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# Retardation of melanosis and quality loss of pre-cooked Pacific white shrimp using epigallocatechin gallate with the aid of ultrasound

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ABSTRACT

Melanosis and quality changes of pre-cooked Pacific white shrimp treated with epigallocatechin gallate (EGCG) with the aid of ultrasound were monitored during refrigerated storage at 4 °C for 10 days. Shrimps subjected to pre-cooking (70 °C, 30 s), followed by soaking in 0.25% (w/v) EGCG solution with sonication (ultrasound: frequency of 20 kHz, intensity power of 750 W) showed lower melanosis score than those treated with EGCG prior to pre-cooking. Longer sonication time (10–15 min) enhanced the efficacy of melanosis inhibition in treated shrimp ($P < 0.05$). Microbiological analyses revealed that pre-cooked shrimps treated with EGCG solution using ultrasound showed lower total viable count, psychrophilic bacteria, *Pseudomonas* spp., $H_2S$-producing bacteria and Enterobacteriaceae counts than the control and those treated with EGCG or ultrasound alone, throughout 10 days of storage. Lower total volatile base (TVB) content was detected in shrimps treated with EGCG and ultrasound, and these shrimps also showed higher scores for color and overall likeness with coincidentally lower melanosis score than others ($P < 0.05$) at the end of storage. Therefore, soaking of pre-cooked shrimps in EGCG solution with the aid of ultrasound for sufficient time could be a promising method to prevent melanosis and deterioration in pre-cooked Pacific white shrimp during the extended refrigerated storage.



## 1. Introduction

Pacific white shrimp (*Litopenaeus vannamei*) has been of increasing demand worldwide. Shrimp and its products of Thailand have been known for their prime quality and delicacy (Rattanasatheirn, Benjakul, Visessanguan, & Kijroongrojana, 2008). Shrimp is highly perishable with a limited shelf-life, mainly associated with melanosis (discoloration), chemical and microbial deterioration (Gokoglu & Yerlikaya, 2008). Melanosis, triggered by biochemical reaction mediated by polyphenoloxidase, drastically reduces its market value, leading to considerable financial loss (Martínez-Alvarez, Montero, & Gómez-Guillén, 2005). Among shrimp products, the pre-cooked shrimp becomes more popular due to its desirable appearance to consumers, especially reddish color (Manheem, Benjakul, Kijroongrojana, & Visessanguan, 2012). Generally, pre-cooking is classified as a simple process with low cost, which can be used to extend the shelf-life of some food products. Pre-cooking can deactivate some endogenous enzymes, especially polyphenoloxidase (PPO) and proteases, causing quality loss of shrimp (Kim, Marshall, & Wei, 2000). Pre-cooking can also destroy some microorganisms such as *Flavobacterium*, etc. (Harrison & Lee, 1969), thereby increasing storage stability of products. However, pre-cooking at high temperature may cause the enhanced cooking loss of resulting shrimp (Manheem et al., 2012). Pre-cooking of shrimp at lower temperature in combination with the use of PPO inhibitor can be a means to maintain the quality of pre-cooked shrimp.

To overcome or alleviate melanosis in shrimp and other crustaceans, sulfiting agents and 4-hexylresorcinol have been widely used (Montero, Lopez-Caballero, & Perez-Mateos, 2001). Nevertheless, the increasing consumer awareness of the health risks of chemicals and their resistance to the use of chemicals in foods, have compelled regulatory agencies to stringently limit their uses in foods. This brings about an urgent need to discover novel and safer alternatives (McEvily, Iyengar, & Otwell, 1991). Moreover, sulfiting agents, commonly used for melanosis control, elicit allergic type reactions in some individuals (Gunnison, Jacobsen, & Schwartz, 1987). Phenolic compounds or plant extracts have been used to prevent melanosis and quality loss in shrimp. Rosemary extract (Cadun, Kışla, & Çaklı, 2008) and ferulic acid (Nirmal & Benjakul,

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2009a, 2009b) were used to retard quality deterioration in deep-water pink and Pacific white shrimp, respectively. Recently, epigallocatechin gallate (EGCG) has been reported to show the highest PPO inhibitory activity, compared to catechin (C), epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin (EGC) (Sae-leaw, Benjakul, & Simpson, 2017). Sae-leaw et al. (2017) also found that raw Pacific white shrimp treated with EGCG showed the decreased melanosis throughout the storage of 10 days at 4 °C.

Ultrasound is an emerging technology, which has various promising applications in food processing, preservation as well as safety (Chemat, Zill-e, & Khan, 2011). Ultrasound modifies the food properties by inducing mechanical, physical and chemical/biochemical changes through cavitation, which reduces processing time under mild conditions, compared to conventional method. Ultrasound is able to inhibit some enzymes and microbial growth in foods (Chemat et al., 2011). Nevertheless, no information regarding the use of EGCG for maintaining the quality of pre-cooked shrimp exist. Furthermore, the method for EGCG treatment might also determine the efficacy for quality maintenance of pre-cooked shrimp. Sonication via ultrasonic process might be able to enhance the migration of EGCG solution into shrimp, particularly underneath the shell or carapace. The aim of this study was to investigate the effect of EGCG in combination with ultrasound under various conditions on the retardation of melanosis and quality changes in pre-cooked Pacific white shrimp during refrigerated storage.

## 2. Materials and methods

### 2.1. Chemicals

(−)-Epigallocatechin gallate (EGCG) was obtained from Chengdu Biopurify Phytochemicals Ltd. (Sichuan, China). Trichloroacetic acid (TCA) was procured from Merck (Darmstadt, Germany). Potassium carbonate was obtained from Fisher Scientific (Loughborough, UK). Standard plate count agar, triple sugar iron agar, *Pseudomonas* isolation agar and eosin methylene blue agar were purchased from Oxoid Ltd. (Hampshire, UK). All chemicals were of analytical grade.

### 2.2. Shrimp collection

Pacific white shrimps (*L. vannamei*) with the size of 55–60 shrimps/kg were purchased from a local supplier in Songkhla, Thailand. The freshly caught shrimp, completely free of additives, were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, shrimps were washed in iced water (1–3 °C) and stored in ice until used (less than 1 h).

### 2.3. Preparation of pre-cooked Pacific white shrimp treated with EGCG using different processes

Whole shrimps (head-on) were immersed in 0.25% (w/v) EGCG solution using a shrimp/solution ratio of 1:2 (w/v) at 4 °C. Thereafter, the mixtures were subjected to ultrasonic treatment at a frequency of 20 kHz with high intensity power of 750 W using the ultrasonic equipment (Sonics, Model VC750, Sonics & Materials, Inc., Newtown, CT, USA). Different sonication times (0, 5, 10 and 15 min) were used. To reduce heat generated, the iced bath containing water, ice and NaCl (1: 5: 1, w/w/w) was used to maintain the temperature of mixture at 5–8 °C. After sonication for a designated time, the treated shrimps were placed in the stainless cooking pot filled with boiling water (100 °C). A shrimp/boiling water ratio of 1:3 (w/v) was used. The heating was proceeded until the core temperature of the second segment of the shrimp reached 70 °C and held for 30 s (Manheem et al., 2012). To measure the core temperature, the thermo-couple (Union, Kowloon, Hong Kong), disinfected with 70% ethanol, was inserted into the middle of the second segment of abdomen. After heating, the samples were cooled rapidly in iced water for 5 min and then the shrimps were drained on the stainless steel screen for 5 min at 4 °C. The samples soaked in EGCG solution in combination with ultrasound for 0, 5, 10 and 15 min, followed by pre-cooking were referred to as 'EU0 + P', 'EU5 + P', 'EU10 + P' and 'EU15 + P', respectively.

Another portion of fresh shrimps was pre-cooked at 70 °C for 30 s as previously described. After pre-cooking, shrimps were soaked in 0.25% (w/v) EGCG solution with the aid of ultrasound for 0, 5, 10 and 15 min at 4 °C. The samples were drained for 5 min at 4 °C. The samples subjected to pre-cooking, followed by soaking in EGCG solution in combination with ultrasound for 0, 5, 10 and 15 min were referred to as 'P + EU0', 'P + EU5', 'P + EU10' and 'P + EU15', respectively.

Shrimp pre-cooked at 70 °C for 30 s, followed by cooling in iced water for 5 min was also prepared and used as the control. All samples were placed on a polystyrene tray, covered with shrink film and stored at 4 °C. Samples were monitored for melanosis every 2 days up to 10 days.

### 2.4. Melanosis assessment

Melanosis or blackening of pre-cooked shrimp was evaluated through visual inspection using 10-point scoring test by ten trained panelists, who were familiar with the scoring or rating of black spot (melanosis) intensity in pre-cooked Pacific white shrimp (Montero et al., 2001). Prior to the evaluation, the panelists were trained three times a week for 2 weeks. Panelists 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).

### 2.5. Quality changes and melanosis of pre-cooked Pacific white shrimp treated with EGCG using the selected process

Shrimps were pre-cooked as described previously. Pre-cooked samples were then immersed in 0.25% EGCG without and with ultrasound under the aforementioned condition for 10 min and the obtained samples were referred to as 'EGCG' and 'EGCG + US, respectively. Another portion of pre-cooked shrimps were soaked in distilled water at a ratio of 1:2 (w/v) for 10 min at 4 °C without and with ultrasound applied for 10 min. The samples were named as 'CON' and 'US', respectively. All shrimps were drained on a screen for 5 min at 4 °C. The samples were placed on a polystyrene tray, covered with shrink film and stored at 4 °C. Samples were randomly taken and subjected to analyses every 2 days up to 10 days.

### 2.6. Analyses

#### 2.6.1. Microbiological analyses

Microbiological analyses were performed by the spread plate method (Sallam, 2007). Whole shrimps (25 g) were mixed with 225 mL of 0.85% saline buffer, followed by homogenization in a Stomacher blender (Model 400, Seward Ltd., West Sussex, England) for 1 min at 220 rpm. Homogenate was used to prepare ten-fold serial dilutions in 0.85% saline buffer and appropriate dilutions (0.1 mL) were used for the microbiological analyses.

Total viable count (TVC) was determined using plate count agar