months |
| Black tiger shrimp | 0$^{d}$ | 2$^{c}$ | 3$^{b}$ | 3$^{a}$ |
| White leg shrimp | 0$^{c}$ | 1$^{b}$ | 2$^{a}$ | 2$^{a}$ |
| Giant prawn | 0$^{c}$ | 0$^{c}$ | 1$^{b}$ | 2$^{a}$ |
| **Way 3** | 0 month | 1 month | 2 months | 3 months |
| Black tiger shrimp | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| White leg shrimp | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| Giant prawn | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |

$^{a-c}$Each value is the mean of three samples (n = 3). The same characters (denoted above), the difference between them was not significant ($\alpha$ = 5%)

**Effectiveness of optimal parameters of ultrasonic combined PAA on different quality and hygiene criteria of different shrimps:** Optimal parameters of ultrasonic combined PAA: amplitude (120 μm), chemical concentration (40 ppm), temperature (12°C) and time (9 min). We measured effectiveness of optimal parameters of ultrasonic combined PAA on different quality and hygiene criteria of different shrimps (Table 11).

**Melanosis apperance (melanosis score) after thawing:** We measured melanosis apperance (melanosis score) after thawing. Freeze samples at -18°C in 1 month then thaw products under three different ways:

**Way 1:** Thaw the non-treated samples (non-ultrasonication) in cooling water 4°C without ultrasonication.

**Way 2:** Thaw the pre-treated samples (ultrasonication) in cooling water 4°C without ultrasonication.

**Way 3:** Thaw the pre-treated samples (ultrasonication) in cooling water 4°C with ultrasonication.

Then evaluate melanosis formation by numbering (score). After evaluating, re-freezing-thawing-evaluating for more 2 cycles (one cycle each month) (Table 12).

## CONCLUSION

The development of melanosis or blackspot during the postharvest period of crustaceans is a well-known postmortem phenomenon attributed to the polymerization of phenol into an insoluble black pigment, melanin. Microorganisms are present on the external surfaces and in the gut and head of shrimp. Upon death, the microorganisms or the enzymes are free to invade or diffuse into the flesh where they react with the complex substances. Our research could be considered as an potential approach to prevent melanosis, microbial elimination, thus extending the shelf life and acceptability of the product.

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