# ORIGINAL PAPER

# Changes in K Value, Microbiological and Sensory Acceptability of High Pressure Processed Indian White Prawn (Fenneropenaeus indicus)

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Abstract Indian white prawns headless and with shell on were vacuum packed in ethylene-vinyl alcohol pouches and subjected to high pressure treatment of 100, 270, 435 and 600 MPa at 25 °C for 5 min. Subsequently the treated samples were stored in ice  $(2\pm1 \, ^{\circ}\text{C})$  along with control. The samples were periodically analysed to study the changes in total viable count, total Enterobacteriaceae count, K value and overall acceptability. Total viable count, total Enterobacteriaceae count and K value reduced proportionately with increasing high pressure treatment. However, K value and total viable count showed an increasing trend during chill storage whereas total Enterobacteriaceae decreased. No significant difference in K value was observed between control and 100 MPa, while higher pressure treated samples showed a significant difference (P<0.05). However, among the treatments 270 MPa was found to have better sensory acceptability.

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#### Introduction

High pressure (HP) processing is an alternative non-thermal processing method used to preserve food in fresh condition. This technology has the ability to inactivate microorganisms and enzymes responsible for spoilage and hence retains the nutritional qualities of food. Shellfish like oysters and crustaceans are commercially pressure treated in countries like USA, Canada, New Zealand, Australia, South Korea and Greece (Leadley 2009). Moreover, HP is very effective for shucking raw meat from rigid shell of crustaceans and molluscs without cooking (Errol 2007). This technology reduces the microbial load and protects the health of consumers who prefer raw or minimally cooked seafood.

In global food markets, prawn is a commercially traded important seafood item having high market value. It is highly perishable because of the enzymes and microflora which accelerates the spoilage mechanisms after rigor mortis. Moreover, the habitat of prawns is demersal and they are filters feeders which lead to accumulation of several bacteria and viruses in their body. HP destroys pathogenic and spoilage bacteria without greatly affecting vitamins, colour and flavor of the product (Kimura et al. 1994). Generally, gram-negative bacteria are more pressure sensitive (Farkas and Hoover 2000) and can be eliminated by high pressure processing (Gram and Huss 2000). HP treatment in fish and shellfish has shown remarkable reduction of overall microflora. This is due to changes taking place in cell membrane,



cell wall and genetic mechanisms of the microorganisms (Hoover et al. 1989). Most of the vegetative bacteria can be inactivated by 300–600 MPa pressure (Smelt 1998). Lopez-Caballero et al. (2000) reported that a pressure level of 200–400 MPa at 7 °C for 10 min is sufficient for reduction of all targeted microorganisms in prawn.

Dalgaard (2000) reported a short shelf life in raw fish and shellfish during chilled storage due to high water activity, neutral pH, high amino acid content, bacteria and autolytic enzymes. During storage of prawn adenosine triphosphate (ATP) present in the muscle becomes degraded into different compounds like adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) by enzymatic action. These enzymes are normally present inside the cell and reflect autolytic changes during storage. The ratio of these nucleotides is expressed as a percentage called K value. It is used as a chemical indicator of freshness of the product (Ehira 1976). The rate of nucleotide degradation differs with species, processing method and storage condition (Ryder 1985). HP treatment inactivates enzymes and slows down nucleotide degradation thus extending freshness of the product.

Sensory methods are universally applied in estimating the quality in the market, in the retail shop and on the table (Nair and Lahiry 1968). Characteristic sensory changes occur in the appearance, odour, taste and texture of fish and shellfish when they deteriorate (Shewan et al. 1953). These sensory changes occur due to storage conditions, autolytic changes and microbial activity.

Various processing methods inactivate enzymes and reduce microbial load, thereby preventing spoilage and extends shelf life. However, thermal processing techniques affect the freshness of the product. HP, being a non-thermal technique, extends freshness without affecting it.

The present study was undertaken to find out the effect of HP treatment on changes in K value, total viable count (TVC), total Enterobacteriaceae and sensory acceptability at various pressure levels in headless shell-on Indian white prawns during chill storage ( $2\pm1$  °C).

## **Materials and Methods**

Raw Material

Fresh prawns were procured from the fish landing center at Fort Cochin, India, and brought to the laboratory in iced condition. Prawns had an average length of 17.09 cm and weight of 30 g. The raw material was washed in potable water and head was removed manually. The prawns were given a dip treatment in chilled chlorine (2 ppm) water for 10 min. The headless prawns were then vacuum-packed in

EVOH multilayer films for HP treatment. The packed prawns were then immediately stored in chilled condition  $(2\pm1~^{\circ}\text{C})$  in insulated boxes with ice to prawns in a 1:1 ratio and transported to the Defence Food Research Laboratory (DFRL), Mysore, for HP treatment. Samples were divided into five lots, of which one was kept as control and the remaining lots were subjected to four different pressure treatments.

# High Pressure Treatment of Samples

HP processing was carried out at the DFRL using FPG 9400:922 (Stansted Fluid Power, Stansted, Essex, UK). The pressure vessel had a 2-l capacity (570 mm height and 70 mm diameter). Thirty percent propylene glycol in water was used as the pressure transmitting fluid. The technical specifications of the packaging material have been reported (Kamalakanth et al. 2011). Four different pressures 100, 270, 435 and 600 MPa with a holding time of 5 min at a temperature of 25 °C and a pressurization rate of 600 MPa/min were applied to the four lots. After depressurization the samples were stored in insulated boxes with a prawn/ice ratio of 1:1. Sampling was done in triplicate and mean values were taken.

#### Total Viable Count

Ten grams of control and treated prawns was homogenized with 90 ml sterile normal saline (0.85%) for 1 min in a stomacher at 230 rpm (Seward Stomacher 400 Circular, London, UK). The homogenized sample was serially diluted using 9 ml sterile saline (0.85%) and 0.1 ml aliquots of the appropriate dilutions were plated on plate count agar (Sigma). Plates were incubated for 48 h at 37 °C. Average counts were calculated and expressed as colony forming units per gram (cfu g<sup>-1</sup>) of the sample.

# Total Enterobacteriaceae

The method of Koutsoumanis and Nychas (1999) was used to determine the total Enterobacteriaceae count. Volumes of 1 ml from each dilution were pour plated in Violet Red Bile Glucose Agar (VRBGA) (Oxoid, CM 485B). The plates were left to solidify and then incubated at 37 °C for 18–24 h. Large colonies with purple haloes were counted as Enterobacteriaceae and reported in colony forming units of Enterobacteriaceae per gram (cfu g<sup>-1</sup>).

## Estimation of *K* Value

ATP and its degradation products were analysed using highperformance liquid chromatography (HPLC) as per Ryder (1985). Sample for injection was extracted in 0.6 M



**Table 1** Mean comparison of effect of pressure, storage period and their interaction on TVC

TVC						
Days of storage	Control	100 MPa	270 MPa	435 MPa	600 MPa	Mean
0	6.85	6.53	5.07	4.80	2.83	5.21 <sup>E</sup>
7	7.87	7.04	5.42	5.53	3.31	$5.83^{\mathrm{D}}$
14	8.31	7.86	6.80	6.33	5.30	6.92 <sup>C</sup>
21	8.67	8.66	7.90	6.35	5.80	$7.48^{B}$
28	9.81 8.34 <sup>A</sup>	9.12 7.84 <sup>B</sup>	8.87 6.81 <sup>°</sup>	7.80 6.16 <sup>D</sup>	6.70 4.79 <sup>E</sup>	8.46 <sup>A</sup>

Means with common alphabets are statistically homogenous

perchloric acid at 4 °C, centrifuged at 3,000×g for 10 min. Supernatant was neutralized with 1 M KOH and filtered through a 0.45-um syringe filter. Nucleotide standards were purchased from Sigma. Mobile phase consisted of 0.06 M dipotassium hydrogen phosphate and 0.04 M potassium dihydrogen phosphate dissolved in HPLC grade water and pH adjusted to 7.2. Jasco HPLC equipped with quaternary gradient pump (CO-2059 plus), multiwavelength detector (MD-2015 plus) and reverse phase Lichrospher<sup>TM</sup> C-18 stainless steel column (4 mm I.D. × 25 cm) having particle size of 10 μm, was used for separation of these nucleotides. Flow rate was maintained at 1 ml min<sup>-1</sup> and absorbance of eluent was monitored at 254 nm. Twenty microliters of each reference compound of 0.0276 mM was used to calibrate the system. The method described by Saito et al. (1959) was used to compute the K value from the result. The K value of pressure treated samples was initially estimated after 3 days of storage.

## Sensory Assessment

Sensory analysis of the samples was done by 12 panelists using the 9-point hedonic scale as described by Meilgaard et al. (1999). Sensory acceptability of the prawn was conducted after cooking in 2% salt solution for 10 min.

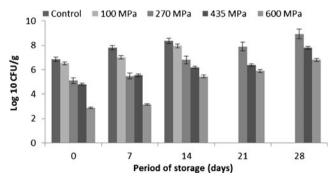


Fig. 1 Total viable count (TVC) in control and pressure treated F indicus during chill storage. Mean values of triplicate samples; standard deviations (SD) are denoted as bars. TVC in control and 100 MPa were too high on the 21st and 28th days of storage, so it is not mentioned

Panelists were provided with sensory evaluation sheet containing sensory attributes (Appendix 1) to assist them in the evaluation of overall acceptability. Average overall acceptability value of the 12 panelists for each sample was taken into account. A value of 4 was taken as the borderline of acceptability.

### Statistical Analysis

Two-way analysis of variance (ANOVA) was carried out to find the direct and interaction effect of pressure and period of storage days on TVC, total Enterobacteriaceae count, K value and sensory acceptability. SAS version 9.2 for windows was used for statistical analysis. Once ANOVA was found significant at 5% level (P<0.05), Tukey's test was performed to compare the means of different levels of pressure and period of storage days.

#### **Results and Discussion**

# Total Viable Count

The initial bacterial load of fresh prawn was 6.5  $\log_{10}$  cfu g<sup>-1</sup>. TVCs reported in prawns from India range from  $10^4$  to  $10^7$  (Pillai et al. 1961; Jacob et al. 1962). After

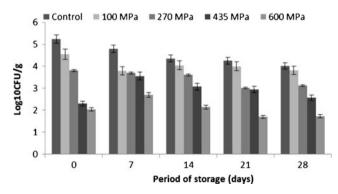


Fig. 2 Total enterobacteriaceae count in control and pressure treated F. indicus during chill storage. Mean values of triplicate samples; SDs are denoted as bars



Table 2 Mean comparison of effect of pressure, storage days and their interaction on total Enterobacteriaceae

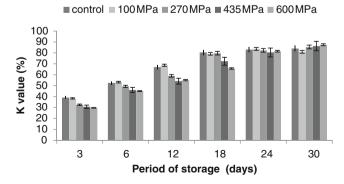
Total Entrobacteriaceae						
Days of storage	Control	100 MPa	270 MPa	435 MPa	600 MPa	Mean
0	5.23	4.49	3.82	2.21	2.06	3.56 <sup>B</sup>
7	4.84	3.76	3.73	3.55	2.67	3.71 <sup>A</sup>
14	4.35	4.07	3.61	3.06	2.12	3.44 <sup>C</sup>
21	4.22	4.00	3.01	2.95	1.68	$3.17^{\mathrm{D}}$
28	4.02 4.53 <sup>A</sup>	3.86 4.04 <sup>B</sup>	3.14 3.46 <sup>C</sup>	2.56 2.87 <sup>D</sup>	1.74 2.05 <sup>E</sup>	3.06 <sup>E</sup>

Means with common alphabets are statistically homogenous

pressure treatment of 100, 270, 435 and 600 MPa, initial TVC reduced to 6.3, 5.1, 4.8 and 2.9 log<sub>10</sub> cfu g<sup>-1</sup>, respectively. Statistical analysis revealed that there is a significant reduction in the initial bacterial load with increasing pressure (Table 1). Cruz-Romero et al. (2008) reported a reduction in the bacterial count for 260, 400 and 600 MPa treatment for 5 min in oyster (Crassostera gigas). Bacterial load reduction is due to breakdown of plasma membrane, denaturation of proteins and alteration in the permeability of the cell wall of microorganisms (Chong and Cossius 1983). TVC showed an increasing trend in all samples during chill storage. Control and 100 MPa was reached the rejection level before day 7 of storage, whereas 270 and 435 MPa pressure treated samples reached the limit before the 21st and 28th days of storage (Fig. 1). The samples treated at 600 MPa were still acceptable after 28 days of storage. This result is in agreement with that reported by Montero et al. (2001) for HP treated Penaeus japonicus after 35 days of storage. According to the International Commission of Microbiological Standards for Foods (ICMSF 1978), the maximum acceptable microbial limit in fresh and frozen fish is  $10^7 \, \text{cfu g}^{-1}$ .

## Total Enterobacteriaceae

The initial Enterobacteriaceae count in fresh prawn was  $5.38 \log_{10}$  cfu g<sup>-1</sup>. After HP processing, the count was reduced to



**Fig. 3** Changes of *K*-value in control and pressure treated *F. indicus* during chill storage. Mean values of triplicate samples; SDs are denoted as *bars* 

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4.55 log<sub>10</sub> cfu g<sup>-1</sup> in 100 MPa, 3.82 log<sub>10</sub> cfu g<sup>-1</sup> in 270 MPa, 2.30 log<sub>10</sub> cfu g<sup>-1</sup> in 435 MPa and 2.03 log<sub>10</sub> cfu g<sup>-1</sup> in 600 MPa (Fig. 2). Jose et al. (2001) reported a remarkable reduction of total Enterobacteriaceae count in octopus muscle after a pressure treatment of 200, 300 and 400 MPa for 15 min at 7 °C and 40 °C. Similar results were reported by Lopez-Caballero et al. (2000) in oysters pressurized at 400 MPa and by Carballo et al. (1997) in meat product pressurized at 300 MPa. Tukey's test at 5% level of significance revealed that there is a significant reduction in total Enterobacteriaceae count in all samples during chill storage and also found that total Enterobacteriaceae count was decreased with increasing pressure (Table 2).

# Changes in K Value

The K value of prawn before HP treatment was 21.25%. Initially after HP treatment, decrease in K value with increasing pressure was noticed in all samples. In carp muscle, Shoji and Saeki (1989) noticed similar decreasing trend in K value with pressure levels of 200, 350 and 500 MPa for 30 min and subsequent storage at 5 °C. A lower K value was reported in pressurized fresh tilapia fillets (50-300 MPa for 2–12 h) by Ko and Hsu (2001). Reduction in K value is due to suppression of IMP decomposition brought about by inactivation of dephosphorylases involved in the degradation of ATP and related compounds (Shoji and Saeki 1989; Ko and Hsu 2001). Increasing trend in K value with storage time was observed in control and treated samples (Fig. 3). Lakshmanan et al. (1996) also reported a linear increase in K value with storage time. The K value for control and 100 MPa was 39.05% and 38.36% after 3 days of storage and reached a value of 84% and 80.91% after 30 days of storage. There is no significant difference in K value between control and 100 MPa samples (Table 3). Similar result was reported with 135 MPa, 170 MPa and 200 MPa pressure for 3 s in salmon muscle during chill storage (Ignacio-Ortea et al. 2010), whereas there is a significant difference in K value between 270, 435 and 600 MPa pressure treated samples (P<0.05). The rejection point of K value as per Ehira (1976) is 60%. This was attained before 12 days of

**Table 3** Mean comparison of effect of pressure, storage days and their interaction on *K* value

K value						
Days of storage	Control	100 MPa	270 MPa	435 MPa	600 MPa	Mean
3	39.02	37.86	32.37	30.44	29.37	33.81 <sup>F</sup>
6	52.33	53.68	49.28	46.83	44.98	$49.42^{\mathrm{E}}$
12	66.89	68.16	58.77	53.97	54.92	60.54 <sup>D</sup>
18	80.25	78.89	79.73	72.01	65.77	75.33 <sup>C</sup>
24	83.01	83.44	82.61	80.35	81.38	$82.16^{B}$
30	83.89	80.68	85.34	86.20	87.21	84.66 <sup>A</sup>
	67.57 <sup>A</sup>	67.12 <sup>A</sup>	$64.68^{\mathrm{B}}$	61.63 <sup>C</sup>	$60.60^{\mathrm{D}}$	

Means with common alphabets are statistically homogenous

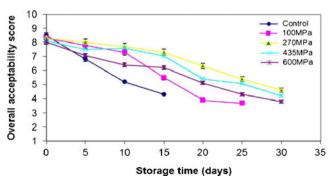
storage in control, and 100 MPa and in 270, 435 and 600 MPa, this was attained before 18 days of storage.

#### Sensory Assessment

Changes in overall sensory acceptability of prawn in control and pressure treated samples during storage is shown in Fig. 4. There is a significant difference in control and pressure treated samples at 5% level of significance. Moreover, 435 and 600 MPa samples had a slightly cooked, whitish appearance after pressure treatment. A similar observation was reported in oyster treated with 600 MPa pressure (Juan et al. 2011). Hence, 270 MPa has a better sensory acceptability.

#### **Conclusions**

Significant reduction in TVC, total Enterobactriaceae and *K* value were observed after HP treatment. Microbiologically, control and 100 MPa treated prawns reached rejection limit early, whereas at 270, 435 and 600 MPa there was an extension in rejection levels due to the destruction of bacteria. There is also a significant reduction in total Enterbacteriaceae by increased pressure levels and during storage



**Fig. 4** Changes in sensory score of control and pressure treated *F. indicus* during chill storage. Mean values of triplicate samples; SDs are denoted as *bars* 

period. The *K* value in all treated samples decreased with increasing pressure due to the inactivation of enzymes. In control and 100 MPa, there is no significant difference on *K* value during storage, while in other pressures there is a significant difference. Slightly cooked whitish appearance was observed in 435 and 600 MPa; hence, 270 MPa has better sensory acceptability. This study concludes that HP technology can improve the quality of prawns during chill storage.

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#### Appendix 1

Sensory Evaluation

Cooked prawn:

Assessor: \_\_\_\_\_ Date: \_\_\_\_

Attributes Sample Sample Sample Sample Sample I II III IV V

Appearance

Colour

Odour

Flavour

Taste

Texture

- CATUIC
- i. Firmnessii. Succulence
- iii. chewiness
- iv. Toughness

Overall acceptability



Please score the sample characteristics according to the following scale

Quality grade description	Score
Like extremely	09
Like very much	08
Like moderately	07
Like slightly	06
Neither likes nor dislikes	05
Dislike slightly	04
Dislike moderately	03
Dislike very much	02
Dislike extremely	01

Comments:

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