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Inhibitory effect of glucose oxidase from *Bacillus* sp. CAMT22370 on the quality deterioration of Pacific white shrimp during cold storage

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ABSTRACT

The retarding effect of glucose oxidase (GOD) from *Bacillus* sp.CAMT22370 on the quality degradation of *Litopenaeus vannamei* was investigated in comparison with the commonly used preservatives of sodium sulfite (SS), phytic acid (PA) and vitamin C (Vc) during 5 days of storage at 4 °C. The quality parameters of sensory (appearance, color, odd, elasticity and overall sensory score), physicochemical (total volatile basic nitrogen (TVB-N), pH, protease and polyphenol oxidase (PPO) activity, texture (hardness, gumminess, chewiness, adhesiveness, springiness and cohesiveness) and bacteriological characteristics (total aerobic counts (TAC) and *Pseudomonas* spp. counts (PBC)) were periodically detected and analyzed. The results indicated that the selected preservatives of SS, PA, Vc and GOD could improve the overall sensory score, reduce the increase of TVB-N, pH, proteinase and PPO activity, enhance the texture properties and inhibit the microbial growth at remarkable or significant level ($p < 0.05$) versus the control group of distilled water. Moreover, GOD treatment displayed more desirable retarding effect on quality degradation than that of SS, PA and Vc. Therefore, GOD treatment may be a promising alternative for maintaining the storage quality and extending shelf life of *L. vannamei* during cold storage.

1. Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is an economically important species worldwide with the proportion of 90% global aquaculture shrimp production (Okpala, Choo, & Dykes, 2014; Xu, Wang, Sun, Liu, & Li, 2013). Freshness is an important quality indicator of *L. vannamei* and restoring the freshness of *L. vannamei* after harvesting is consequently the primary task for shrimp industry. Quality deterioration of *L. vannamei*, however, occurs rapidly after postmortem owing to the microbial and enzymatic breakdown (Encarnacion et al., 2012). To retard the deterioration and extend shelf life of shrimp species, many measures, such as high temperature treatment (Manheem, Benjakul, Kijroongrojana, & Visessanguan, 2012), immersing in ergothioneine-rich extract and tea extracts (Encarnacion et al., 2012; Nirmal & Benjakul, 2011), and even treatment with dense phase carbon dioxide (Zhang et al., 2011) have been reported. Preservation at low temperature, however, is still the primary measure to maintain freshness and extend shelf life of shrimp as the reduction of microbiological, chemical and biochemical reaction at low temperature. Synthetic preservatives (e.g., antioxidants, chelating agents, antimicrobial compounds etc.) have been applied as food additives to extend shelf life, but they are strictly regulated due to toxicological concerns and some health problems. Therefore, it is increasingly attractive to find out safety and effective alternatives to extend the shelf life of shrimp.

Glucose oxidase (β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.2.3.4, GOD) catalyzes the oxidation of β-D-glucose to gluconic acid by utilizing molecular oxygen as an electron acceptor and the hydrogen peroxide was simultaneously produced (Bankar, Bule, Singhal, & Ananthanarayan, 2009). Generally, GOD exhibited remarkable oxygen and glucose removal and proved safety (Konishi, Aoshima, Mizuhashi, Choi, & Roberts, 2013). Thus it has been widely put into use of glucose determination in the body fluids and of enzyme antibody conjugates for enzyme immunoassays. Moreover, GOD has been widely used in the food industry for glucose removal from powdered eggs (Sisak, Csanadi, Ronay, & Szajani, 2006), oxygen removal from fruit juices (Parpinello, Chinnici, Versari, & Riponi, 2002) and yogurt (Cruz et al., 2012), improvement of color, flavor, and shelf life of bread and other sample quality (Bonet et al., 2006; Gujral & Rosell, 2004; Rasiah, Sutton, Low,

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Lin, & Gerrard, 2005). Additionally, GOD possesses remarkable antibacterial activity against different food-borne pathogens such as *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus* and *Listeria monocytogens* and thus is used as a food preservative (Pluschkell, Hellmuth, & Rinas, 1996). In view of these excellent maintaining and improving effect of GOD on food quality during storage, screening the novel strain with high production of GOD is imperative. After continuous isolation from the marine environment, a strain of *Bacillus* sp. with high production of GOD was screened in our laboratory and was name after *Bacillus* sp. CAMT22370. Moreover, little information on the use of GOD for enhancing the shelf life of shrimp is available. Therefore, the objective of this study was to investigate the effects of GOD from Bacillus sp. CAMT22370 on the sensory, physicochemical and bacteriological characteristics of *L. vannamei* during cold storage.

2. Materials and methods

2.1. Liquid fermentation of Bacillus sp. CAMT22370 and preparation of preservatives

The strain of *Bacillus* sp. CAMT22370 was isolated from the marine soil and the stock cultures were maintained at −20 °C in 5 mL headspace vials containing 20% (v/v) glycerol until use. Cells from the stock cultures were transferred to 100 mL of growth medium in 250 mL Erlenmeyer flasks, followed by incubation at rotation of 150 rpm and 28 °C for 48 h. The growth medium was comprised of the following components (g L^{-1}): tryptone 10, yeast extract 5 and NaCl 10. The exponentially grown cultures were used as an inoculum for further experiments. Then this cultures was inoculated aseptically at a level of 2% (v/v) into a fresh 250 mL Erlenmeyer flask containing 100 mL of the growth medium and fermented on a shaking incubator (THZ-100; Yiheng Co., Ltd., Shanghai of China) at rotation of 150 rpm and 28 °C for 48 h. After fermentation, the bacterial cells were precipitated and separated from fermentation broth with cold centrifugation at the speed of 8000 × g and 4 °C for 10 min and then the culture supernatant from the fermentation broth was used as the source of enzyme. The enzyme activity was measured to be 12.5 U/ml according to the method described by Vikartovsk et al. (2007) and Guiseppi-Elie, Choi, and Geckeler (2009). The commonly used preparative in fishery products of SS (Taosheng chemical Co. LTD, Guangzhou of China), PA (Jinhua chemical reagent Co. LTD, Guangzhou of China) and Vc (Sihewei chemical industry Co. LTD, Shanghai of China) were purchased and 2% (w/v) of preparative solutions were prepared by dissolving the preservatives into distilled water. The retarding effect of SS, PA, Vc and GOD on the quality degradation of shrimps during cold storage was compared with distilled water.

2.2. Shrimp collection and treatment

500 of live full-grown *L. vannamei* with the average weight of 12–16 g and length of 12–15 cm were purchased from a local aquatic products wholesale market in Zhanjiang of China and were immediately transported to the laboratory alive within 3 h. Upon arrival, the shrimp were maintained in an aerated and brackish water tank at 28 °C for 2 days to stabilize their condition before storage. Then the 500 shrimps were randomly and equally divided into five groups: control samples (CK), samples treated with SS, PA, Vc and GOD, respectively. Samples of SS, PA, Vc and GOD were treated with preservatives by soaking into the solution for 5 min and drained at 4 °C for 10 min, and samples CK were soaked into distilled water instead. After that, all of the healthy shrimps without visual defects or breakage in each group were sacrificed on the ice and placed into the sterile polyethylene bags for storage at 4 °C in a refrigerator (KG23F1830W, Simens, made in China). At intervals 0, 1, 2, 3, 4 and 5 days of storage, approximately 10 of shrimps were taken randomly for analysis and the retarding efficacy of each preventive on quality degradation was compared with the control samples.

2.3. Sensory analysis

To evaluate the sensory quality, a sensory panel consisted of nine experienced and trained assessors were recruited from the laboratory staff aged from 30 to 55 years. Evaluation was performed immediately after the sample removal from storage conditions. Each assessor was requested to grade the appearance, color, odor, elasticity, and overall sensory score. Appearance was evaluated using the 4-point scale, 4 = like extremely, 3 = like moderately, 2 = neither like nor dislike, 1 = dislike. The color was assessed with the melanosis development and the 10-point scoring test was performed according to the method of Montero, Lopez-Caballero, and Perez-Mateos (2001). The odor was graded with method of Arancibia, López-Caballero, Gómez-Guilléna, and Montero (2015). The elasticity from 0 to 4 was as follows: 4 = typical elasticity characteristic in fresh shrimp, 3 = slight decrease in elasticity but still being acceptable, 2 = remarkable decrease in elasticity, 1 = loss of elasticity. The acceptability limit of appearance, color, odor and elasticity were 2, 4, 3 and 3, respectively. Consequently, the overall acceptability limit of sensory score was determined at 12.

2.4. Biochemical analysis

The determination of total volatile basic nitrogen (TVB-N) was carried out according to the semi-micro-titration method proposed by Okpala et al. (2014) with some modifications Approximately 5 g of shrimp sample with head and shell removed was homogenized with 15 mL of 4% trichloroacetic acid (TCA) (w/v) and briefly centrifuged at 3000 × g for 3 min then filtered through Qualitative CirclesR filter paper (125 mm diameter) (GE Healthcare, Buckinghamshire, UK). 5 mL of aliquot was removed and mixed with 5 mL of 2 M NaOH. For TVB -N determinations, the mixture was poured into a semi-micro-distillation tube and steam distillation was performed. The distillate was collected in a beaker containing 15 mL of 0.01 M HCl standard to a final volume of 50 mL. Rosolic acid (1%) in 10% (v/v) ethanol served as indicator. Titration was performed using 0.01 M NaOH to a pale pink end point. The levels of TVB-N was calculated and expressed in mg N/100 g of shrimp. The value of pH was measured according to the method of Wang et al., (2014). pH was determined at room temperature on homogenates of filleted samples in distilled water (1/10 w/w) and the value was monitored using a pH meter (PHS-3DW, Liangyan Smart Tech. LTD., Shanghai of China). For activity analysis of protease and PPO, 2 g of shrimp flesh was mixed with two volumes of 0.01 M sodium phosphate buffer (pH 7.6). The mixture was homogenized for 5 min using a homogenizer (IKA labortechnik, Selangor, Malaysia) at a speed of 10,000 × g. The homogenate was stirred for 30 min at 4 °C, followed by centrifugation at 10,000 × g at 4 °C for 30 min using a refrigerated centrifuge. The supernatant was referred to as crude enzyme extract. Protease activity of crude enzyme extract was determined using 2% casein as substrate according to the method of Manheem et al. (2012) and PPO activity was assayed using L-DOPA as substrate according to the method of Nirmal and Benjakul (2010).

2.5. Texture analyses

The texture parameters of hardness, gumminess, chewiness, adhesiveness, springiness, cohesiveness, and resilience of shrimp were determined using Texture Analyser (TA. XT. plusR, Stable Micro System, Godalming GU7 1YL, UK). The shrimp shell was removed and the muscle were cut between the first and second segments, then were placed on the flat plate of texturometer. Compression was applied using a cylindrical plunger of 25 mm diameter (TA3/1000). The determination was equipped with a 5 mm cylindrical probe (P/5) and the trigger force was 0.05. N. The cell loading capacity was set at 5 kg. The test was replicated five times at pre-test speed of 2.0 mm/s, test speed of