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# Inactivation effects and kinetics of polyphenol oxidase from *Litopenaeus vannamei* by ultra-high pressure and heat

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Using heat treatment (40–100 °C) as the control, the effects of ultra-high pressure (UHP, 100–600 MPa) on activity of polyphenol oxidase (PPO) from *Litopenaeus vannamei* (whiteleg shrimp, also known as Pacific white shrimp) were investigated, and the kinetic models of PPO inactivated by UHP were established. The results showed that PPO activity was significantly reduced ($p < 0.05$) with increasing temperature. For UHP treatment, PPO was activated and increased maximally by 57.61% when the pressure was lower than 300 MPa, and PPO activity reduced significantly ($p < 0.05$) when the pressure was above 300 MPa. PPO was completely inactivated using 2-min exposures to 500 MPa. Inactivation of PPO by heat (60–100 °C) and UHP (400–600 MPa) all followed first-order reaction kinetics. At 400–600 MPa PPO from the crude extracts was effectively inactivated, but PPO was activated directly in shrimp. If the PPO in shrimp was completely inactivated, higher pressure would probably be needed or the pressure would need to be combined with temperature or other melanosis inhibitors. It is a reference for the application of high pressure to inactivate PPO of *Litopenaeus vannamei*.

*Industrial relevance:* Ultra-high pressure (UHP) has the potential to destroy food-borne microorganisms and inactivate enzymes while minimally affecting the sensory and nutritional quality of food products. *Litopenaeus vannamei* is one of the aquatic products with high nutritional value and accounts for 90% of global aquaculture shrimp production. However, melanosis induced by polyphenol oxidase (PPO) from shrimp has been of great concern to food scientists and food processors. UHP is a good technology to inactivate PPO. However, little work has been reported on PPO from shrimp processed by UHP. The available data are provided for the evaluation and application of UHP, and the present work is inactivation effects and kinetics of polyphenol oxidase from *Litopenaeus vannamei* by ultra-high pressure in order to explore the feasibility of processing shrimp using UHP.



## 1. Introduction

Melanosis is a chemical process that occurs in the membrane between the shell and the meat in the metamorphic process triggered by polyphenol oxidase (PPO) in crustaceans, such as shrimp. This change, as one of the most important causes of shrimp quality loss, decreases the marketability of the shrimp during storage. The pigmentation is harmless for consumers, but seriously affects the organoleptic quality of the shrimp. Therefore, it is especially important to prevent shrimp from melanosis during processing and storage. PPO is a copper-containing enzyme, widely distributed in plants, microorganisms and crustaceans (Kumar, Mohan, & Murugan, 2008; Roman, Ulrike, & Dietrich, 2009). PPO easily catalyzes phenolic substances and polymerize to form insoluble melanin that are not attractive to consumers (Begona, Oscar, Pilar, & Maria, 2010).

By inhibiting or removing the PPO activity, reducing action of tyrosine, or isolating the shrimp from oxygen, it is possible to control shrimp melanosis effectively during processing and storage (Ling & Xie, 2009). Currently, the technologies for preventing shrimp melanosis can be divided into two categories: the first is to add some inhibitors, such as a reducing agent, antioxidant, metal ion-chelating agent, oxygen insulation film formers or structural analogues of the phenol oxidase for preventing shrimp melanosis, and the second is to utilize process technology, such as heat treatment, low-temperature preservation, UHP processing, dense high carbon dioxide processing or pulsed electric field to inhibit or inactivate PPO activity.

UHP is a non-thermal technology and has a good potential as an alternative for traditional preservation methods like heat treatment. UHP is widely used for vegetables, meat and seafood because of the inactivation of microorganisms and enzymes, and the technology better retains the nutritional and sensory characteristics of fresh food (Jantakoson, Kijroongrojana, & Benjakul, 2012; Kaur, Kaushik, Rao, & Chauhan, 2013; Roman, Ulrike, & Dietrich, 2009). UHP is used for inactivating enzymes in food, mainly for inactivating PPO, peroxidase (POD), lipoxidase (LOX) and protease from fruit and vegetable. PPO is a

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pressure-resistant enzyme and the relative activity of PPO is still greater than 88% while pressure is less than 600 MPa (Yi et al., 2012), even PPO is activated at lower pressure (Chang, Li, Xue, & Zhang, 2008; Gomes & Ledward, 1996). These phenomena are related to the sources and characteristics of PPO and the treatment conditions of UHP.

Much research on PPOs from fruits and vegetables inactivated by UHP has been reported, including apple PPO (Anese, Nicoli, Dallaglio, & Lerici, 1995; Keenan, Rossle, Gormley, Butler, & Brunton, 2012; Roman, Ulrike, & Dietrich, 2009), strawberry PPO (Garcia-Palazon, Suthanthangjai, Kajda, & Zabetakis, 2004; Keenan et al., 2012; Sulaiman & Silva, 2013), red raspberry PPO (Garcia-Palazon et al., 2004), mushroom PPO (Gomes & Ledward, 1996; Yi, Zhang, Liao, Zhang, & Hu, 2012), plum purée PPO (González-Cebrino, Durán, Delgado-Adámez, Contador, & Ramírez, 2013), pear PPO (Liu et al., 2009) and beverages like apple juice PPO (Keenan et al., 2012), carrot juice PPO (Kim, Park, Cho, & Park, 2001), strawberry puree PPO (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010), and banana puree PPO (Palou, López-Malo, Barbosa-Cánovas, Welti-Chanes, & Swanson, 1999). However, there are few reports concerning UHP inactivation of shrimp PPO. *Litopenaeus vannamei* accounts for 90% of global aquaculture shrimp production (Nirmal & Benjakul, 2012a), but it is susceptible to deterioration during storage. Unfavorable color change that is associated with melanosis on the surface of shrimp products has been of great concern to food scientists and food processors (Nirmal & Benjakul, 2012b; Zhang et al., 2011).

The objective of this work was to investigate and compare the effects of UHP and heat treatment on PPO activity of *Litopenaeus vannamei* and to establish inactivation kinetic models. It will provide a reference for the application of high pressure to inactivate PPO of *Litopenaeus vannamei*.

## 2. Materials and methods

### 2.1. Materials

Live *Litopenaeus vannamei* (whiteleg shrimp, also known as Pacific white shrimp) with an average size of 55–60 shrimp/kg was purchased from the Dongfeng seafood wholesale market in Zhanjiang, China. The shrimp was kept in ice with a shrimp/ice ratio of 1:3 (w/w) and transported to the laboratory within 1 h. Upon arrival, shrimp was washed in cold water and stored in ice until used (not more than 5 h).

The 3-(3,4-Dihydroxyphenyl)-L-alanine(L-DOPA) and Brij-35 were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

### 2.2. Ultra-high pressure treatment system

Pressure treatments were conducted using a UHP experimental system (Tianjin Huatai Senmiao Limited Company, Tianjin, China). The system was composed of a control panel, pressure vessel, hydraulic intensifier and pumping system. All parts of the system exposed to high pressure were made of stainless steel. The pressure vessel was 0.6 L, which was designed to withstand a pressure of up to 600 MPa. A copper tube jacket in thermal contact with the outer surface of the vessel wall was connected to the circulator to allow temperature control. The treated temperature was set to 25 °C,which fluctuation ranged between 0 °C and 1 °C. The maximum working pressure of the UHP unit was 600 MPa. Distilled water was used as the pressure transmission medium in the UHP unit. The pressure increase and release rates were approximately 25 MPa/s and 60 MPa/s, respectively. The pressures and treatment times were displayed on a control panel and were continuously recorded by a computer during the pressure treatment.

### 2.3. UHP treatment

Pressure treatment of PPO extracts from *Litopenaeus vannamei* was at 100–600 MPa at 25 °C for 0.5–10 min and an untreated sample was used as a control. Two mL of sample was filled into a polyethylene (PE) bag and vacuum-sealed. Then, the sealed bag was placed into the UHP vessel and pressurized to the required pressure level, and the pressure was held for the required treatment time. The treatment time in this study did not include the come-up time and release time. After UHP treatment, the sample was taken out from the vessel and immediately cooled in the ice bath. The PPO activity measurement was performed in 1 h.

### 2.4. Heat treatment

Heat treatment was performed at 40, 50, 60, 70, 80, 90 and 100 °C for 0.5–30 min. A 2.0-mL volume of PPO extracts from *Litopenaeus vannamei* was filled into a polyethylene bag and vacuum-sealed. Samples were then heated for preset times in a water bath with a thermostat. The time period to reach the treatment temperature was not included in calculations. The PPO sample was taken out from the water bath and immediately cooled in an ice bath after heat treatment. The PPO activity measurement was performed in 1 h.

### 2.5. Enzyme extraction

The isolation of PPO was carried out according to the method of Simpson, Marshall, and Otwell (1987) with slight modification. The cephalothoraxes of the shrimp (150 g) were mixed with 450 mL of 0.05 M sodium phosphate buffer, pH 7.2, containing 0.5 M NaCl and 0.2% Brij35. The mixture was stirred continuously for 30 min at 4 °C, followed by centrifugation at 8000 g at 4 °C for 30 min using a refrigerated centrifuge (Hitachi, Kyoto, Japan). The supernatant was collected and precipitated with ammonium sulfate to obtain 40% - 70% saturation and allowed to stand for 30 min at 4 °C. After subsequent centrifugation at 12,500 g for 30 min at 4 °C, the pellet obtained was dissolved in a minimum volume of 0.05 M sodium phosphate buffer, pH 7.2, and dialyzed against three changes of distilled water. The insoluble materials were removed by centrifugation at 3000 g for 30 min at 4 °C, and the supernatant was used as "crude PPO extracts".

### 2.6. Measurement of PPO activity

PPO activity was assayed using L-DOPA as a substrate according to the method of Simpson et al. (1987) with a slight modification. Measurements were made in a mixture of 0.3 mL of crude PPO extracts, 1 mL of 15 mM L-DOPA in deionized water, 1 mL of 0.05 M phosphate buffer, pH 6.0, and 1 mL of deionized water. The PPO activity was determined for 3 min at 25 °C by monitoring the formation of dopachrome at 475 nm using a UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan). One unit of PPO activity was defined as an increase in the absorbance by 0.001 at 475 nm/min/mL. The blank was prepared by excluding the enzyme from the reaction mixture and deionized water was used instead.

### 2.7. Data analysis

The inactivation kinetics of PPO were analyzed by using a conventional zero-order and first-order reaction (Eqs. (1)-(3)).

$$A_t = A_0 \pm k_0 * t \tag{1}$$

$$A_t = A_0 * \exp\left(\pm k1 * t\right) \tag{2}$$

$$D = \frac{2.303}{k} \tag{3}$$