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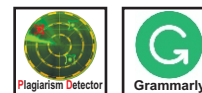
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Effect of different rearing conditions on the shelf-life of Pacific white shrimp (*Litopenaeus vannamei*) during ice storage

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Abstract

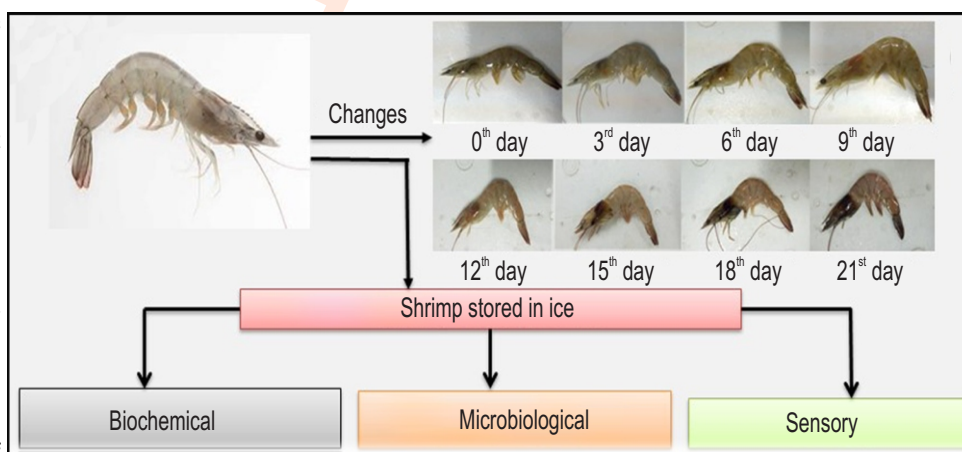
Aim: The present study aimed to investigate the shelf life of Pacific white shrimp (*Litopenaeus vannamei*) reared in inland saline water (ISRV) with those reared in natural brackish water (BWRV) during ice storage.

Methodology: Freshly harvested *L. vannamei* shrimp cultured in inland saline water and brackish water were collected and biochemical parameters, microbial analysis and sensory parameters were evaluated for 21 days during ice storage with sampling interval of 3 days.

Results: Total volatile basic nitrogen content increased up to 9th day in shrimps reared in BWRV and up to 3rd day in ISRV reared shrimps. Similarly, tri methyl amine content increased up to 3rd day for ISRV, there after the values decreased. The values of lipid oxidation such as peroxide value, free fatty acid and thiobarbituric acid reactive substance increased. Sensory scores for ice stored BWRV and ISRV showed a decreasing trend with increasing storage period.

Interpretation: On the basis of microbiological parameters, pacific white shrimp (*Litopenaeus vannamei*) reared in brackish water and inland saline water can be ice-stored up to 12 days in fresh conditions, while other biochemical and sensory parameters are acceptable up to 18 days.

Key words: Brackish water, Inland saline aquaculture, Ice storage, Sensory analysis, Shelf life, *Vannamei* shrimp



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Introduction

Shrimp culture is one of the most profitable culture ventures in different regions of the world, including India. Saline groundwater is the most potent available resource for increasing aquaculture production in India and many countries like USA, China, Vietnam, Thailand, etc. (Javith *et al.*, 2020). Saline groundwater may be present in regions with high rainfall, as a result of connate water of marine origin, underground salt deposits and saltwater intrusion in coastal areas (Boyd *et al.*, 2009). Inland saline water resources are not appropriately used resources in the world so there is an existing need to develop a technology for using these resources in food production (Kumar *et al.*, 2016). In India, salinization of inland waters, particularly in the North-west region is increasing at a startling rate due to both natural and anthropogenic activities (Dhawan *et al.*, 2010) causing serious threat to agriculture. Increasing inland salinity in the country has major economic, social and environmental consequences; threatening the viability of numerous rural communities (Chand *et al.*, 2015). India has tremendous potential to increase the shrimp production by utilizing the intact resources for commercial aquaculture. In India, around 8.62 million ha of agricultural land has been critically affected due to soil salinity and around 40% of total inland saline soils are located in the states of Haryana, Uttar Pradesh, Punjab and Rajasthan (Lakra *et al.*, 2014; Javith *et al.*, 2020).

The inland saline soils are not suitable for agriculture and often considered as barren land. These lands have been converted into ponds for aquaculture practices and different salt tolerant freshwater and brackish water species have been standardized as good candidate species for inland saline aquaculture (Singh *et al.*, 2019; Iffat *et al.*, 2020). Among those Pacific white shrimp (*Litopenaeus vannamei*) is the most commonly reared aquaculture species around the western hemisphere that can be grown at a salinity range of 1 to 40 ppt (Bray *et al.*, 1994). It grows well in inland saline waters having a salinity range between 0.5 to 28.3 ppt (Samocha *et al.*, 2002). The remarkable ability of this hardy species to grow in less ideal environments has made this species a suitable candidate for culturing in inland saline water. Presently, *L. vannamei*, are reared in low-saline inland waters of Alabama, Arizona, Florida, Indiana, Illinois and Texas both experimentally and commercially (Samocha *et al.*, 2002).

In addition, Ecuador and Thailand, also produce marine shrimp commercially in low-saline waters. More than 30% of shrimp culture in Thailand is contributed alone by inland low-saline waters (Saoud *et al.*, 2003). Similar to other food products, spoilage in shrimps is a major concern for shrimp farmers and shrimp processing industry (Don *et al.*, 2018). Icing is considered as the best choice for preservation and quality maintenance of shrimps unlike other processing methods such as freezing, drying and canning (Bhat *et al.*, 2017). Iced shrimp during storage undergoes many quality changes, which generally occur due to the combined action of enzymes from tissues and microbial

contamination (Flores and Crawford, 1973). Studies on the quality of seafood have entailed the quantification of total volatile basic nitrogen, Tri Methyl Amine, microorganisms and lipid oxidation products including peroxide value, free fatty acid and Thiobarbituric Acid Reactive Substances (TBARS) value. Apart from this, pH also influences the quality of meat which indicates increased bacterial action (Okeyo *et al.*, 2009).

The physico-chemical and sensory parameters also play a major role in deciding the acceptability of the product. Seafood industries at present utilise odour and visual observation as criterion for shrimp quality evaluation (Iyengar *et al.*, 1960). It was hypothesized that the compromised environment like inland saline water affects the shelf life of shrimp. As there is plenty of salt affected soil in the interior parts of India which is lying idle at present and can be used for rearing salt tolerant shrimp, which can be a profitable venture for shrimp farmers. However, before recommending this, it is important to study the quality changes and shelf life during storage of shrimp meat as effect of salinity. Nevertheless, there is no information or record available about the same. Therefore, the present work was designed to assess the meat stability of *Litopenaeus vannamei* reared in inland saline waters under ice conditions.

Materials and Methods

Collection of sample: Freshly harvested Pacific white shrimp (*L. vannamei*) cultured in brackish water having 24 ppt salinity were collected from Valsad district of Gujarat. Shrimp was immediately iced in a plastic polystyrene insulated container with a shrimp: ice ratio of 1:1 (w/w) and brought to the laboratory within 4 hrs from Valsad. Freshly harvested *L. vannamei* reared in inland saline water with 14 ppt salinity were collected from Rohtak district of Haryana, India and transported in iced condition to the laboratory within 5 hrs. The average count of both shrimp samples was 40-50 per kg. Shrimp samples were cleaned thoroughly with chilled potable water and stored in direct contact with ice in the ratio of 1:1 (Shrimp: Ice). The ice was changed daily and sensory, biochemical, microbiological analysis were carried out at an interval of 3 days.

Estimation of pH and non-protein nitrogen: Shrimp muscle (10 g) was mixed with distilled water and the mixture was homogenised for 30 sec with a homogenizer (Polytron system PT 2100, Germany). Digital pH meter (Eutech tutor pH/°C meter, Eutech Instruments, Singapore) standardized with buffers (pH 4 and 7) was used for measuring the pH of shrimp homogenate. Non-protein nitrogen (NPN) content was estimated as per AOAC methods (2019).

Estimation of protein degradation products: TVB-N and TMA-N were estimated following the method of EU/EC (2008). Approximately, 10 g of shrimp muscle was homogenised with 7.5% trichloroacetic acid and filtered using Whatman no.1 filter paper until 100 ml of aliquot was collected. A 25 ml aliquot was taken and mixed with 6 ml of 10% NaOH. For TMA-N estimation,

20 ml of 35% (v/v) formaldehyde was used. For estimating TVB and TMA-N, the mixture was poured into distillation tube for steam distillation. The distillate was collected in a beaker containing Tashiro's indicator in 15 ml of 4% boric acid to a final volume of 100 ml. The collected distillate was titrated against 0.025 N H₂SO₄ until the colour of the solution changed to pale pink. TVB-N and TMA-N levels were calculated and expressed in mg N 100 g⁻¹ of shrimp flesh sample.

Estimation of lipid oxidation products: The peroxide value was determined following the method of Kirk and Sawyer (1991). Approximately, 10 g of shrimp muscle mixed in 15 g of anhydrous sodium sulphate was homogenised with chloroform and filtered through Whatman no. 1 filter paper. A 10 ml of chloroform extract was taken in iodine flask and 15 ml of glacial acetic acid was added to it. The homogenate was vigorously shaken for 30 sec and after adding 1 ml of fresh saturated potassium iodide solution, the mixture was kept in dark condition for 30 min. In order to release the iodine, 50 ml of distilled water was added to the mixture and titrated against 0.01 N sodium thiosulphate solution. Peroxide value (PV) was calculated and expressed as meq O₂ kg⁻¹ lipids. For free fatty acid (FFA) estimation, chloroform extract was taken in a clean pre-weighed beaker and kept in a hot air oven maintained at 50°C until chloroform evaporated. A 10 ml of neutral alcohol was added to the remaining fat and titrated against 0.01 N NaOH (AOAC, 2005). Thiobarbituric acid reactive substances (TBARS) content was measured spectrophotometrically by the method of Tarladgis et al. (1960).

Microbiological analysis: Total viable count (TVC) of fresh and ice stored shrimp muscle was determined by the method described in BAM (2004). Approximately, 10g of shrimp meat was taken aseptically and homogenized in 90 ml of 0.85% physiological saline. Serial tenfold dilutions of homogenate were

made for inoculation and 0.1ml of sample from each dilution was spread over the sterile agar petriplates and incubated at 35±2°C for 24 hrs. Bacterial colonies developed after incubation were enumerated manually and expressed as log CFU g⁻¹.

Sensory evaluation: The sensory evaluation for overall acceptability of shrimp sample was done by non trained panel members which included staff and students from the Post-harvest Technology Department, CIFE (n=30) using 9 point hedonic scales with 1 being the lowest and 9 being the highest score.

Statistical analysis: Statistical package of SPSS 16.0 (SPSS, 2000) was used for analyzing the experimental results. Duncan's Multiple Range Test was used for Post hoc comparison to assess statistical significance (P<0.05) between the triplicates and the results were expressed as mean ± S.D.

Results and Discussion

The pH and Non-protein nitrogen (NPN) value of ice stored *Litopenaeus vannamei* reared in different system are given in Table 1. pH is a vital index for determining the quality of seafood. The pH of fresh brackish water reared *vannamei* and Inland saline reared *vannamei* was 6.14 and 6.65, respectively. pH of the samples increased significantly (P<0.05) with the increase in storage period and reached a maximum of 7.21 and 7.80 in BWRV and ISRV samples, respectively, at the end of storage. This increasing trend in pH of shrimp may be due to the accumulation of basic compounds such as TVB-N and TMA due to bacterial activity or enzymatic actions. Generally, the shellfish products are suitable for consumption when the pH values range between 7 and 8 (Büyükcan et al., 2009). Non-protein nitrogen contributes significantly to the unique taste of seafood as well as to its spoilage. In the present study, Fresh BWRV and ISRV had a

Table 1: Effect of different rearing conditions on biochemical quality parameters of *Litopenaeus vannamei* during ice storage

Parameters	Samples	0 th day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day	21 st day
pH	BWRV	6.14±0.02 ^{aa}	6.53±0.06 ^{ba}	6.67±0.14 ^{ba}	7.26±0.13 ^{ca}	7.52±0.23 ^{da}	7.38±0.05 ^{cdA}	7.36±0.10 ^{cdA}	7.21±0.10 ^{ca}
	SRV	6.65±0.04 ^{ab}	7.43±0.01 ^{ab}	7.65±0.03 ^{bb}	7.73±0.08 ^{bb}	7.77±0.07 ^{ba}	7.78±0.03 ^{bb}	7.99±0.18 ^{cb}	7.80±0.04 ^{bb}
NPN	BWRV	0.18±0.00 ^{aa}	0.39±0.05 ^{ca}	0.41±0.05 ^{cdA}	0.45±0.04 ^{cdA}	0.46±0.05 ^{cdA}	0.28±0.02 ^{ba}	0.25±0.00 ^{ba}	0.15±0.01 ^{aa}
	ISRV	0.39±0.00 ^{bb}	0.47±0.02 ^{bb}	0.39±0.00 ^{ba}	0.30±0.02 ^{cb}	0.28±0.01 ^{cb}	0.21±0.00 ^{eb}	0.20±0.01 ^{eb}	0.14±0.01 ^{fa}
TVB-N	BWRV	11.43±1.07 ^{abcA}	12.60±1.40 ^{bcdA}	13.53±0.40 ^{deA}	14.47±0.81 ^{eA}	13.07±0.81 ^{cdB}	11.20±1.21 ^{abA}	10.03±0.40 ^{abA}	10.03±0.81 ^{abA}
	ISRV	21.00±1.40 ^{ab}	22.45±1.47 ^{ab}	21.00±1.40 ^{ab}	18.57±0.32 ^{bb}	16.10±0.70 ^{cb}	15.45±1.47 ^{cb}	15.63±1.73 ^{cb}	17.50±0.70 ^{bcB}
TMA	BWRV	ND	ND	ND	ND	ND	ND	2.24±0.14 ^{ba}	4.20±0.7 ^{ca}
	ISRV	5.60±0.7 ^{bb}	7.51±0.84 ^{ab}	5.60±1.40 ^{bb}	5.51±0.16 ^{bb}	4.90±0.70 ^{bcb}	4.81±0.16 ^{cb}	3.87±0.35 ^{cdB}	3.13±0.35 ^{db}
PV	BWRV	0.12±0.03 ^{aa}	0.16±0.02 ^{ba}	0.17±0.06 ^{ba}	0.30±0.00 ^{ba}	0.37±0.06 ^{bca}	0.40±0.09 ^{ca}	0.45±0.09 ^{ca}	0.57±0.03 ^{da}
	ISRV	0.12±0.02 ^{aa}	0.14±0.02 ^{abA}	0.16±0.01 ^{abA}	0.19±0.04 ^{bcB}	0.22±0.03 ^{cdB}	0.28±0.03 ^{db}	0.25±0.05 ^{db}	0.40±0.05 ^{eb}
FFA	BWRV	0.002±0.00 ^{aa}	0.003±0.00 ^{aa}	0.004±0.01 ^{abA}	0.011±0.00 ^{ba}	0.015±0.00 ^{ba}	0.026±0.01 ^{ca}	0.038±0.00 ^{da}	0.040±0.00 ^{da}
	ISRV	0.002±0.00 ^{aa}	0.005±0.00 ^{ba}	0.006±0.00 ^{ba}	0.007±0.00 ^{bb}	0.010±0.00 ^{ca}	0.012±0.00 ^{bb}	0.015±0.00 ^{db}	0.016±0.00 ^{db}
TBARS	BWRV	0.08±0.01 ^{aa}	0.11±0.02 ^{abA}	0.14±0.01 ^{bcA}	0.16±0.04 ^{ca}	0.17±0.04 ^{ca}	0.18±0.04 ^{ca}	0.18±0.02 ^{ca}	0.19±0.02 ^{ca}
	ISRV	0.05±0.00 ^{ab}	0.10±0.01 ^{ba}	0.13±0.03 ^{ca}	0.15±0.01 ^{cdA}	0.16±0.00 ^{deA}	0.17±0.00 ^{deA}	0.18±0.00 ^{ea}	0.19±0.00 ^{ea}

BWRV- Brackish water reared *vannamei*; ISRV-Inland saline reared *vannamei*; Data expressed as mean±SD (n=3), the mean value in the same row with different superscripts are significantly different (P<0.05). The mean value in the same column with different capital letters superscripts are significantly different (P<0.05)

Table 2: Effect of different rearing conditions on total viable count of *Litopenaeus vannamei* during ice storage

	Samples	0 th day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day	21 st day
Total viable count (log CFU g ⁻¹)	BWRV	4.62	4.57	5.32	5.43	6.50	7.80	8.85	NA
	ISRV	4.51	4.32	5.40	5.49	6.60	7.82	8.92	NA

BWRV- Brackish water reared *vannamei*; ISRV-Inland saline reared *vannamei*; NA-Not analyzed

Table 3: Effect of different rearing conditions on sensory score of *Litopenaeus vannamei* during ice storage

Parameters	Samples	0 th day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day	21 st day
Appearance	BWRV	9.00±0.00 ^{aA}	8.44±0.48 ^{aA}	7.67±0.52 ^{bA}	7.00±0.00 ^{cA}	6.50±0.53 ^{cdA}	6.33±0.52 ^{dA}	4.83±0.76 ^{eA}	3.67±0.58 ^{fA}
	ISRV	9.00±0.00 ^{aA}	7.88±0.35 ^{bB}	7.25±0.46 ^{cAbA}	7.20±0.45 ^{abA}	7.00±0.76 ^{bA}	5.86±0.90 ^{cA}	4.40±0.49 ^{dA}	4.00±0.61 ^{dA}
Shell colour	BWRV	8.96±0.10 ^{aA}	8.46±0.50 ^{abA}	7.92±0.66 ^{bcA}	7.20±0.45 ^{cdA}	6.56±0.82 ^{deA}	6.33±0.52 ^{EA}	5.83±0.29 ^{EA}	4.67±0.58 ^{fA}
	ISRV	9.00±0.00 ^{aA}	8.00±0.53 ^{bA}	7.19±0.37 ^{bB}	7.00±0.71 ^{cA}	6.88±0.64 ^{cA}	5.14±0.90 ^{dB}	4.00±0.63 ^{EB}	3.40±0.42 ^{EB}
Meat colour	BWRV	8.83±0.40 ^{aA}	8.43±0.71 ^{abA}	7.79±0.40 ^{bA}	7.00±0.71 ^{cA}	6.94±0.78 ^{cA}	6.92±0.2 ^{cA}	5.50±0.50 ^{dA}	5.00±0.58 ^{dA}
	ISRV	8.90±0.22 ^{aA}	8.00±0.00 ^{bA}	7.38±0.52 ^{bcA}	7.20±0.45 ^{cA}	6.88±0.83 ^{cA}	5.71±0.95 ^{dB}	4.20±0.75 ^{EB}	3.80±0.45 ^{EB}
Odour	BWRV	9.00±0.00 ^{aA}	8.50±0.46 ^{aA}	7.75±0.42 ^{bA}	7.40±0.55 ^{bA}	7.13±0.64 ^{bA}	7.08±0.49 ^{bA}	6.17±0.29 ^{cA}	4.67±0.58 ^{dA}
	ISRV	9.00±0.00 ^{aA}	8.13±0.64 ^{bA}	7.38±0.52 ^{bcA}	7.10±0.55 ^{ca}	6.88±0.64 ^{cA}	5.29±0.95 ^{dB}	4.80±0.68 ^{deB}	4.20±0.76 ^{eA}
Texture	BWRV	9.00±0.00 ^{aA}	8.44±0.50 ^{aA}	7.42±0.79 ^{bA}	7.00±0.00 ^{bcA}	6.63±0.92 ^{cA}	6.42±0.49 ^{cA}	5.17±0.29 ^{dA}	4.33±0.58 ^{eA}
	ISRV	9.00±0.00 ^{aA}	8.13±0.35 ^{bA}	7.63±0.52 ^{bcA}	7.00±0.71 ^{cdA}	6.75±0.46 ^{dA}	5.43±0.98 ^{EB}	4.80±0.75 ^{EA}	4.00±0.35 ^{EA}
Taste	BWRV	9.00±0.00 ^{aA}	8.50±0.46 ^{aA}	7.52±0.65 ^{cA}	7.10±0.55 ^{dA}	6.50±0.53 ^{EA}	5.86±0.90 ^{EA}	4.40±0.49 ^{GA}	NA
	ISRV	8.86±0.22 ^{aA}	8.43±0.71 ^{bA}	7.19±0.37 ^{bB}	6.56±0.82 ^{EB}	5.71±0.95 ^{EB}	5.14±0.90 ^{EB}	4.00±0.63 ^{GA}	NA
Flavour	BWRV	9.00±0.00 ^{aA}	8.70±0.42 ^{bA}	7.92±0.66 ^{ca}	7.20±0.45 ^{dA}	6.88±0.64 ^{EA}	6.33±0.52 ^{EA}	5.50±0.50 ^{GA}	NA
	ISRV	8.86±0.22 ^{aA}	8.51±0.35 ^{bA}	7.38±0.52 ^{cb}	7.00±0.71 ^{dA}	6.33±0.52 ^{EB}	5.71±0.95 ^{EB}	5.00±0.58 ^{GA}	NA
Overall acceptability	BWRV	9.00±0.00 ^{aA}	8.41±0.46 ^{aA}	7.79±0.40 ^{bA}	7.00±0.00 ^{cA}	6.75±0.71 ^{cA}	6.67±0.52 ^{cA}	5.50±0.50 ^{dA}	4.33±0.58 ^{eA}
	ISRV	9.00±0.00 ^{aA}	8.00±0.00 ^{bA}	7.31±0.46 ^{ca}	7.20±0.45 ^{cA}	6.81±0.37 ^{ca}	5.71±0.86 ^{dB}	4.90±0.49 ^{EA}	3.30±0.45 ^{EB}

BWRV- Brackish water reared *vannamei*; ISRV-Inland saline reared *vannamei*; Data expressed as mean± SD (n=30), the mean value in the same row with different superscripts are significantly different (p<0.05). The mean value in the same column with different capital letters superscripts are significantly different (p<0.05). NA-Not analysed

NPN content of 0.18 and 0.39, respectively. The NPN content of BWRV sample increased up to 12th day (0.46%), and later reduced till the end of the study (0.15%). Whereas, in ISRV, increase in NPN content was observed up to 3rd day (0.47%), after that it showed a decreasing trend up to final day of storage (0.14%). Hydrolysis of protein and other nitrogenous compounds by autolytic enzyme together with bacterial action may lead to an increase in the NPN content during initial days of storage (Yeasmin *et al.*, 2010). Later decrease in NPN content could be attributed to leaching of soluble components when the fish muscle tissue lost its non-permeable nature and started absorbing water from the melting ice (Annamalai *et al.*, 2015). Bhat *et al.* (2018) also found a decrease in NPN contents of *Litopenaeus vannamei* ranging from 0.72% to 0.47% during ice storage. TVB-N and TMA are the most valid biochemical indices used for the assessment of freshness and quality of any seafood product and is related to the activity of spoilage bacteria and endogenous enzymes (Zhang *et al.*, 2015).

The initial TVB-N values of BWRV and ISRV were 11.43 and 21.00 mg N 100 g⁻¹, respectively (Table 1). During ice storage, the TVB-N value of BWRV sample increased up to 14.47 mg N 100 g⁻¹ on 9th day of storage and after that, the value

started decreasing and reached 10.03 mg N 100 g⁻¹ at the end of storage. Similarly, the ISRV sample also increased up to 22.45 mg N 100 g⁻¹ on 3rd day of storage, after that the value started decreasing and reached 17.50 mg N 100 g⁻¹ at the end of storage. The increase of TVB-N in initial days of storage could be related to the activity of spoilage bacteria and endogenous enzymes. The decrease of TVB-N after a certain storage period may be due to leaching of volatile nitrogen materials by ice meltwater (Magunsson and Martinsdottir, 1995). The contact of shrimp with flake ice can result in a loss of volatile compounds, particularly, ammonia that is highly water-soluble. The leaching of volatile compounds can be significant in small fish and shrimp as well as in scallops (Etienne, 2005).

TMA values were not detected in BWRV up to 15th day then the value increased and reached a maximum (4.2 mg N 100 g⁻¹) on the final day of storage (Table 1). In the case of ISRV sample, the TMA value increased up to 3rd day, followed by decrease up to end of storage. Absence of TMA in BWRV sample during initial days of storage may be due to less initial concentration of TMAO. The initial concentration of TMAO depends on the type of species, the origin of species, season, fishing ground and depth of living (Etienne, 2005). The increasing

TMA content is related to the reduction of TMAO by bacterial action. A study by Annamalai *et al.* (2015) showed that the TMA value of *L. vannamei* sample was nil up to third day and thereafter showed 2.70 mg N 100 g⁻¹ during 6th day and it increased to 10.90 mg N 100 g⁻¹ during 12th day of ice storage. In ISRV sample, the reduction of TMA after 3rd day of storage could be attributed to leaching out of volatile amines into melted water (Etienne, 2005). Annamalai *et al.* (2015) and Connell (1995) suggested that 10–15 mg TMA-N 100 g⁻¹ was fit for human consumption. The peroxide value of BWRV and ISRV gradually increased up to 0.57 meq O₂ kg⁻¹ and 0.40 meq O₂ kg⁻¹ of fat, respectively, at the end of ice storage. Oxidation of fatty acids present in the shrimp muscles during storage results in the formation of either hydro peroxide or peroxide that leads increase in peroxide value (Okpala *et al.*, 2014). However, there was only a slight increase in peroxide value during the entire storage period of both the samples in the present study. This could be correlated to the odour parameter from sensory analysis during ice storage. Farajzadeh *et al.* (2016) reported that the initial peroxide value value of *L. vannamei* was 0.15 meq of O₂ kg⁻¹ and it increased up to 2.1 meq of O₂ kg⁻¹ on 8th day under refrigerated storage and then dropped to 1.59 meq of O₂ kg⁻¹ on 12th day of storage. The limit of acceptability of peroxide value is 8-10 meq of O₂ kg⁻¹ (Okpala *et al.*, 2014).

The Free fatty acids value of BWRV and ISRV showed an increasing trend and reached maximum value of 0.040 % oleic acid and 0.016 % oleic acid, respectively, on 21st day (Table 1). Psychrotrophic bacteria, mainly *Pseudomonas* species, produce lipase and phospholipase enzymes that increase free fatty acids, which are highly susceptible to oxidation and form unstable lipid hydroperoxide (Nirmal and Benjakul, 2011). Kaur *et al.* (2013) also reported increased Free fatty acids of black tiger shrimp (*Penaeus monodon*) during chilled storage. Hydroperoxide formed during lipid oxidation is highly unstable and decomposes readily to shorter chain hydrocarbons such as aldehydes; these final products can be detected as TBARS (Nirmal and Benjakul, 2009). The TBARS value of both BWRV and ISRV sample gradually increased up to 0.19 mg MDA kg⁻¹ fat at the end of storage (Table 1). The increasing trend of TBARS value of *L. vannamei* during iced storage was observed by Farajzadeh *et al.* (2016) who found gradual increase in TBARS value from 0.03 to 1.12 mg MDA kg⁻¹. Nirmal and Benjakul (2009) observed the TBARS value of fresh Pacific white shrimp as 0.65 mg MDA kg⁻¹ that continuously increased up to 1.5 mg MDA kg⁻¹ on 10th day of ice storage.

Total viable count of BWRV and ISRV sample during ice storage showed wide changes (Table 2). The initial total viable count of BWRV and ISRV sample was 4.62 log CFU g⁻¹ and 4.51 log CFU g⁻¹ decreased on 3rd day (4.51 and 4.32 log CFU g⁻¹) and that later, a continuous increase was observed and reached maximum of 8.85 and 8.92 log CFU g⁻¹, respectively, for BWRV and ISRV sample on 18th day of storage. The initial decrease of microflora at early stage of storage may be due to the effect of low temperature and the increase at later stages may be due to

microbial tolerance towards cold conditions (Nirmal and Benjakul, 2009). This result seems to be in agreement with the previous observation of Senapati *et al.* (2017) who found that total viable count of fresh *L. vannamei* reduced slightly on 2nd day (3.29 log CFU g⁻¹) and later, a continuous increase was observed till the end of storage. Okpala *et al.* (2014) reported an increase in aerobic plate count of Pacific white shrimps during ice storage.

For both BWRV and ISRV, the bacterial total viable count exceeded the maximal permissible limit of 7.0 log CFU g⁻¹ in fish (ICMSF, 1986) on the 15th day of storage. If the APC value exceeded the critical limit of spoilage, the shrimp samples could lose its consumer acceptability level as well as its value in the global market. Table 3 shows the results of all the sensory parameters (appearance, colour of the shell and meat, odour, taste, flavour, texture and overall acceptability) tested for BWRV and ISRV sample during ice storage based on 9 points hedonic scale. Storage period significantly ($P < 0.05$) affected all the parameters. The scoring for all the attributes during 0th day sampling was excellent, but as time progress, the quality of products reduced and reached an unacceptable level on 18th day for both BWRV and ISRV samples. Farajzadeh *et al.* (2016) found that shrimp was not preferred and evident off odour was reported on the 8th day of storage. Senapati *et al.* (2017) reported the reducing sensory score of regularly farmed *L. vannamei* during ice storage. Thus, from this study it is concluded that *Litopenaeus vannamei* reared in brackish water and inland saline water stored in ice condition up to 12 days are safe for consumption. Further, it is concluded that rearing of shrimp in inland saline water does not affect its shelf life; hence, culturing *Litopenaeus vannamei* in inland saline water can be recommended to the farmers having salt-affected land for enhancing their socio-economic status.

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Add-on Information

Authors' contribution: S.M.A. Javith: Experiment execution and manuscript preparation; K.A.M. Xavier: Monitoring and manuscript editing; B.B. Nayak: Experiment planning and manuscript corrections; H.S. Kumar: Monitoring of microbiological analysis and manuscript corrections; V. Harikrishna: Providing samples for the study; A.K. Balange: Designing of experiment, supervision and manuscript editing; G. Krishna: Project administration.

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