



Effect of hydrostatic pressure and holding time on physicochemical quality and microbial inactivation kinetics of black tiger shrimp (*Penaeus monodon*)

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ABSTRACT

The effects of high pressure processing (HPP), applied within 300–600 MPa for 3–15 min at ambient temperature ($27 \pm 2^\circ\text{C}$) on physicochemical (color, texture, trimethylamine nitrogen and thiobarbituric acid content) attributes and inactivation kinetics of natural microbiota (total aerobic mesophiles, psychrotrophs, *Escherichia coli*, and *Staphylococcus aureus*) in black tiger shrimp (*Penaeus monodon*) were investigated. The color parameters L^* (lightness) and b^* (yellowness) increased but a^* (redness) decreased with pressure and holding time imparting a brighter and mildly cooked appearance to shrimp muscle. Pressure-induced lipid oxidation accelerated at pressure treatments of 400–600 MPa. Hardness was found to be influenced by both pressure level and holding time. Among the group of microorganisms studied, pressure sensitivity of *E. coli* was found to be maximum whereas aerobic mesophiles were least sensitive (z_p values of 421 MPa and 714 MPa; ΔV values of -18.60×10^{-5} and $-9.13 \times 10^{-5} \text{ m}^3 \text{ mol}^{-1}$, respectively).

Industrial relevance: High pressure processing (HPP) is a promising seafood preservation technique and has been successfully applied over a range of seafood. Shrimp is a high value commodity with constant demand in the seafood market, but it is highly perishable. This study will demonstrate the changes induced by HPP in black tiger shrimp which may further be applied to develop the process technology to preserve the valuable catch.

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1. Introduction

Black tiger shrimp (*Penaeus monodon*) is commercially important shrimp species having persistent demand in global market due to its distinct flavor, texture and high nutritive value (Pushparajan & Soundarapandian, 2010). It is evident that the growth environment and feeding regime have strong influence on their quality. Most shrimps are omnivorous, besides some are also filter feeders. Their diet includes both plant and animal matter present in their natural habitats viz. phytoplankton, detritus, crustacean remains and insects (Abu Hena & Hishamuddin, 2012), thus accumulates several bacteria and viruses on their surface and gut. Usually shrimps are eaten whole (including the intestinal tract) either raw or following a mild heat treatment (Gram & Huss, 2000). The consumption of raw or lightly cooked shrimps not

only has implications for disease transmission but also limits processing methods suitable for these products (Murchie et al., 2005). Prevailing thermal preservation technique has detrimental effects on taste and appearance and is therefore unacceptable to many consumers. On the other hand, the freezing technology requires high operational costs because of the necessity of sustaining the cold chain handling of the final product at -18°C throughout the storage, distribution and marketing until consumption, involving the high rates of energy consumption (Büyükcın, Bozoğlu, & Alpas, 2009). Hence, to overcome these limitations, it has become necessary to explore and adopt alternate processing methods such as high pressure processing (HPP).

HPP involves the application of hydrostatic pressure in the range of 100–900 MPa which is instantaneously and uniformly distributed throughout the packaged or non-packaged food product with or without the assistance of thermal treatment. The vast interest in this novel technology is due to its ability to destroy or inactivate foodborne microorganisms and enzymes at ambient or low temperatures with minimal change in organoleptic properties and nutritional quality (Kaur, Kaushik, Rao, & Chauhan, 2013). This technology has been successfully applied over a range of seafood including black tiger shrimp (Kaur,

Abbreviations: ΔV , activation volume; D_p , decimal reduction time; HPP, high pressure processing; MPa, megapascal; K, microbial death rate constant; PE, pressure pulse effect; TBA, thiobarbituric acid number; TMA-N, trimethylamine nitrogen; z_p , pressure sensitivity.

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Kaushik, Rao, & Mishra, 2013), bay scallop (*Argopecten irradians*) (Yi et al., 2013), oyster (*Crassostrea gigas*) (Cruz-Romero, Kelly, & Kerry, 2007) and white prawn (*Fenneropenaeus indicus*) (Ginso et al., 2013).

Seafoods are highly perishable and post mortem changes occur rapidly compared to other muscle foods. Usually these changes result from bacterial metabolism, developing off-odors and off-flavors in the samples. Trimethylamine nitrogen (TMA-N) content is the most commonly used biochemical index to assess the keeping quality of fish and seafood. It is formed from trimethylamine oxide (TMAO), a flavorless non-protein nitrogen compound that acts as osmoprotectant in fish (Gou, Lee, & Ahn, 2010) and its content varies with the fish and seafood species, environment, season, size and age of fish. In the metabolic pathway of facultative anaerobic bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Aeromonas* spp., *Vibrio* spp., and *Enterobacteriaceae*, TMAO degrades to TMA-N in which the former acts as an electron acceptor. This degradation is catalyzed by TMAO reductase, which is inherent to these bacteria thus producing an unpleasant “fishy” odor (Nosedá, Vermeulen, Ragaert, & Devlieghere, 2014). Several studies have reported the effectiveness of HPP in controlling the production of TMA-N and correlated this to inhibition of proteolytic activity (Erkan et al., 2011; Hernández-Andrés, Gómez-Guillén, Montero, & Pérez-Mateos, 2005).

Lipid oxidation is other major problem associated with seafood. High percentage of polyunsaturated fatty acids in fish and shellfish makes it prone to lipid oxidation and develops off-flavor and off-odor compounds. The thiobarbituric acid (TBA) index is a measure of malondialdehyde content, one of the degradation products of lipid hydroperoxides, formed during the oxidation process of polyunsaturated fatty acids (Gomes, Silva, Nascimento, & Fukuma, 2003). Various studies reported that HPP accelerated lipid oxidation in fish and seafoods. It has been suggested that water-soluble components such as low molecular weight iron compounds and myoglobin, hemoglobin and ferritin present in meat and fish play a key role in lipid oxidation following high pressure treatment (Ohshima, Nakagawa, & Koizumi, 1992). Pressure treatment may either induce the exposure of catalytic heme groups to unsaturated fatty acids, or potentiate the release of iron (Fe^{2+}) from heme groups to oxidize unsaturated fatty acids present in fish. It was also reported that denaturation of proteins plays an important role in catalyzing lipid oxidation in pressure-treated meat (Sequeira-Munoz, Chevalier, LeBail, Ramaswamy, & Simpson, 2006). The denaturation of heme proteins by high pressure treatment results in release of metal ions, which facilitates lipid oxidation. However, information related to effects of HPP on lipid oxidation in seafoods is still limited.

HPP has also been investigated for destructive effects on microbes and pressure inactivation kinetics of several strains of microorganisms, both spoilage and pathogenic species, have been studied extensively (Campus et al., 2010). Many studies have reported that first-order kinetics fit well to the high pressure inactivation of microorganisms in food (Houjaj, Ramaswamy, & Idziak, 2004; Ramaswamy, Zaman, & Smith, 2008). These authors have estimated the kinetic parameters such as decimal reduction time (D_p), pressure sensitivity (z_p) and activation volume (ΔV). Both D_p and z_p are analogous to thermobacteriology and ensure a more practical approach to design industrial high pressure processes for food safety and preservation (Kaushik, Kaur, Rao, & Mishra, 2014). Houjaj et al. (2004) studied high pressure destruction kinetics of indigenous microorganisms in shrimp (*Penaeus* sp.) and trout (*Oncorhynchus* sp.), and evaluated their quality changes during storage. Ramaswamy et al. (2008) reported destruction kinetics of pathogens namely *Escherichia coli* (O157:H7) and *Listeria monocytogenes* (Scott A) in fish slurry. The inactivation kinetics of *Listeria innocua* in minced trout (*Oncorhynchus mykiss*) (Basaran-Akgul et al., 2010), *Listeria monocytogenes* CTC1034 in RTE meat products (Hereu, Dalggaard, Garriga, Aymerich, & Bover-Cid, 2012) and *Staphylococcus aureus* in sliced ham (Tassou, Panagou, Samaras, Galiatsatou, & Mallidis, 2008) have also been studied.

However, it was noted that most of the kinetic studies conducted focused on specific microbial species or strains and much data is not available for destruction kinetics of natural microbiota of aquatic foods.

The cost associated with HPP is directly related to the processing conditions necessary to achieve the desired effect (Nienaber & Shellhammer, 2001). Besides pressure, working temperature and holding time also influence the product quality significantly (Amanatidou et al., 2000). Improvements in HPP efficacy will result in reduction of processing cost through optimized operating pressure and holding time. Hence, the present study was undertaken to evaluate the combined effect of pressure and holding time at room temperature on physical (color and texture), biochemical (TMA-N content and thiobarbituric acid) and microbiological counts (total aerobic mesophiles, psychrotrophs, *E. coli*, and *S. aureus*), quality of black tiger shrimp (*Penaeus monodon*) and model pressure-induced microbial destruction kinetics.

2. Materials and methods

2.1. Sample preparation

Freshly harvested black tiger shrimp (*Penaeus monodon*) of size in the range 25–35 g were procured from Shankarpur coast, West Bengal, India and transported to laboratory within 4 h under chilled conditions (sample/ice ratio of 1:1). The samples were washed with chilled water, heads removed, shelled and vacuum packed in LDPE film (thickness: $80 \pm 0.50 \mu\text{m}$; B.B. Chatterjee Company Private Limited, West Bengal, India) with 3 shrimps per packet. Samples were double packed to ensure no direct contact with pressure transmitting fluid during processing.

2.2. High pressure processing

Packed samples were treated in a batch-type high pressure food processor (model: S-IL-100-250-09-W; make: Stansted Fluid Power Ltd., UK) having a 2 L capacity cylindrical vessel with a 100-mm inner diameter and a 250-mm depth which can be operated to a maximum working pressure of 900 MPa. The samples were treated at 300, 400, 500 and 600 MPa pressures for holding times of 3, 6, 9, 12 and 15 min at room temperature ($27 \pm 2^\circ\text{C}$) at a rate of 300 MPa/min. An aqueous solution of 30% monopropylene glycol was used as a pressure transmitting medium (PTM). An average temperature rise of $2 \pm 0.5^\circ\text{C}$ per 100 MPa increase in pressure was recorded for pressurizing fluid due to adiabatic heating of compression. Time to reach the desired pressure was 1.5 ± 0.5 min and depressurization time was less than 10 s. After processing, the samples were cooled in ice and stored at $2 \pm 0.5^\circ\text{C}$ for 2 h until further analysis. Untreated samples were used as controls.

2.3. Trimethylamine nitrogen (TMA-N) analysis

TMA-N was determined according to Conway microdiffusion method as proposed by Beatty and Gibbons (1937) and the results were expressed in mg N/100 g of sample. Briefly, the sample extract was prepared by homogenizing 10 g of shrimp sample with 10 mL of 20% trichloroacetic acid and the homogenate was filtered using Whatman filter paper No. 41. 1 mL of prepared sample extract and 1 mL of formalin followed by 1 mL of saturated potassium carbonate were pipetted into the outer ring of microdiffusion unit, the inner ring of which contained 2 mL of boric acid solution (boric acid, methyl red and bromocresol green indicator). The unit was immediately covered and sealed using silica grease and rotated gently to mix the solutions of outer ring. The inner ring solution (green color) after incubation for 90 min at 37°C was titrated using 0.02 N sulphuric acid until it turned pink. A blank test was carried out using 1 mL of 2% trichloroacetic acid, instead of a sample extract.

2.4. Lipid oxidation measurement

Lipid oxidation was measured as a change in TBA value as per the method proposed by Nielsen, Sørensen, Skibsted, and Bertelsen (1997). Two gram shrimp muscle was blended with 10-mL trichloroacetic acid solution (75 g TCA/L, 1 g propyl gallate/L, 1 g EDTA/L) using a mortar and a pestle and the suspension was filtered through Whatman filter paper No. 1. The filtrate (5 mL) was mixed with 0.5 mL of thiobarbituric acid solution (2.88 g/L) in a screw cap tube and kept in boiling water for 40 min. The tubes were then cooled using ice and the absorbance was measured at 532 nm. TBA values were calculated from the standard curve plotted using malondialdehyde-bis-diethyl acetate as standard. Triplicate analyses were performed for all the samples and the results were expressed as milligrams of malondialdehyde per kilogram (mg MDA/kg) of sample.

2.5. Color measurement

Surface color of black tiger shrimp was measured using a Portable Colorimeter, (model: Spectro-guide 45/0 gloss, make: BYK Gardner, Germany) with a D65 illuminant and a 10° standard observer angle. The color was expressed in L^* (lightness); a^* (+a, red; −a, green) and b^* (+b, yellow; −b, blue) coordinates. The measurements were repeated at three different locations on each shrimp sample and mean values were reported. To illustrate the degree of color change, the total color difference was computed, $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$ where ΔL^* , Δa^* , and Δb^* are the differences in the L^* , a^* , and b^* between treated samples and control.

2.6. Texture profile analysis (TPA)

The texture profile of the samples was evaluated using a texture analyzer (model: TA.XT-2, make: Stable Micro Systems, UK) equipped with a 25 kg load cell capacity. Whole shrimp was compressed by 30% of its original height using a cylindrical probe of 6 mm diameter (SMSP/6) with a test speed of 2 mm/s (Jantakoson, Kijroongrojana, & Benjakul, 2012). Three readings were taken on each shrimp; one at the lateral centre and other two at 1 cm distance on either side of the centre point. From the TPA curves so obtained, the following textural parameters were calculated: hardness (g), cohesiveness, gumminess (g): hardness × cohesiveness, springiness and chewiness (g): gumminess × springiness.

2.7. Microbiological analysis

2.7.1. Sample preparation

A sample of 10 g was macerated with 90 mL of sterilized normal saline (0.85 g NaCl/100 g distilled water) using a mortar and a pestle and appropriate serial dilutions were spread plated on sterile agar plates.

2.7.2. Microbiological media and enumeration

Both treated and untreated samples were analyzed for aerobic mesophiles, psychrotrophs, *E. coli*, and *S. aureus* count as per APHA (2001). For aerobic mesophiles and psychrotrophs, 1 mL of suitable dilution was spread plated on tryptone glucose agar (TGA). Thereafter, the plates were incubated at 37 °C for 36–48 h and 7 °C for 10 days for mesophilic and psychrotrophic counts, respectively. To examine the presence of *E. coli* and *S. aureus*, 1 mL of diluted sample was inoculated on sterile plates of Tergitol 7 and Baird Parker agar and incubated at 37 °C for a period of 18–24 h and 36–48 h respectively. The isolated colonies from Tergitol 7 agar were presumptively identified as *E. coli* on the basis of the Gram stain and IMViC testing and in the case of Baird Parker medium, only black colonies with a clear zone were counted and presumed to be *S. aureus*.

2.8. Data analysis and microbial kinetic estimation

Microbiological analyses were carried out in duplicates and all other analyses were replicated thrice. Results were reported as their mean values ± standard deviation. Two-way analysis of variance (ANOVA) of the data was carried out using the SPSS 17 for Windows (SPSS Statistical Software, Inc., Chicago, IL, USA) software package. The difference between the pairs of means was evaluated by the Tukey's test at a confidence level of 95%.

The pressure destruction of microorganisms was analyzed based on the combination of pressure inactivation due to pressure pulse effect (PE) and pressure–hold effect (Hiremath & Ramaswamy, 2011). The pulse effect represents the destruction achieved during a pressure pulse, which consists of pressurization to the desired level followed by an immediate decompression (zero holding time). As a part of the experimental set-up, a zero holding time was included for estimating the pulsed effect. The PE values were determined by calculating the logarithmic difference between the initial microbial counts in the control samples (N_0) and number of survival cells after single pulse pressurization (N_{PE}) as

$$PE = \log_{10} (N_0) - \log_{10} (N_{PE}). \quad (1)$$

The pressure destruction of microorganisms during the pressure–hold time was analyzed based on first-order rate kinetics and expressed as.

$$\ln (N_t/N_{PE}) = -kt \quad (2)$$

where, N_t is the number of surviving microorganisms after pressure treatment for time t (min) and k is the microbial death rate constant (min^{-1}).

Microbial death rate constant obtained from Eq. (2) was further used to calculate decimal reduction time (D_p) using Eq. (3). D_p is defined as the time required in minutes to reduce the microbial population by 90% at a constant pressure.

$$D_p = 2.303/k. \quad (3)$$

Another term 'z_p value' which indicates the pressure difference required to change D value by ten-fold was determined as the negative reciprocal of the slope of $\log_{10} D_p$ versus pressure plot or using Eq. (4).

$$z_p = (P_2 - P_1) / (\log_{10} (D_1/D_2)). \quad (4)$$

The pressure dependence of k values is computed as the change in activation volume (ΔV) from the regression slope of $\ln k$ versus pressure as suggested by Eyring and Magee (1942).

$$\Delta V = -RT (\text{slope}) \text{ or } -RT (\Delta (\ln k) / \Delta P) \quad (5)$$

where, ΔV is change in activation volume ($\text{m}^3 \text{mol}^{-1}$).

R is gas constant ($8.314 \times 10^{-6} \text{ m}^3 \text{mol}^{-1} \text{MPa}^{-1} \text{K}^{-1}$).

T is absolute temperature (K); and

P is pressure (MPa).

3. Results and discussion

3.1. Trimethylamine nitrogen (TMA-N)

The mean TMA-N content in control was $3.92 \pm 0.03 \text{ mg N/100 g}$. All the treated samples showed significantly ($p < 0.05$) lower TMA-N values compared to control (Fig. 1). With increase in both pressure and holding time, production of TMA-N content was delayed in shrimp. The lowest reduction obtained was 23.73% for samples pressurized at 300 MPa for 3 min and the highest was 69.13% for treatment of 600 MPa for 15 min. This might be due to substantial reduction of TMA-N producers

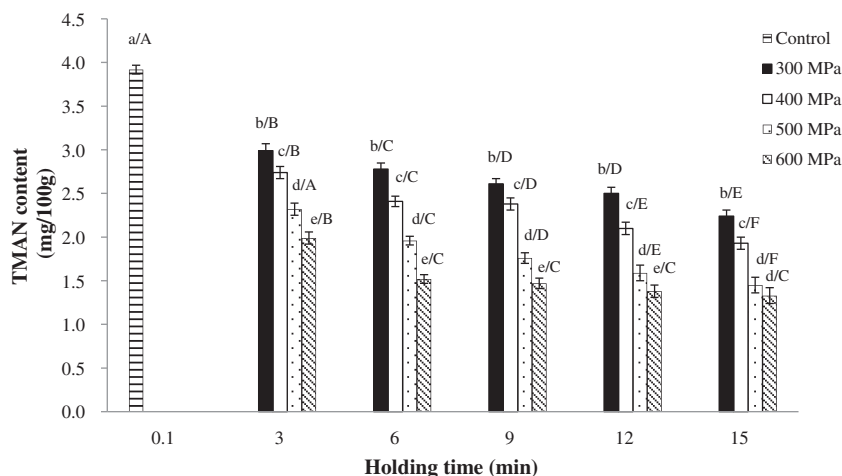


Fig. 1. Changes in TMA-N content (mg N/100 g muscle) of high pressure-treated shrimp. Error bars represent standard deviations of analysis of three replicates. Different letters (a, b, c, d) indicate significant differences in effect of pressure alone on TMA-N content. Different letters (A, B, C, D, E, F) indicate significant differences in effect of holding times at constant pressure.

by high pressure treatments. Gou et al. (2010) found that squid (*Todarodes pacificus*) treated at 300 MPa at 20 °C for various holding times (0, 5, 10 and 20 min) effectively lowered the amount of TMA-N content, although holding time had no significant effect on TMA-N concentrations. Another study conducted on horse mackerel (*Trachurus trachurus*) by Erkan et al. (2011) also showed significant reduction in TMA-N content by high pressure treatments of 220, 250 and 330 MPa for 10 min at 25 °C and 330 MPa for 5 min at 7 °C. However, Erkan and Üretener (2010) reported unchanged TMA-N values in gilthead bream (*Sparus aurata*) for pressure treatments up to 250 MPa for 10 min at 7 °C while a treatment of 330 MPa resulted in significantly lower values compared to control.

3.2. Lipid oxidation

Changes in lipid oxidation of raw and treated shrimp samples are presented in Fig. 2. The mean TBA value in control sample was 0.54 ± 0.02 mg N/100 g and found to be influenced both by pressure level and holding time. The higher the pressure level and holding time

used, the higher was the TBA value obtained; however it remained below the critical limit of 2.0 mg MDA/kg (Goulas & Kontominas, 2007). A slight increase in TBA values was recorded in samples pressurized at 300 MPa for 3, 6 and 9 min compared to control. However, for all other treatments a significant ($p < 0.05$) increase was observed. Moreover, no significant change in TBA values was observed with increased pressure from 400 to 600 MPa for holding times of 12 and 15 min. The results obtained in the present study are in agreement with research by Yağiz, Kristinsson, Balaban, and Marshall (2007) who reported increased lipid oxidation at treatments >300 MPa in rainbow trout (*O. mykiss*) pressurized at 150–600 MPa for 15 min. In the study conducted on vacuum-packed slices of dry-cured Iberian ham and loin (Cava, Ladero, González, Carrasco, & Ramírez, 2009) and chilled jack mackerel increased lipid oxidation both by pressure level and holding time was reported by the authors (Maluenda, Roco, Tabilo-Munizaga, Pérez-Won, & Aubourg, 2013). In contrast, in other study on horse mackerel by Torres, Saraiva, Guerra-Rodríguez, Aubourg, and Vázquez (2014) showed an inhibitory effect of high hydrostatic pressurization on lipid oxidation.

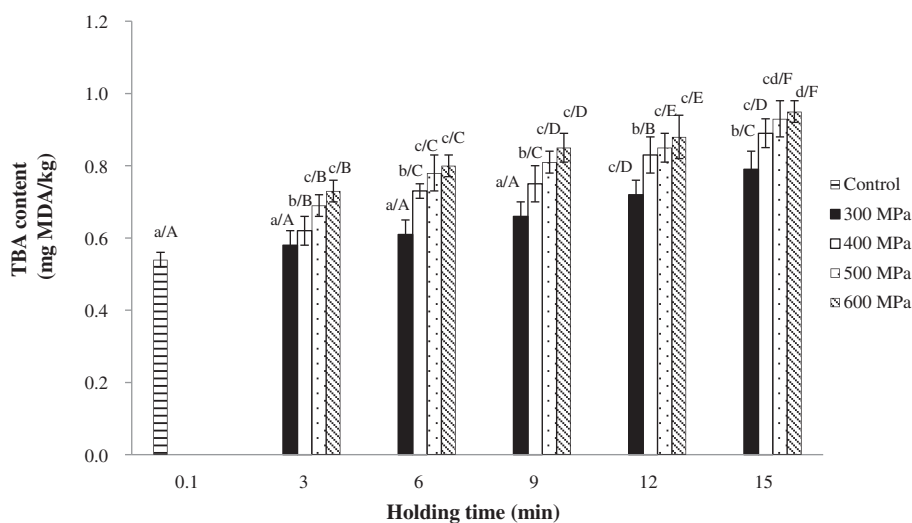


Fig. 2. Changes in TBA content (mg MDA/kg) of high pressure-treated shrimp. Error bars represent standard deviations of analysis of three replicates. Different letters (a, b, c, d) indicate significant differences in effect of pressure alone on TBA content. Different letters (A, B, C, D, E, F) indicate significant differences in effect of holding times at constant pressure.

Table 1
Changes in color of treated and untreated black tiger shrimp.

Holding time (min)	Pressure treatment (MPa)			
	300	400	500	600
<i>L*</i>				
Untreated	53.08 ± 2.74 ^{aA}	53.08 ± 2.74 ^{aA}	53.08 ± 2.74 ^{aA}	53.08 ± 2.74 ^{aA}
3	64.64 ± 3.78 ^{bb}	69.25 ± 5.28 ^{cb}	72.52 ± 6.64 ^{cb}	75.13 ± 5.93 ^{db}
6	70.15 ± 4.94 ^{bc}	72.54 ± 4.94 ^{bb}	75.76 ± 4.22 ^{cc}	80.74 ± 4.11 ^{dbc}
9	72.06 ± 3.32 ^{bc}	75.49 ± 3.68 ^{cc}	79.52 ± 3.97 ^{dd}	82.84 ± 2.38 ^{ec}
12	74.74 ± 5.08 ^{bd}	77.08 ± 1.76 ^{cde}	81.74 ± 4.99 ^{dd}	84.98 ± 2.76 ^{ed}
15	77.31 ± 1.93 ^{bd}	79.93 ± 1.56 ^{ce}	83.16 ± 4.30 ^{dd}	86.43 ± 1.11 ^{ed}
<i>a*</i>				
Untreated	6.83 ± 0.48 ^{aA}	6.83 ± 0.48 ^{aA}	6.83 ± 0.48 ^{aA}	6.83 ± 0.48 ^{aA}
3	6.12 ± 0.30 ^{bb}	4.52 ± 0.31 ^{cb}	3.45 ± 0.28 ^{db}	2.50 ± 0.17 ^{eb}
6	5.90 ± 0.58 ^{bb}	3.98 ± 0.23 ^{cb}	3.05 ± 0.62 ^{db}	2.15 ± 0.10 ^{eb}
9	5.81 ± 0.37 ^{bb}	3.87 ± 0.24 ^{cb}	2.87 ± 0.38 ^{db}	2.09 ± 0.13 ^{ebc}
12	5.72 ± 0.58 ^{bb}	3.83 ± 0.14 ^{cb}	2.60 ± 0.46 ^{db}	2.01 ± 0.31 ^{dc}
15	5.38 ± 0.86 ^{bb}	3.70 ± 0.20 ^{cb}	2.39 ± 0.60 ^{db}	1.72 ± 0.18 ^{dc}
<i>b*</i>				
Untreated	5.14 ± 0.10 ^{aA}	5.14 ± 0.10 ^{aA}	5.14 ± 0.10 ^{aA}	5.14 ± 0.10 ^{aA}
3	6.39 ± 0.65 ^{bb}	8.47 ± 0.87 ^{cb}	9.59 ± 0.68 ^{db}	11.12 ± 0.67 ^{eb}
6	7.07 ± 0.07 ^{bb}	9.17 ± 0.58 ^{cb}	10.40 ± 0.49 ^{db}	11.58 ± 0.26 ^{eb}
9	7.33 ± 0.22 ^{bb}	9.59 ± 0.58 ^{cb}	10.63 ± 0.39 ^{db}	11.81 ± 0.67 ^{eb}
12	7.79 ± 0.73 ^{bb}	9.67 ± 0.38 ^{cb}	10.78 ± 0.64 ^{db}	11.91 ± 0.59 ^{eb}
15	9.14 ± 0.95 ^{bc}	10.06 ± 0.52 ^{cb}	10.97 ± 0.35 ^{cb}	12.54 ± 0.77 ^{dc}
ΔE^*				
Untreated				
3	11.68 ± 3.24 ^{aA}	16.71 ± 2.06 ^{bA}	20.33 ± 6.31 ^{cA}	23.32 ± 5.64 ^{dA}
6	17.21 ± 2.38 ^{aB}	20.09 ± 2.49 ^{bb}	23.63 ± 4.01 ^{cb}	28.81 ± 3.92 ^{db}
9	19.14 ± 2.89 ^{aB}	23.04 ± 1.17 ^{bc}	27.63 ± 3.88 ^{cb}	30.88 ± 2.20 ^{dc}
12	21.87 ± 2.90 ^{aC}	27.61 ± 1.72 ^{bcd}	29.20 ± 4.95 ^{bc}	32.97 ± 2.75 ^{cC}
15	24.62 ± 2.00 ^{aD}	27.48 ± 1.56 ^{abd}	30.98 ± 4.14 ^{bcc}	34.55 ± 1.19 ^{cC}

All values are means ± standard deviations of data from three independent experiments. Different superscripts (a, b, c, d, e) in the same row indicate significant difference ($p < 0.05$).

Different superscripts (A, B, C, D) in the same column indicate significant difference ($p < 0.05$).

3.3. Color

Significant changes were observed in the surface color of shrimp muscle following HPP (Table 1). The shrimp samples lost their transparency with increased pressure intensity and holding time as clearly indicated by increased L^* values. The muscle appeared cooked and this effect accentuated with increase in pressure. The increase in lightness in shrimp samples by HPP was might be due to coagulation of proteins and loss of active pigments. Astaxanthin (64–98% of the total carotenoids) is one of the major pigments in shrimp and is primarily located on the shrimp surface (Latscha, 1989). The protein coagulation changes sample surface properties; increases light reflection and results in white color (Kruk et al., 2011). Redness (a^* value) decreased and yellowness (b^* value) increased as pressure level was incremented; however holding time did not significantly affected these parameters. These changes might be due to globin denaturation and/or heme displacement or release as stated by Cheftel and Culioli (1997). Lipid oxidation is another possible reason suggested for color changes in fish products, due to degradation of highly unsaturated carotenoids such as astaxanthin (Cruz-Romero, Kerry, & Kelly, 2008). Similar results for changes in L^* and chromatic parameters (decreased a^* and increased b^*) have been reported for different seafood species such as red mullet (Erkan, Üretener, & Alpas, 2010) and hilsa filets (Chouhan, Kaur, & Rao, 2015).

Further statistical analysis of ΔE^* values, also reflected the progressive color changes in pressure-treated samples. Cruz-Romero et al. (2007) reported that ΔE^* values of 3.0–6.0, 6.0–12.0 and >12 correspond to very distinctive, strong, and very strong differences

and according to this scale high pressure-treated shrimps exhibit very strong color differences.

3.4. Texture

The textural properties of high pressure-treated shrimp were evaluated based on the textural attributes: hardness, cohesiveness, gumminess, springiness, and chewiness (Table 2). All the high pressure processed samples showed significantly ($p < 0.05$) higher hardness values compared to untreated samples. The effect of pressure and holding time on samples followed a direct relationship, i.e., the higher the pressure and holding time used, the higher the gel hardness obtained. The improvement in gel texture was observed immediately after HPP, with the lowest being 2.38 times (300 MPa × 3 min) and the highest 7.26 times (600 MPa × 15 min) harder compared to control. In the present work, a significant ($p < 0.05$) increase was observed in springiness and cohesiveness with pressure increment; however holding time had no significant effect on these parameters. Similar findings have been reported by Chen et al. (2010) for cohesiveness and springiness of duck muscle gel pressurized at 100–500 MPa for 10–40 min. Truong, Buckow, Minh, and Costas (2015) also obtained increased springiness in barramundi (*Lates calcarifer*) muscle with pressurization at 150–300 MPa for 15 min and reported that application of HPP results in compaction of muscle fibers which enhance protein and protein interactions leading to formation of disulphide bonds (formed under pressure) and hydrogen bonds (formed upon release of pressure). These chemical bonds result in improved tissue hardness, cohesiveness and springiness.

Chewiness and gumminess of shrimp samples also showed increasing trend with increased pressure level and holding time. However, these textural properties showed slight change with holding time at pressure treatment of 300 MPa. This increased chewiness in treated shrimp may be correlated to improved cohesion between the meat particles following HPP (Kaur, Kaushik, Rao et al., 2013b). Yağiz et al. (2007) studied the effect of high pressures on rainbow trout (*O. mykiss*) and also reported higher cohesiveness values for samples subjected to pressures between 300 and 600 MPa compared to control. The results of the current study indicate that high pressure treatments lead to noticeable changes in textural parameters of black tiger shrimp.

3.5. Microbiological quality

The initial aerobic mesophilic and psychrotrophic counts in the control sample were 5.9 log cfu/g and 5.5 log cfu/g, respectively. *E. coli* and *S. aureus* population was 4.3 log cfu/g and 4.7 log cfu/g respectively, which were higher than the acceptable limits (3 and 4 log cfu/g respectively; Yousef et al., 2008). In the present study the inactivation kinetics of natural microbiota in high pressure-treated shrimp samples was modeled assuming a dual mechanism of pressure inactivation (as discussed in Section 2.8). This type of biphasic behavior was observed earlier for high pressure destruction of *E. coli* O157:H7 and *L. monocytogenes* Scott A in fish slurry (Ramaswamy et al., 2008) as well as different species of spoilage microorganisms in mango pulp (Kaushik et al., 2014).

3.5.1. Pressure pulse effect (PE)

The pressure pulse effect for the group of microbiota studied is presented in Table 3. PE can be observed from the survivor curves in which the linear curves do not originate from the initial population of microorganisms but at lower values (Fig. 3). A significant ($p < 0.05$) effect of single pressure pulse was observed (ranging from 0.38 to 1.72 log₁₀ cycles) as the operating pressure changed from 300 to 600 MPa, depicting the efficacy of pulse pressurization in lowering microbial counts. Both aerobic mesophiles and psychrotrophs showed similar sensitivity to high pressure treatment of 300 MPa. However, at pressures above 300 MPa psychrotrophs showed greater sensitivity to pulse pressurization than mesophiles. Moreover, *E. coli* which has considerable baroresistance

Table 2
Texture profile analysis (TPA) parameters of high pressure processed black tiger shrimp.

		Holding time (min)				
		3	6	9	12	15
Hardness (g)	Untreated	171.70 ± 19.21 ^{aA}	171.70 ± 19.21 ^{aA}	171.70 ± 19.21 ^{aA}	171.70 ± 19.21 ^{aA}	171.70 ± 19.21 ^{aA}
	300	408.98 ± 25.03 ^{bB}	417.30 ± 16.32 ^{bBC}	429.90 ± 23.90 ^{bC}	434.36 ± 25.01 ^{bC}	519.08 ± 14.56 ^{bD}
	400	505.22 ± 21.33 ^{cB}	543.09 ± 28.19 ^{cC}	628.69 ± 34.31 ^{cD}	685.49 ± 34.06 ^{cE}	763.94 ± 42.44 ^{cF}
	500	667.88 ± 14.51 ^{dB}	767.88 ± 42.33 ^{dC}	954.40 ± 76.84 ^{dD}	985.17 ± 80.04 ^{dE}	1158.29 ± 96.83 ^{dF}
	600	799.43 ± 23.66 ^{eB}	919.47 ± 58.47 ^{eC}	1153.33 ± 90.60 ^{eD}	1210.28 ± 72.32 ^{eE}	1245.70 ± 93.25 ^{eF}
Cohesiveness	Untreated	0.418 ± 0.01 ^{aA}	0.418 ± 0.01 ^{aA}	0.418 ± 0.01 ^{aA}	0.418 ± 0.01 ^{aA}	0.418 ± 0.01 ^{aA}
	300	0.461 ± 0.03 ^{bB}	0.463 ± 0.06 ^{bB}	0.469 ± 0.09 ^{bB}	0.474 ± 0.01 ^{bB}	0.490 ± 0.04 ^{bB}
	400	0.474 ± 0.01 ^{bB}	0.486 ± 0.01 ^{bBC}	0.494 ± 0.04 ^{bBC}	0.514 ± 0.06 ^{cC}	0.526 ± 0.03 ^{bCC}
	500	0.489 ± 0.01 ^{bB}	0.508 ± 0.04 ^{bBC}	0.523 ± 0.01 ^{cC}	0.540 ± 0.04 ^{cC}	0.543 ± 0.04 ^{cC}
	600	0.494 ± 0.01 ^{bB}	0.525 ± 0.01 ^{cBC}	0.537 ± 0.06 ^{cC}	0.542 ± 0.02 ^{cC}	0.552 ± 0.03 ^{cC}
Springiness	Untreated	0.456 ± 0.01 ^{aA}	0.456 ± 0.01 ^{aA}	0.456 ± 0.01 ^{aA}	0.456 ± 0.01 ^{aA}	0.456 ± 0.01 ^{aA}
	300	0.521 ± 0.03 ^{bB}	0.547 ± 0.06 ^{bB}	0.550 ± 0.09 ^{bB}	0.558 ± 0.01 ^{bB}	0.563 ± 0.04 ^{bB}
	400	0.590 ± 0.01 ^{cB}	0.598 ± 0.01 ^{cB}	0.611 ± 0.04 ^{cB}	0.614 ± 0.06 ^{cB}	0.624 ± 0.03 ^{cB}
	500	0.629 ± 0.01 ^{cDB}	0.639 ± 0.04 ^{dB}	0.631 ± 0.01 ^{cB}	0.647 ± 0.04 ^{dB}	0.652 ± 0.04 ^{dB}
	600	0.674 ± 0.01 ^{dB}	0.714 ± 0.01 ^{EC}	0.752 ± 0.06 ^{dC}	0.762 ± 0.02 ^{ED}	0.784 ± 0.03 ^{ED}
Gumminess (g)	Untreated	71.75 ± 6.11 ^{aA}	71.75 ± 6.11 ^{aA}	71.75 ± 6.11 ^{aA}	71.75 ± 6.11 ^{aA}	71.75 ± 6.11 ^{aA}
	300	188.57 ± 15.13 ^{bB}	193.52 ± 19.23 ^{bB}	201.66 ± 13.67 ^{bB}	205.78 ± 11.35 ^{bB}	254.68 ± 14.23 ^{bC}
	400	239.35 ± 11.21 ^{cB}	263.46 ± 19.34 ^{cC}	310.62 ± 24.98 ^{cD}	352.36 ± 26.53 ^{cE}	402.40 ± 23.33 ^{cF}
	500	326.88 ± 15.01 ^{dB}	390.52 ± 20.21 ^{dC}	498.78 ± 27.01 ^{dD}	531.71 ± 34.56 ^{dE}	629.20 ± 41.45 ^{dF}
	600	394.90 ± 20.21 ^{eB}	482.37 ± 19.43 ^{eC}	619.36 ± 46.34 ^{eD}	656.44 ± 32.45 ^{eE}	687.69 ± 30.13 ^{eF}
Chewiness (g)	Untreated	32.79 ± 2.21 ^{aA}	32.79 ± 2.21 ^{aA}	32.79 ± 2.21 ^{aA}	32.79 ± 2.21 ^{aA}	32.79 ± 2.21 ^{aA}
	300	98.26 ± 7.32 ^{bB}	105.93 ± 10.15 ^{bBC}	110.89 ± 9.55 ^{bC}	114.83 ± 9.41 ^{bC}	143.00 ± 8.35 ^{bD}
	400	141.35 ± 20.01 ^{cB}	157.53 ± 11.04 ^{cB}	189.86 ± 12.84 ^{cC}	216.36 ± 16.36 ^{cD}	250.95 ± 14.37 ^{cE}
	500	205.70 ± 17.65 ^{dB}	249.50 ± 15.40 ^{dC}	314.75 ± 21.35 ^{dD}	344.06 ± 24.32 ^{dE}	410.11 ± 20.64 ^{dF}
	600	265.10 ± 22.16 ^{eB}	344.43 ± 16.66 ^{eC}	465.78 ± 25.76 ^{eD}	500.23 ± 22.23 ^{eE}	539.92 ± 20.23 ^{eF}

All values are means ± standard deviations of data from three independent experiments.

Different superscripts (A, B, C, D, E, F) in the same row indicate significant difference ($p < 0.05$).

Different superscripts (a, b, c, d, e) in the same column indicate significant difference ($p < 0.05$).

among Gram-negative bacteria was found to be sensitive to pulse pressurization compared to *S. aureus*, a Gram-positive facultative anaerobe, one of the most baroresistant bacteria (Alpas, Kalchayanand, Bozoglu, & Ray, 2000). In the present study, 600 MPa pulse was most effective in reducing the microbial load within the pressure range studied. The reduction in microbial load by pulse pressurization was due to pressurization (adiabatic compression) and depressurization (rapid expansion) that leads to rupturing of cells and microbial death (Hiremath & Ramaswamy, 2011). The cell rupture was more in *E. coli* (Gram-negative bacteria) than in *S. aureus* (Gram-positive bacteria) by high pressure treatment due to the presence of thinner cell wall in *E. coli* which resulted in higher inactivation (Wang, Pan, Xie, Yang, & Lin, 2010). In the

present study, *E. coli* was found to be most sensitive to pulse pressure and least affected were aerobic mesophiles with PE values of 1.3 and 1.7 log₁₀ cycles at 600 MPa, respectively. Since, inactivation of microorganisms obtained was not too high as indicated by PE values; therefore, the application of pulse pressure alone is not sufficient to obtain significant reduction in microbial loads.

3.5.2. Pressure hold time kinetics

The survival curves of *E. coli* indicated that destruction was influenced by both pressure level and holding time (Fig. 3). They are steeper at higher pressures indicating that the destruction rate is higher at higher pressures. The linearity of the survival curves and the associated high R^2 values (0.89 to 0.95) support the appropriateness of the first-order model for describing the pressure hold destruction of microorganisms. An increase in pressure level resulted in lowering of D_p value indicating that the cells were destroyed more rapidly with increased pressure intensity (Table 3). Total aerobic mesophiles had higher D_p values (5.5 to 1.7 min) for all the pressure treatments used in this study than psychrotrophs (3.3 to 1.6 min) demonstrating the former

Table 3
Estimated kinetic parameters for pressure inactivation of natural microbiota in black tiger shrimp.

Group of microorganisms	Pressure (MPa)	PE (Log cfu/g)	D value (min)	k value (min ⁻¹)	R ²
Aerobic mesophiles	300	0.43 ± 0.04 ^a	5.45 ± 0.13 ^a	0.20 ± 0.03 ^a	0.90
	400	0.68 ± 0.04 ^b	3.37 ± 0.21 ^b	0.31 ± 0.03 ^b	0.91
	500	1.25 ± 0.07 ^c	2.37 ± 0.11 ^c	0.42 ± 0.05 ^c	0.89
	600	1.32 ± 0.05 ^d	1.67 ± 0.12 ^d	0.60 ± 0.04 ^d	0.92
Psychrotrophs	300	0.38 ± 0.03 ^e	3.29 ± 0.16 ^e	0.30 ± 0.04 ^b	0.93
	400	0.73 ± 0.03 ^f	2.52 ± 0.12 ^f	0.40 ± 0.03 ^e	0.91
	500	1.07 ± 0.02 ^g	2.09 ± 0.12 ^g	0.48 ± 0.03 ^f	0.89
	600	1.37 ± 0.02 ^h	1.58 ± 0.08 ^h	0.69 ± 0.03 ^g	0.93
<i>E. coli</i>	300	0.53 ± 0.02 ⁱ	2.89 ± 0.11 ⁱ	0.37 ± 0.02 ^h	0.95
	400	0.91 ± 0.03 ^j	1.82 ± 0.09 ^j	0.55 ± 0.02 ⁱ	0.90
	500	1.43 ± 0.06 ^k	1.41 ± 0.08 ^k	0.71 ± 0.02 ^j	0.91
	600	1.72 ± 0.06 ^l	1.11 ± 0.08 ^l	0.87 ± 0.04 ^k	0.94
<i>S. aureus</i>	300	0.48 ± 0.03 ^m	3.26 ± 0.13 ^m	0.32 ± 0.02 ⁱ	0.89
	400	0.75 ± 0.02 ^f	2.37 ± 0.06 ⁿ	0.45 ± 0.02 ^m	0.94
	500	1.12 ± 0.06 ⁿ	1.86 ± 0.04 ^o	0.54 ± 0.02 ⁱ	0.93
	600	1.40 ± 0.06 ^o	1.49 ± 0.03 ^p	0.68 ± 0.03 ⁿ	0.94

All values are means ± standard deviations of data from two independent experiments (N = 4).

Different superscripts (a, b, c, ..., p) in the same column indicate significant difference ($p < 0.05$).

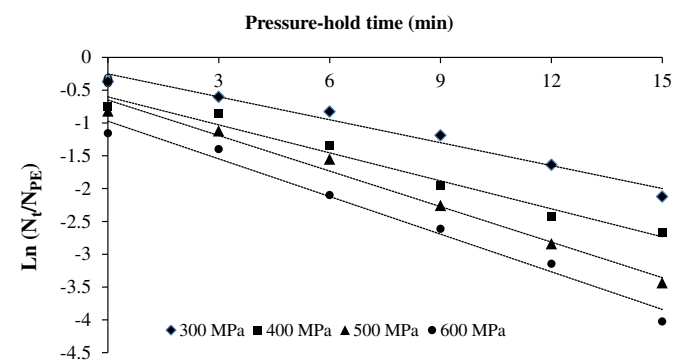


Fig. 3. Effect of high pressure processing on the survivor curve of *E. coli* in black tiger shrimp.

to be more resistant to static pressurization. The marked pressure sensitivity of psychrotrophs compared to aerobic mesophiles has also been reported by other researchers (Garriga, Gre'bol, Aymerich, Monfort, & Hugas, 2004; Suárez-Jacobo, Gervilla, Guamis, Roig-Sagués, & Saldo, 2010). As indicated by D_p values, for the similar treatment the reduction obtained was higher for *E. coli* count (2.9 to 1.1 min) compared to *S. aureus* (3.3 to 1.5 min).

Dogan and Erkmen (2004) studied destruction kinetics of indigenous microbiota present in milk, peach and orange juices at 300–600 MPa and Kaushik et al. (2014) in mango pulp at 100–600 MPa. They reported significantly ($p < 0.05$) higher D_p values than those obtained in the present study. Pressure sensitivity of microorganisms not only depends on the type/strain of microorganism but also on the composition of the dispersion medium as well as rate of pressurization & depressurization, cell growth stage and handling procedures (Basak, Ramaswamy, & Piette, 2002). Since, the working conditions reported in literature were different from the one used in the present study, hence the results obtained are not identical. Moreover, the survivor curves showed the possibility of achieving a sizeable reduction in the number of survivors even at relatively low pressure by prolonging the holding time.

3.5.3. Pressure sensitivity of microorganisms

To analyze the sensitivity of microorganisms towards pressurization, pressure sensitivity (z_p values, MPa) and activation volume (ΔV value, $\text{m}^3 \text{mol}^{-1}$) were calculated from slope of $\log_{10} D_p$ versus pressure (Fig. 4a) and $\ln k$ versus pressure (Fig. 4b). The higher magnitude of ΔV (positive or negative) and lower z_p indicate greater pressure sensitivity of microorganisms. The high R^2 values (0.95 to 0.97) for all the

Table 4

Estimated pressure sensitivity (z_p) and change in activation volume (ΔV) of natural microbiota in high pressure processed black tiger shrimp.

Group of microorganisms	z_p value (MPa)	$\Delta V (\times 10^{-5} \text{ m}^3 \text{mol}^{-1})$	R^2
Aerobic mesophiles	714.00 ± 23.15^a	-9.13 ± 0.76^a	0.97
Psychrotrophs	526.00 ± 13.56^b	-11.81 ± 0.80^b	0.95
<i>E. coli</i>	421.00 ± 16.89^c	-18.60 ± 1.05^c	0.96
<i>S. aureus</i>	494.00 ± 10.34^d	-13.96 ± 1.21^d	0.96

All values are means $\pm$ standard deviations of data from two independent experiments ($N = 4$). Different superscripts (a, b, c, d) in the same column indicate significant difference ($p < 0.05$).

microorganisms studied indicate that the pressure death time approach is applicable to describe the pressure sensitivity parameters (Table 4).

Substantially different z_p values were obtained among groups of microbes studied, indicating varied sensitivity towards pressure destruction. *E. coli* was found to be most sensitive to changes in pressure (z_p value of 421 MPa and ΔV value of $-18.60 \times 10^{-5} \text{ m}^3 \text{mol}^{-1}$), whereas, total aerobic mesophiles were least sensitive (z_p value of 714 MPa and ΔV value of $-9.13 \times 10^{-5} \text{ m}^3 \text{mol}^{-1}$) followed by psychrotrophs (z_p value of 526 MPa and ΔV value of $-11.81 \times 10^{-5} \text{ m}^3 \text{mol}^{-1}$) and *S. aureus* (z_p value of 494 MPa and ΔV value of $-13.96 \times 10^{-5} \text{ m}^3 \text{mol}^{-1}$).

4. Conclusions

The present study highlights the effective use of HPP for enhancing microbiological safety and quality of seafood. This study revealed that

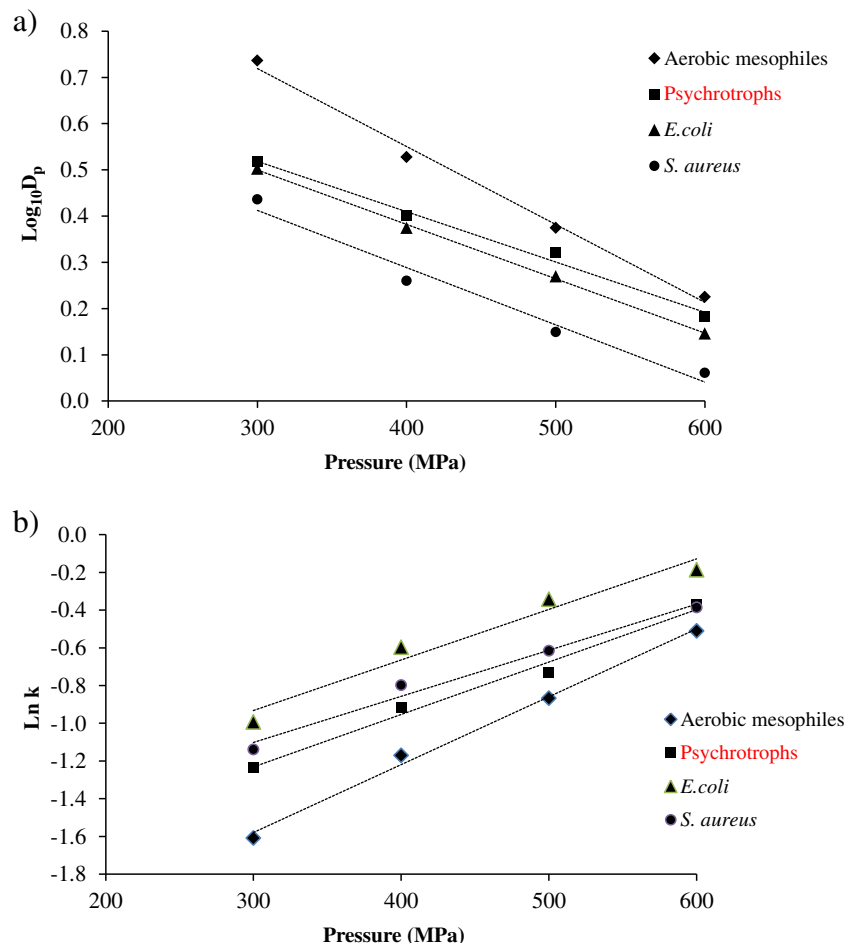


Fig. 4. (a) $\log D_p$ versus pressure; and (b) $\ln k$ versus pressure plot for high pressure-treated black tiger shrimp.

HPP is very effective in reducing microbial loads in shrimp with a maximum reduction at 600 MPa for 15 min. High pressure treatment resulted in cooked appearance with harder texture in shrimp which can be exploited as a ready-to-eat (RTE) product. Processing at 500 and 600 MPa resulted in significant alterations in these parameters which may not be acceptable by the consumers. All the treatments above 300 MPa resulted in higher TBA values compared to control, however it was still within the acceptable limits. Based on the results obtained in the present study, the recommended HPP conditions for treating black tiger shrimp are 300 MPa for 3–9 min and 400 MPa for 3 min which give texture and color values close to control but significantly lower microbial loads, TMA-N and TBA content.

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