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Shelf Life of High Pressure Treated Indian White Prawn (*Fenneropenaeus indicus*) During Chilled Storage

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Abstract

Indian white prawn (*Fenneropenaeus indicus*) was vacuum packed in ethylene vinyl alcohol (EVOH) pouches and subjected to high pressure (HP) treatment of 250 MPa for 6 min at a ramp rate of 400 MPa min^{-1}. Treated sample was stored in ice (2±1°C) along with untreated (control) sample to evaluate the changes in physicochemical parameters, mesophilic count and shelf life. Lightness (L* value) and yellowness (b*) value increased; whereas, redness decreased after pressure treatment. Significant increase of pH, hardness, FFA and TBA of prawn was noticed immediately after HP-treatment; however, TMA, TVB-N, K-value, relative activity of PPO and mesophilic count were reduced ($p<0.05$). During storage, hardness, pH, TMA, TVB-N, FFA, TBA values and mesophilic count increased; whereas, K-value and PPO were reduced in HP-treated sample ($p<0.05$). Tensile strength, elongation at break, oxygen transmission rate (OTR), carbon dioxide transmission rate (CO_2-TR), water vapour transmission rate (WVTR), water extractives, *n*-heptane of EVOH packing material have showed marginal changes after HP-treatment. Based on overall acceptability, control sample and HP-treated sample revealed a shelf life of 9 and 21 days, respectively during chilled storage.

Keywords: High pressure treatment, Indian white prawn, EVOH pouch, chilled storage

Introduction

Indian white prawn is a major commercial sea food item in the export market because of its good source of protein, and is low in fat and calories. However, prawn is highly perishable in nature due to high water activity, high amino acid content, pH, bacteria and autolytic enzymes (Dalgaard, 2000). Significant quantities of free amino acids content in prawn muscles make it more susceptible to bacterial spoilage (Simidu, 1962). Quality changes in chill stored prawns are mainly due to the combined action of tissue enzymes and microbial activity (Flick & Lovell, 1972). Various processing techniques are practiced to preserve the prawn. However, consumers demand and prefer fresh and minimally processed food having natural flavour, taste and fresh like appearance. High pressure (HP) technology has the capability to reduce processing time and retain freshness, flavour, texture and colour without losing vitamins and nutrients in the product (Kadam et al., 2012). Food preservation by high pressure is a promising technique in food industry as it offers numerous opportunities for developing new food products with high nutritional value and excellent organoleptic characteristics. Recently the advances in various designs for high pressure processing machine ensure worldwide recognition of the potential for such a technology in food processing (Balci & Wilbey, 1999).

Packaging of the food material is not only for preserving and conserving the food from external contaminants but also for getting good consumer attraction. Several authors have been reported that ethylene vinyl alcohol (EVOH) packaging material is suitable for HP treatment of food (Amparo et al., 2005). EVOH material is a multilayer structure, sandwiched between at least two layers of polyethylene or polypropylene which are hydrophobic material and excellent barrier to oxygen without compromising flexibility or transparency (Ozen & Floros, 2001).

Present study was carried out to understand the changes in the physical and biochemical parameters

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of headless (HL) *F. indicus* packed in EVOH pouches under vacuum condition pressurized at optimum processing condition (250 MPa pressure, 400 MPa ramp rate and 6 min holding time) and also to evaluate its shelf life during chilled storage.

Materials and Methods

Fresh prawns of an average length of 15±1 cm and weight of 25±2 g were procured from the fish landing centre at Fort Cochin, India, and transported to the laboratory in iced condition. The raw material was washed in potable water and head was removed manually. The headless (HL) prawns were then vacuum-packed in EVOH multilayer films for HP treatment. Multilayered ethylene vinyl alcohol (EVOH) film (nylon, EVOH and polypropylene) (Sealed Air Pvt. Ltd, Bangalore, India) packaging material of 72 μm thickness was used for the study. EVOH pouches having the dimension of 200 mm length and 150 mm width were used to pack the HL prawn for the experiment. Pressure treatment was carried out in high pressure processing (HPP) machine (Stansted Fluid Power, Stansted, Essex, UK). Thirty percent propylene glycol in distilled water was used as the pressure transmitting fluid. Samples were subjected to optimized high pressure processing condition 250 MPa with a holding time of 6 min and a pressurization rate of 400 MPa min^{-1} at a temperature of 25°C (Ginson, 2015). Both pressure treated and untreated samples (control) were stored in insulated boxes with a prawn/ice ratio of 1:1 for chill storage.

The colour of the sample was measured by using Hunter lab Colorimeter Model No D/8-S (Miniscan XE Plus) (Plate 3.5) with geometry of diffuse /8°C (sphere 8 mm view) and an illuminant of D 65/10°C (Shah, 1991). Texture profile analysis (TPA) of prawn samples was determined by the method of Andersen et al. (1994) by using universal testing machine (Lloyd instruments LRX plus, UK), equipped with a load cell of 50 N. The pH of the homogenized sample was measured by using glass electrode digital pH meter15 (Cyberscan 510, Eutech Instruments, Singapore) as per APHA (1998) method. TMA and TVB-N were determined by micro diffusion method as per Conway (1962). FFA content in the prawn meat was determined by AOCS (1989) method. TBA was measured according to the method of Tarladgis et al. (1960). K-value of the sample was analysed using high performance liquid chromatography (HPLC) as per Ryder (1985). Polyphenol oxidase was estimated by the method described by Esterbauer et al. (1977).

Sensory analysis of the samples was carried out by the method described by Meilgaard et al. (1999). Determination of O_2 and CO_2 permeability of EVOH film was carried out using gas permeability apparatus (gas and steam permeability, AtsFaar, Societa' Per Azioni, Milano, Italia) (ASTM- D 1434 (1975)) and is expressed as mL m^{-2} 24 h^{-1} at 1 atm. pressure at 21°C. Water vapour transmission rate (WVTR) was determined by ASTM E 96-80 (1987) method and expressed as g m^{-2} 24 h^{-1} at 90 ± 2 % RH and 37°C. Overall migration residue of EVOH packing material was determined by using IS: 9845 (1998). Overall migration test was performed by using the food stimulants such as distilled water, and *n*-heptane. The amount of extractives was as expressed as mg L^{-1} or ppm. Universal Testing Machine (Lloyd instruments LRX plus, UK) was used for determination of tensile strength and elongation at break as per IS: 2508 (1984). The mean of five results was expressed for the machine direction (MD) and cross direction (CD). Two-way ANOVA was carried out to find the direct and interaction effect of pressure and storage days on physical and biochemical parameters of Indian white prawn. SAS version 9.2 for windows was used for statistical analysis. Once ANOVA was found to be significant at 5% level ($p<0.05$), Tukey's test was performed to compare the means of treatments and storage days. Independent *t*-test was used to compare the physicochemical properties of EVOH packing at a significance level of 5% (pd" 0.05).

Result and Discussion

The change in colour values of control and HP-treated samples are given in Table 1. The lightness (L*) and yellowness of the sample significantly increased, whereas redness got significantly reduced after HP-treatment ($p<0.05$). Colour changes in HP-treated seafood were due to several factors such as denaturation of myofibrillar and sarcoplasmic proteins, denaturation of myosin (Angsupanich & Ledward, 1998), formation of metmyoglobin (Carlez et al., 1995), oxidation of haemoprotein (Cheah & Ledward, 1996) and stabilization of ferrous nitrosomyoglobin (Brunn & Skibsted, 1996). A significant increase of colour values was observed in both samples during chilled storage. Similar trend was noticed in black tiger prawn (Barjinder et al., 2012). Colour values in control and HP-treated

Table 1. Changes in L*, a* & b* value in control and HP-treated *F. indicus* during chilled storage

Storage Days	L* value		a* value		b* value	
	Control	250 MPa treated	Control	250 MPa treated	Control	250 MPa treated
0	41.68 ± 0.01^{aA}	51.42 ± 0.02^{bA}	-2.57 ± 0.01^{aA}	-3.66 ± 0.10^{bA}	1.08 ± 0.01^{aA}	1.49 ± 0.01^{bA}
3	43.14 ± 0.03^{aB}	53.39 ± 0.02^{bB}	-2.11 ± 0.05^{aB}	-2.60 ± 0.02^{bB}	1.48 ± 0.04^{aB}	1.77 ± 0.02^{bB}
6	43.47 ± 0.04^{aB}	62.14 ± 0.04^{bC}	-1.80 ± 0.02^{aC}	-2.57 ± 0.03^{bB}	2.59 ± 0.04^{aC}	1.81 ± 0.02^{bC}
9	47.16 ± 0.04^{aC}	67.50 ± 0.02^{bD}	-1.66 ± 0.04^{aD}	-2.47 ± 0.04^{bC}	2.21 ± 0.01^{aD}	2.42 ± 0.01^{bD}
12	45.31 ± 0.05^{aD}	68.19 ± 0.07^{bD}	-1.24 ± 0.04^{aE}	-2.41 ± 0.06^{bC}	2.91 ± 0.03^{aE}	2.37 ± 0.01^{bE}
15	51.07 ± 0.05^{aE}	68.58 ± 1.00^{bD}	-0.97 ± 0.03^{aF}	-2.21 ± 0.02^{bD}	3.31 ± 0.03^{aF}	2.53 ± 0.01^{bF}
18	52.61 ± 0.08^{aF}	67.90 ± 0.05^{bD}	-0.88 ± 0.01^{aG}	-1.72 ± 0.02^{bE}	3.77 ± 0.01^{aG}	2.61 ± 0.02^{bG}
21	55.11 ± 0.01^{aG}	69.92 ± 0.01^{bE}	-0.82 ± 0.04^{aG}	-1.66 ± 0.07^{bE}	3.96 ± 0.01^{aH}	2.76 ± 0.01^{bH}

Treatment means having common lower case in rows and upper case in columns are homogenous. Each value represents the mean ± SD (n= 3), $p<0.05$

sample were significantly different during storage ($p<0.05$). However, L* value in control sample showed a significant difference with each period of storage except on 3rd and 6th day of storage. Whereas, it was homogenous on 9th, 12th, 15th and 18th day of storage in HP-treated sample ($p>0.05$). Redness of the control sample revealed no significant difference on 18th and 21st day of storage; whereas, HP-treated sample showed a homogenous value between 3rd and 6th, 9th and 12th, and 18th & 21st day of storage ($p>0.05$). Effect of period of storage on b* value in control and HP-treated sample were found to be significant ($p<0.05$).

Textural hardness of prawn significantly increased after HP-treatment ($p<0.05$) (Table 2). Lopez-Caballero et al. (2000) suggested that HP-treatment at 200 and 400 MPa increases hardening of prawns. Hardness values were significantly different in control and HP-treated samples during storage ($p<0.05$). However, hardness values were homogenous on 3rd, 6th, 9th, 15th and 21st day of storage in control samples ($p<0.05$) and 12th & 15th day of storage for HP-treated sample ($p<0.05$). Hardness increased in control and HP-treated sample till 12th and 15th day of storage, respectively and then it remained unchanged or reduced with days of storage. Similar trend was reported in HP-treated albacore tuna minced muscle during refrigerated storage (Ramirez-Suarez & Morrissey, 2006) and authors suggested that it could be due to the enhancement of hydrogen-bonds among the proteins at low temperature which reverts at the end of the storage time. At the end of storage, hardness was found to be 66.15 N and 83.14 N in control and HP-treated samples, respectively. Significant increase in pH of prawn was noticed after HP-treatment (Table 2). Initial pH of *F. indicus* was 6.86 and it increased to 6.98 after HP-treatment. Cruz-Romero et al. (2008) found higher pH values in HP-treated oyster. Similar findings were reported in HP-treated cod (Angsupanich & Ledward, 1998). The increase in pH is due to pressure induced unfolding of protein and ionisation of denatured protein (Yamamoto et al., 1994). Significant difference in pH was observed in control and HP-treated samples during storage ($p<0.05$). Period of storage days have significant effect on pH in control sample except 12th and 15th day of storage ($p>0.05$). Similarly, pH values in HP-treated samples were homogenous ($p>0.05$) between 0th and 3rd day; 6th, 9th and 12th day and 15th, 18th and 24th day of storage, respectively.

Pressure has significant effect on trimethylamine ($p<0.05$) in *F. indicus*. Reduction of 1.89 mg N_2 100 g^{-1} of TMA value was observed in Indian white prawn immediately after HP treatment (Table 3). Similar result was found in vacuum packed hake muscle subjected to 400 MPa at 7°C (Hurtado et al., 2000). TMA value showed an increasing trend in treated and untreated sample during storage. Reduction of TMAOase activity was noticed in HP treated squid (Gou et al., 2010). Limit of acceptability of TMA value is 10-15 mg N_2 100 g^{-1} (Dalgaard et al., 1993). In untreated it exceed on 12th day of storage whereas in treated sample it reached a value

Table 2. Changes in hardness (N) and pH in control and HP-treated *F. indicus* during chilled storage

	Hardness		pH	
Storage days	Control	250 MPa treated	Control	250 MPa treated
0	60.33 ± 0.03^{aA}	73.62 ± 0.02^{bA}	6.86 ± 0.01^{aA}	6.98 ± 0.01^{bA}
3	65.69 ± 0.03^{aB}	76.01 ± 0.01^{bB}	6.93 ± 0.01^{aA}	7.02 ± 0.01^{bA}
6	66.68 ± 0.01^{aB}	77.92 ± 0.01^{bC}	7.25 ± 0.01^{aB}	7.15 ± 0.01^{bB}
9	67.14 ± 0.04^{aB}	79.15 ± 0.04^{bD}	7.58 ± 0.01^{aC}	7.22 ± 0.02^{bB}
12	68.44 ± 0.04^{aC}	83.46 ± 1.65^{bE}	7.70 ± 0.10^{aD}	7.30 ± 0.10^{bB}
15	67.24 ± 0.10^{aB}	84.66 ± 0.03^{bE}	7.71 ± 0.01^{aD}	7.48 ± 0.01^{bC}
18	66.15 ± 0.04^{aD}	82.10 ± 0.05^{bF}	7.91 ± 0.01^{aE}	7.55 ± 0.02^{bC}
21	66.15 ± 0.01^{aB}	83.14 ± 0.02^{bG}	8.03 ± 0.01^{aF}	7.57 ± 0.03^{bC}

Treatment means having common lower case in rows and upper case in columns are homogenous. Each value represents the mean ± SD (n= 3), $p<0.05$

Table 3. Changes in TMA and TVB-N (mg N_2 $100g^{-1}$) in control and HP-treated *F. indicus* during chilled storage

	TMA		TVB-N	
Storage days	Control	250 MPa treated	Control	250 MPa treated
0	8.54 ± 0.10^{aA}	6.65 ± 0.02^{bA}	13.84 ± 0.00^{aA}	12.16 ± 0.10^{bA}
3	10.44 ± 0.03^{aB}	8.99 ± 0.01^{bB}	23.93 ± 0.01^{aB}	18.59 ± 0.03^{bB}
6	13.52 ± 0.01^{aC}	9.27 ± 0.02^{bC}	29.77 ± 0.04^{aC}	23.43 ± 0.03^{bC}
9	14.22 ± 0.01^{aD}	10.46 ± 0.02^{bD}	32.43 ± 0.02^{aD}	27.16 ± 0.04^{bD}
12	15.66 ± 0.03^{aE}	10.86 ± 0.04^{bE}	36.06 ± 0.03^{aE}	30.26 ± 0.04^{bE}
15	16.87 ± 0.02^{aF}	12.45 ± 0.01^{bF}	41.88 ± 0.02^{aF}	34.36 ± 0.01^{bF}
18	18.08 ± 0.01^{aG}	13.5 ± 0.10^{bG}	46.58 ± 0.02^{aG}	38.36 ± 0.01^{bG}
21	19.28 ± 0.01^{aH}	14.88 ± 0.01^{bH}	51.27 ± 0.03^{aH}	42.12 ± 0.01^{bH}

Treatment means having common lower case in rows and upper case in columns are homogenous. Each value represents the mean ± SD (n= 3), $p<0.05$

of 14.88 on 21st day of storage. Significant reduction of TVB-N value was noticed after HP treatment ($p<0.05$) (Table 3). During storage both samples showed an increasing trend and was significantly different with storage days ($p<0.05$). There was a delay for TVB-N formation in HP-treated sample throughout the storage. Result agrees with Buyukcan et al. (2009 Reduction of TVB-N value may be due to the inhibition of proteolytic activity. Hernández-Andrés et al. (2005) revealed that HP treatment can inhibit the proteolytic activity. Acceptability level of TVB-N value is considered to be 30-35 mg N_2 100 g^{-1} (Connel, 1995). In control it exceeds (36.06 mg N_2 100 g^{-1}) on 12th day of storage. In treated sample TVB-N value exceeded (38.36 mg N_2 100 g^{-1}) on the 18th day of storage. Increasing trend of TVB-N content during chill storage be due to the endogenous enzymatic activity (Botta et al., 1984).

Free fatty acid content significantly increased with pressure treatment ($p<0.05$). During storage FFA value was found to have an increasing trend (Table 4). Treated and untreated samples had significant difference with storage days ($p<0.05$). Pressure treated samples observed higher FFA values and similar trend was observed in HP treated black tiger shrimp (Barjinder et al., 2012). Formation of FFA value during initial days of storage is due to the activity of lipases and phospholipases (Whittle et al., 1990) and later stage it is due to the microbial

Table 4. Changes in FFA (mg % oleic acid) and TBA (mg malonaldehyde kg-1) in control and HP-treated *F. indicus* during chilled storage

	FFA		TBA	
Storage days	Control	250 MPa treated	Control	250 MPa treated
0	15.34 ± 0.05^{aA}	20.68 ± 0.10^{bA}	0.11 ± 0.00^{aA}	0.34 ± 0.01^{bA}
3	16.43 ± 0.05^{aB}	21.31 ± 0.06^{bB}	0.48 ± 0.01^{aB}	0.92 ± 0.01^{bB}
6	20.26 ± 0.06^{aC}	24.41 ± 0.06^{bC}	0.63 ± 0.00^{aC}	1.45 ± 0.01^{bC}
9	20.29 ± 0.02^{aC}	24.82 ± 0.04^{bD}	1.36 ± 0.01^{aD}	2.32 ± 0.01^{bD}
12	22.48 ± 0.04^{aD}	28.29 ± 0.02^{bE}	1.98 ± 0.01^{aE}	2.41 ± 0.01^{bE}
15	23.46 ± 0.02^{aE}	30.42 ± 0.02^{bF}	2.29 ± 0.01^{aF}	2.51 ± 0.01^{bF}
18	24.11 ± 0.05^{aF}	33.16 ± 0.03^{bG}	2.42 ± 0.01^{aG}	2.61 ± 0.01^{bG}
21	25.46 ± 0.04^{aG}	34.12 ± 0.06^{bH}	2.6 ± 0.02^{aH}	2.72 ± 0.01^{bH}

Treatment means having common lower case in rows and upper case in columns are homogenous. Each value represents the mean ± SD (n= 3), $p<0.05$

activity. Lipase, lipoxygenase, peroxidase, phosphatase and catalase were found to be more resistant even at higher pressures (Syderhelm et al., 1996). At the end of storage, FFA value in untreated and treated samples reached 25.46 and 34.12 mg% oleic acid, respectively. Pressure significantly increased TBA value in Indian white prawn ($p<0.05$) (Table 4). Increase in TBA value was mainly due to the high pressure release of the iron (Fe^{2+}) from heme groups, which increased the oxidation of unsaturated fatty acids (Igene et al., 1979). The increase in TBA values may also be due to damage to the cell membrane and the lipids are more exposed and easily available for oxidation. Studies also suggest that the accelerated oxidation in pressurized fishes may be due to the denaturation of hemeprotein (Ohshima et al., 1992). TBA value increased with storage days in treated and untreated samples and both samples showed a significant difference with storage days ($p<0.05$). Similar results were observed in HP treated vacuum packed cold-smoked salmon (Lakshmanan et al., 2005). Limit of acceptability for TBA values is 1–2 mg malonaldehyde kg-1 (Goulas & Kontominas, 2007). In treated and untreated sample TBA value exceeded on 9th and 6th day of storage, respectively.

Changes in PPO relative activity in shell and telson of *F. indicus* after HP-treatment are presented in Table 5, respectively. Relative activity of PPO in telson and shell of prawn significantly reduced after HP-treatment ($p<0.05$). Montero et al. (2001) revealed that HP-treatment could inactivate PPO activity of tiger prawn (*P. japonicus*) pressurized at 0.1-400 MPa for 10 min and also found that PPO activity apparently reduced with increasing pressure. PPO activity of shell and telson of control and HP-treated samples declined and showed a significant difference during chilled storage except 12th, 14th and 16th day of storage for shell and 16th day of storage for telson, respectively ($p>0.05$) (Table 5). However, effect of period of storage on the relative activity of prawn shell was not significant in control and HP-treated samples, except 0th, 2nd, 4th and 6th day of storage for control and 0th, 4th and 8th day of storage for HP-treated samples, respectively. Similarly, PPO activity of telson was not significantly different between 10th and 12th day of storage, and 14th and 16th day of storage, respectively in control sample ($p<0.05$). Whereas, 0th and 2nd, 4th and 6th day of storage were homogenous ($p>0.05$) and remaining period of storage showed no significant change in HP-treated sample ($p<0.05$).

K-value reduced significantly in *F. indicus* after HP-treatment ($p<0.05$). Initial K-value of the sample was 23.01% which declined to 12.01% after HP-treatment (Table 6). Lower K-value in prawns after different pressure treatment was reported by Ginson et al. (2013). Reduction of K-value could be due to the suppression of IMP decomposition, denaturation of proteins and deactivation of enzymes, mainly dephosphorylases, responsible for the degradation of ATP and related compounds (Ko & Hsue, 2002). Significant difference in K-value was perceived in both samples during chilled storage. Likewise,

period of storage also revealed significant effect on K-value in both samples ($p<0.05$). K-value showed an increasing trend in both samples; however, lower values were observed in HP-treated samples during storage. As per Ehira (1976), the rejection point of K-value is 60% which exceeded on 9th and 15th day of storage in control and HP-treated samples, respectively. At the end of storage, K-value in control and HP-treated samples was 85.12 and 78.12%, respectively. Initial bacterial load of fresh prawn was 4.3 Log_{10} CFU g^{-1} which reduced to 3.82 Log_{10} CFU g^{-1} after HP-treatment (Table 6). Reduction of microbial flora after pressurization was reported in prawns and clams (Linton et al., 2003). Reduction of bacterial load after HP-treatment was due to break down of phospholipid molecules, denaturation of protein, and alteration in the permeability of the cell wall of microorganisms (Chong et al., 1983). Mesophilic count in control and HP-treated samples showed an increasing trend with storage time. Mesophilic count in control and HP-treated sample were 7.36 Log_{10} CFU g^{-1} and 5.42 Log_{10} CFU g^{-1} on 24th day of storage, respectively. Similar result was found in HP-treated *P. japonicus*

Table 5. Changes in PPO relative activity (%) in shell and teleson of control and HP-treated F. indicus during chilled storage

	PPO relative activity (%) in shell		PPO relative activity (%) in telson	
Storage days	Control	250 MPa treated	Control	250 MPa treated
0	100.0 ± 00^{aA}	66.25 ± 5.30^{bA}	100.00 ± 00^{aA}	51.67 ± 2.357^{bA}
2	83.75 ± 5.3^{aB}	53.50 ± 4.94^{bB}	83.96 ± 3.83^{aB}	45.21 ± 2.062^{bA}
4	55.00 ± 7.07^{aC}	36.695 ± 3.45^{bC}	54.79 ± 2.062^{aC}	28.96 ± 3.241^{bB}
6	38.75 ± 1.76^{aD}	27.285 ± 1.64^{bC}	35.63 ± 6.187^{aD}	22.50 ± 3.536^{bB}
8	27.50 ± 3.53^{aE}	16.18 ± 1.329^{bD}	25.83 ± 1.179^{aE}	12.92 ± 0.589^{bC}
10	19.22 ± 1.09^{aE}	11.69 ± 0.636^{bD}	19.50 ± 0.714^{aF}	7.800 ± 0.919^{bC}
12	11.68 ± 0.60^{aE}	8.795 ± 0.926^{aD}	14.73 ± 0.735^{aF}	3.000 ± 0.778^{bC}
14	7.67 ± 0.31^{aE}	3.75 ± 0.7071^{aD}	6.685 ± 0.615^{aG}	1.500 ± 0.552^{bC}
16	1.89 ± 0.34^{aE}	0.04 ± 0.0212^{aD}	2.000 ± 0.156^{aG}	0.083 ± 0.007^{aC}

Treatment means having common lower case in rows and upper case in columns are homogenous. Each value represents the mean ± SD (n= 3), $p<0.05$

Table 6. Changes in K-value (%) and mesophilic count (Log_{10} CFU g^{-1}) in control and HP-treated *F. indicus* during chilled storage

	K-value		Mesophilic count	
Storage days	Control	250 MPa treated	Control	250 MPa treated
0	23.05 ± 0.04^{aA}	12.01 ± 0.03^{bA}	4.30 ± 0.01^{aA}	3.82 ± 0.08^{bA}
3	33.21 ± 0.11^{aB}	21.15 ± 0.05^{bB}	4.62 ± 0.01^{aB}	3.92 ± 0.04^{bB}
6	53.66 ± 0.11^{aC}	33.45 ± 0.10^{bC}	4.87 ± 0.02^{aC}	4.28 ± 0.01^{bC}
9	67.45 ± 0.10^{aD}	40.91 ± 0.08^{bD}	5.01 ± 0.03^{aD}	4.49 ± 0.05^{bD}
12	73.55 ± 0.10^{aE}	50.57 ± 0.10^{bE}	6.03 ± 0.08^{aE}	4.65 ± 0.04^{bE}
15	80.88 ± 0.10^{aF}	74.03 ± 0.07^{bF}	6.47 ± 0.03^{aF}	4.82 ± 0.01^{bF}
18	85.12 ± 0.10^{aG}	78.12 ± 0.10^{bG}	6.91 ± 0.03^{aG}	4.94 ± 0.04^{bG}
21	-	-	7.36 ± 0.03^{aH}	5.08 ± 0.03^{bH}

Treatment means having common lower case in rows and upper case in columns are homogenous. Each value represents the mean ± SD (n= 3), $p<0.05$

after 35 days of storage (Montero et al., 2001). As per ICMSF (1986), mesophilic count in control and HP-treated sample reached the acceptable limit on 9th and 21st day of storage.

Changes in sensory evaluation score of control and HP-treated sample during chilled storage are presented in Table 7. Overall acceptability of control and HP-treated sample was significantly different during chilled storage ($p<0.05$), except on initial day of storage ($p>0.05$). Similarly, changes of sensory score with storage period were significant in control and HP-treated samples. Sensory acceptability score of both samples was reduced with days of storage; whereas, HP-treated samples maintained better overall acceptability during the entire period of storage. There was a significant alteration in the sensory attributes like colour, odour, hardness and taste in HP-treated samples compared to control sample. Sensory score of 4 was taken as the acceptable limit for determining the shelf life of HP-treated prawn. Initial sensory score of control and HP-treated sample were 8.6 and 8.5, respectively; which reached acceptable limit on 9th and 21st day of storage. Shelf life of HP-treated sample was found to be extended up to 21 days of storage; whereas, control sample was rejected on 9th day of storage. An extended shelf life of 28 and 35 days was noticed in prawn (*P. japonicus*) pressurized at 200 and 400 MPa during chilled storage (Lopez-Caballero et al., 2000). HP-treated prawn (*P. longirostris*) showed 16 days of shelf life extension at refrigerated temperature (Buyukcan et al., 2009).

Table 7. Changes in sensory score in control and HP-treated *F. indicus* during chilled storage

Storage days	Control	250 MPa treated
0	8.60 ± 0.10^{aA}	8.50 ± 0.10^{aA}
3	6.13 ± 0.06^{aB}	7.83 ± 0.06^{bB}
6	4.20 ± 0.10^{aC}	7.53 ± 0.06^{bC}
9	4.00 ± 0.06^{aD}	7.20 ± 0.06^{bD}
12	3.25 ± 0.01^{aE}	6.50 ± 0.10^{bE}
15	2.61 ± 0.03^{aF}	5.70 ± 0.10^{bF}
18	1.62 ± 0.01^{aG}	5.10 ± 0.06^{bG}
21	1.12 ± 0.06^{aH}	4.00 ± 0.06^{bH}

Treatment means having common lower case in rows and upper case in columns are homogenous. Each value represents the mean ± SD (n= 3), $p<0.05$

Pressure has a significant effect on tensile strength and elongation at break both in MD and CD of the packing material (p*d"*0.05). The value of tensile strength and elongation at break in both MD and CD direction is presented in Table 8. Numerous folds and wrinkles were found on the films after HP treatment. Tensile strength of untreated EVOH film has showed a value of 294.44 kg cm^{-2} and 291.58 kg cm^{-2} in MD and CD respectively. Whereas slight reduction of tensile strength was observed in the EVOH pouches after HP treatment and it showed a value of 292.44 kg cm^{-2} and 285.51 kg cm^{-2} in MD and CD respectively. Elongation at break of EVOH material increased after HP treatment. It showed a value of 141 and 189 % in HP treated EVOH material both in MD and CD respectively. Increase of elongation at break and slight reduction of tensile strength packing material treated with high pressure have been reported by Masuda et al. (1992). Slight reduction in the tensile strength and elongation at break may due to the physical change on the structure of packing material caused by compression and expansion during pressure treatment. HP treatment had a significant effect of OTR, WVTR and CO_2-TR in EVOH material (p*d"*0.05) (Table 8). HP treatment slightly altered oxygen transmission rate and showed a value of 2.24 cc m^{-2} 24 h^{-1} at 1 atm. compared with untreated EVOH material (1.92 cc m^{-2} 24 h^{-1} at 1 atm.). Pastorelli (1997) reported that an increase of O_2 permeability in EVOH material (LLDPE/EVA/EVOH/EVA/LLDPE) after an HP treatment of 400 MPa for 60°C at 30 min. Lambert et al. (2000) suggested that 12% change in oxygen barrier properties of packaging materials after HP treatment is an allowable deviation. Galotto et al. (2010) found that permeability of polyethylene/ethylene-vinyl-alcohol/polyethylene (PE/EVOH/PE) packing material increased after HP treatment compared to control and this may due the structural damages caused by pressure treatment. However, Amparo et al. (2005) suggested that HP treatment does not significantly alter the oxygen permeability of EVOH packing material. Masuda et al. (1992) found that the high pressure treatment did not change initial oxygen barrier properties of PP/EVOH/PP and PP/EVOH/ PE. Co_2-TR of EVOH material showed a slight increase after HP treatment. WVTR of EVOH pouches showed slight increase after HP treatment from 19.61 g m^{-2} 24 h^{-1} at 37°C and 92 % RH to 20.89 g m^{-2} 24 h^{-1} at 37°C and 92 % RH. Halim et al. (2009) reported that slight increase of WVTR after HP treatment in PE/N6/EVOH/PE film but which is

Table 8. Changes in physicochemical properties of untreated and HP-treated EVOH pouches

Physicochemical properties of EVOH	Untreated		250 MPa		Significance
	Mean ± SD	SE	Mean ± SD	SE	level
Tensile strength MD (kg cm^{-2})	294.44±0.40	0.23	292.44±0.38	0.22	0.003
Tensile strength CD (kg cm^{-2})	291.58±0.41	0.24	285.51±0.48	0.28	0.000
Elongation at break MD (%)	138.33±0.58	0.33	141.00±1.00	0.58	0.016
Elongation at break CD (%)	176.33±1.53	0.88	189.00±1.00	0.58	0.000
OTR (cc m^{-2} 24 h-1 at 1 atm. pressure)	1.92±0.03	0.017	2.24±0.10	0.06	0.007
CO_2-TR (cc m^{-2} 24 h-1 at 1 atm. pressure)	6.83±0.19	0.11	8.23±0.12	0.07	0.000
WVTR (g m^{-2} 24 h^{-1} at 37 ^{0}C and 92 % RH)	19.58±0.32	0.18	20.58±0.36	0.21	0.023
Water extractives (mg L^{-1})	4.69±0.19	0.11	8.55±0.44	0.26	0.000
n-heptane (mg L^{-1})	3.91±0.25	0.14	6.26±0.53	0.31	0.002

Each value represents the mean ± SD (*n*= 3), p*d"*0.05. (MD=Machine direction; CD=Cross Direction; OTR=Oxygen transmission rate; WVTR=Water vapour transmission rate; CO_2-TR= CO_2 transmission rate; SD=Standard deviation; SE= Standard error)

not statistically significant. Lambert et al. (2000) suggested that 12% change in water vapour barrier properties of packaging materials after high pressure treatment is an allowable deviation. Migration of water extractives and *n*-heptane significantly increased after HP treatment (Table 8) even though it is in the acceptable range. Galotto et al. (2010) reported that the migration of olive oil increased in polyethylene/ethylene-vinyl-alcohol/polyethylene (PE/EVOH/PE) material after HP treatment. This may be due to the damage occurred to the structure of the packing material after pressurization. However, Ochiai & Nakagawa (1992) reported no significant changes in the total migration of PP/PVDC/PP packages after pressure treatment. The reason may be due to the pressure treatment reduces the free volume of plastic matrix and loses the capacity for absorbing flavour compounds from food (Caner et al., 2004). With pressure release, some plastic material recovers their original dimensions, and thus ensue the sorption and diffusion (Caner et al., 2004); whereas, some packing materials failed to regain their free volume which reduces the migration of flavor compounds from the materials.

High pressure treatment had a significant effect on physical and biochemical properties of HL *F. indicus*. Colour values of the prawn moderately changed after HP-treatment. Significant increase of pH, FFA and TBA of prawn were noticed after HP-treatment and during storage ($p<0.05$). There was a reduction of TMA, TVB-N, K-value, relative activity of PPO and mesophilic count after HP-treatment ($p<0.05$). During storage, TMA and TVB-N values were enhanced whereas; K-value and PPO were reduced in HP-treated sample. Tensile strength, elongation at break, OTR, CO_2-TR, WVTR, water extractives, *n*-heptane of EVOH packing material have showed significant difference after HP treatment. Sensory score reveals that shelf life of HP-treated sample was found to be extended up to 21 days of storage, but control sample rejected on 9th day of storage. Hence, the study concluded that HP-treatment cause significant effect on physical and biochemical parameters of *F. indicus*, therefore it can be suitably used to augment shelf life of seafood and provide better quality product to consumers.

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Reference

Amparo-Lopez-Rubio, Jose, M., Lagaron, Pilar-Hernandez-Munoz, Eva-Almenar, Ramon-Catala, Rafael-Gavara

and Pascall, M. A. (2005) Effect of high pressure treatments on the properties of EVOH-based food packaging materials. Innov. Food Sci. Emerg. Technol. 6: 51-58

Andersen, U.B., Stromsnes, A.N., Steinsholt, K. and Thomassen, M.S. (1994) Fillet gaping in farmed Atlantic salmon (*Salmo Salar*). Norweg. J. Agric. Sci. 8: 165-179

Angsupanich, K. and Ledward, D.A. (1998). High pressure treatment effects on cod (*Gadus morhua*) muscle. J. Food Chem. 63(1): 39-50

AOCS (1989) Official methods and recommended practices of American Oil Chemists Society, 5th edn., AOCS, Champaign, USA

APHA (1998) Standard methods for examination of water and water waste. 20th edn., American Public Health Association, American Water Works Association, Water Environmental Federation, Washington, DC.

ASTM (1975) Standard test methods for gas transmission rate of plastic film sheeting, D-1434, American Society for Testing and Materials, Philadelphia, USA

ASTM (1987) Standard operating procedure for the determination of water vapour transmission rate of packaging materials, E 96-80, American Society for Testing and Materials, Philadelphia, USA

Balci, A.T. and Wilbey, R.A. (1999) High pressure processing of milk the first 100 years in the development of new technology. Int J Dairy Technol. 52: 149-155

Barjinder, P.K., Neelima, K., Srinivasa, P.R. and Chauhan, O.P. (2012) Effect of high-pressure processing on physical, biochemical, and microbiological characteristics of black tiger shrimp (*Penaeus monodon*). Food Bioprocess Tech. 6 (6): 1390-1400

Botta, J.R., Lauder, J.T. and Jewer, M.A. (1984) Effect of methodology on total volatile basic nitrogen (TVB-N) determination as an index of quality of fresh Atlantic cod (*Gadus morhua*). J Food Sci. 49 (734-736): 750

Brunn, J. L. and Skibsted, L. H. (1996) High-pressure effects on oxidation of nitrosylmyoglobin. Meat Sci. 44: 145-149

Buyukcan, M., Bozoglu, F. and Alpas, H. (2009) Preservation and shelf-life extension of shrimps and clams by high hydrostatic pressure. Int. J. Food Sci. Technol. 44: 1495-1502

Caner, C., Hernandez, R.J., Pascall, M., Balasubramaniam, V.M. and Harte, B.R. (2004) The effect of high-pressure food processing on the sorption behavior of selected packaging materials. Packag. Technol. Sci. 17(3): 139-153

Carlez, A., Borderias, A. J., Dumay, E. and Cheftel, J.C. (1995) High pressure gelation of fish myofibrillar proteins. In: Food Macromolecules and Colloids (Dickinson, E. and Lorient, D., Eds), pp 400-409, Royal Society of Chemistry, Cambridge, U.K

Cheah, P. B. and Ledward, D. A. (1996) High pressure effects on lipid oxidation in minced pork. Meat Sci. 43(2): 123-134

Chong, P. L., Cossins, A.R. and Weber, G. (1983) A differential polarized phase fluorometric study of the effects of high hydrostatic pressure upon the fluidity of cellular membranes. Biochemistry. 22(2): 409-415

Connel, J. J. (1995) Control of fish quality, 4th 4th edn., London: Fishing News Books Limited

Conway, E. J. (1962) Micro-diffusion analysis and volumetric error, Crosby, Lockwood and Son Ltd., London

Cruz-Romero, M.C., Kerry, J.P. and Kelly, A.L. (2008) Fatty acids, volatile compounds and colour changes in high-pressure-treated oysters (*Crassostrea gigas*). Innov. Food Sci. Emerg. Technol. 9: 54-61

Dalgaard, P. (2000) Fresh and lightly preserved seafood. In: Shelf life Evaluation of Foods (Man, C. M. D. and Jones, A. A., Eds), pp 110–139. Maryland: Aspen Publishers

Dalgaard, P., Gram, L. and Huss, H. H. (1993) Spoilage and shelf-life of cod ûllets packed in vacuum or modiûed atmospheres. Int. J. Food Microbiol. 19: 83-94

Ehira, S. (1976) A biochemical study on the freshness of fish. Bulletin of Tokai Regional Fisheries Research Laboratory. 88: 1-132

Erkan, N. and Uretener, G. (2010) The effect of high hydrostatic pressure on the microbiological, chemical and sensory quality of fresh gilthead sea bream (*Sparus aurata*). Eur. Food Res. Technol. 230: 533-542

Esterbauer, H., Schwarzl, E. and Hayn, M. (1977) A rapid assay for catechol oxidase and laccase using 2-nitro-5-thio benzoic acid. Anal. Biochem. 77: 486-494

Finne, G. (1992). Non-protein nitrogen compounds in fish and shellfish. In: Advances in Seafood Biochemistry Composition and Quality (Flick, G. J. and Martin, R. E., Eds), pp: 393-401, Technomic Publishing, Lancaster, Pennsylvania

Flick, G. J. and Lovell, R. T. (1972) Post-mortem biochemical changes in the muscle of gulf shrimp (*Penaeus aztecus*). J. Food Sci. 32: 609-611

Galotto, M. J., Ulloa, P., Escobar, R., Guarda, A., Gavara, R. and Miltz, J. (2010) Effect of high pressure food processing on the mass transfer properties of selected packaging materials. Packag. Technol. Sci. 23(5): 253-266

Gennari, M., Tomaselli, S. and Cotrona, V. (1999) The microflora of fresh and spoiled sardines (*Sardina*

pilchardus) caught in Adriatic (Mediterranean sea) and stored in ice. Food Microbiol. 16: 15-28

Ginson J. (2015) Optimization of High Pressure Processing Parameters for Indian White Prawn (*Fenneropenaeus indicus*) and Its Shelf Life Evaluation during Chilled Storage. Ph.D Thesis. CUSAT

Ginson, J., Kamalakanth, C.K., Bindu, J., Venkateswarlu, R., Das, S., Chauhan, O.P. and Srinivasa, G. (2013) Changes in K-value, microbiological and sensory acceptability of high pressure processed Indian white prawn (*Fenneropenaeus indicus*). Food Bioprocess Tech. 17: 37-42

Gomes, H.A., Silva, E.N., Nascimento, M.R.L. and Fukuma, H.T. (2003) Evaluation of the 2-thiobarbituric acid method for the measurement of lipid oxidation in mechanically deboned gamma irradiated chicken meat. Food Chem. 80(3): 433-437

Gou, J., Lee, H.Y. and Ahn, J. (2010) Effect of high pressure processing on the quality of squid (*Todarodes pacificus*) during refrigerated storage. Food Chem. 119: 471-476

Goulas, A. E. and Kontominas, M. G. (2007) Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): biochemical and sensory attributes. Food Chem. 100: 287-296

Halim, L., Pascall, M.A., Lee, J. and Finnigan, B. (2009) Effect of pasteurization, high pressure processing, and retorting on the barrier properties of Nylon 6, Nylon 6/Ethylene Vinyl Alcohol and Nylon 6/Nanocomposites films. J Food Sci. 74(1): 9-15

Harada, K. (1991) Fish colour. Aust Fish. 50: 18-19

He, H., Adams, R.M., Farkas, D.F. and Morrissey, M.T. (2002) Use of high-pressure processing for oyster shucking and shelf-life extension. J. Food Sci. 67(2): 640-645

Hebard, C.E., Flick, G.J. and Martin, R.E. (1982) Occurrence and Significance of trimethylamine oxide and its derivatives in fish and shellfish. Westport, CT, AVI Publishing, USA

Hernández-Andrés, A., Gómez-Guillén, C., Montero, P. and Pérez-Mateos, M. (2005) Partial characterization of protease activity in squid (*Todaropsis eblanae*) mantle: Modification by high pressure treatment. J. Food Sci. 70(4): 239-245

Hurtado, J. L., Montero, P. and Borderias, A. J. (2000) Extension of shelf life of chilled hake (*Merluccius capensis*) by high pressure. Food Sci Technol Int. 6: 243-249

ICMSF (1986) Microorganisms in foods 2. Sampling for food microbiological analysis: Principle and Specific Applications, 2nd Edition. Toronto, Canada: University of Toronto Press

Igene, J.O., King, J.A., Pearson, A.M. and Gray, J.I. (1979) Influence of heme pigments, nitrite and non-heme iron on development of warmed over flavour in cooked meat. J. Agric. Food Chem. 27: 838-842

IS: 2508, (1984) Specification for low-density polyethylene film. (2nd revision), pp 15, Indian Standards Institution, New Delhi

IS: 9845, (1998) Determination of overall migration of constituents of plastics materials and articles intended to come in contact with foodstuffs –methods of analysis, New Delhi, India

Kadam, P.S., Jadhav, B.A., Salve, R.V. and Machewad, G.M. (2012) Review on the high pressure technology (HPT) for food preservation. J. Food Process. Technol. 3(1): 2-5

Ko, W.C. and Hsue, K.C. (2002) Effect of high-pressure storage on the processing quality of tilapia meat. Proceedings International on Trends in High Pressure Bioscience and Biotechnology. 19: 411-416

Lakshmanan, R., Miskin, D. and Piggot, J.R. (2005) Quality of vacuum packed cold-smoked salmon during refrigerated storage as affected by high-pressure processing. J. Sci. Food Agric. 85: 655-661

Lambert, Y., Demazeau, G., Largeteau, A., Bouvier, J.M., Laborde- Croubit, S. and Cabannes, M. (2000) Packaging for high-pressure treatments in the food industry. Packag. Technol. Sci. 13(2): 63-71

Linton, M., Mc Clement, J. M. J. and Patterson, M. F. (2003) Changes in the microbiological quality of shellfish, brought about by treatment with high hydrostatic pressure. Int. J. Food Sci. Technol. 38: 713-727

López-Caballero, M.E., Pérez-Mateos, M., Borderías, J.A. and Montero, P. (2000) Extension of the shelf life of prawns (*Penaeus japonicus*) by vacuum packaging and high pressure treatment. J. Food Protect. 63(10): 1381-1388

Lopez-Galvez, D., Hoz. L. and Ordonez, J.A. (1995) Effect of carbon dioxide and oxygen enriched atmospheres on microbiological and chemical changes in refrigerated tuna (*Thunnus alalunga*) streaks. J. Agric. Food Chem. 43: 483-490

Masuda, M., Saito, Y., Iwanami, T. and Hirai, Y. (1992) Effect of hydrostatic pressure on packaging materials for food. In: High Pressure and Biotechnology (Balny, C., Hayashi, R., Heremans, K. and Masson, P., Eds), pp:545–548, Inserm/John Libbey Eurotex, Montrouge

Meilgaard, M., Civille, G. V. and Carr, B.T. (1999) Sensory Evaluation Technique, 3rd edn., 387p, Boca Raton: CRC

Montero, P., Lopez-Caballero, M. E. and Perez-Mateos, M. (2001) The effect of inhibitors and high pressure treatment to prevent melanosis and microbial growth

on chilled prawns (*Penaeus japonicus*). J. Food Sci. 66(8): 1201-1206

Montgomery, W. A., Sidhu, G.S. and Vale, G. L. (1970) The Australian prawn industry, 1. Natural resources and quality aspects of whole cooked fresh prawns and frozen prawn meat. CSIRO Food Preservation Quarterly. 30(2): 21

Ochiai, S. and Nakagawa, Y. (1992) Packaging of high pressure food processing. In: High pressure and biotechnology (Balny, C., Hayashi, R., Heremans, K. and Masson, P., Eds), pp 515-519. Colloque INSERM/ John Libberry: London

Ohshima, T., Nakagawa, T. and Koizumi, C. (1992) Effect of high pressure on the enzymatic degradation of phospholipids in fish muscle during storage. In: Seafood Science and Technology (Bligh, E., Ed), pp:64-75. Fishing News Books, Oxford

Ozen, B. F. and Floros, J. D. (2001) Effects of emerging food processing techniques on the packaging materials. Trends Food Sci. Technol. 12: 60-67

Pastorelli, S. (1997) Ital. Imbaliaggio. 9: 98-101

Ramirez-Suarez, J. C. and Morrissey, M.T. (2006) Effect of high pressure processing (HPP) on shelf life of albacore tuna (*Thunnus alalunga*) minced muscle. Innov. Food Sci. Emerg. Technol. 7: 19-27

Ryder, J.M. (1985) Determination of adenine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography. J. Agric. Food Chem. 3: 673

Shah, A.J. (1991) Extrusion cooking of corn grits. Ph.D Thesis, School of food and fisheries studies, Humberside Polytechnic, U.K

Sikorski, Z. E. and Borderias, J.,A. (1994) Collagen in the muscles and skin of marine animals. In: Seafood Proteins (Sikorski, Z. E., Pan, B.,S. and Shahidi, F., Eds), pp:58-70. Chapman and Hall, New York

Simidu, W. (1962) Non-protein nitrogenous compounds. In: Fish as Food (Borgstorm, G. Ed), pp:353-375, Academic press, New York

Syderhelm, I., Boguslawski, S., Michaelis, G. and Knorr, D. (1996) Pressure induced inactivation of selected food enzymes. J Food Sci. 61: 308-310

Tarladgis, G. B., Watts, M. B. and Younathan, T. M. (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44-50

Varlik, C., Baygar, T., Özden, Ö., Erkan, N. and Metin, S. (2000) Sensory evaluation and determination of some physical and chemical characteristics of shrimp during gold storage. Turk. J. Vet. Anim. Sci. 24: 181-185

Whittle, K., Hardy, R. and Hobbs, G. (1990) Chilled fish and fishery products. In: Chilled Foods (Gormley, T., Ed), pp 87-116, The State of the Art. Elsevier, New York

Yamamoto, K., Yoshida, Y., Morita, J. and Yasui, T. (1994) Morphological and physicochemical changes in the myosin molecules induced by hydrostatic pressure. J Biochem. 116: 215-220