



Comparative Quality Evaluation of Frozen Stored *Litopenaeus vannamei* Reared in Inland Saline Water and Brackish Water

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Abstract

The present study was aimed to investigate the quality changes of Pacific white shrimp (*Litopenaeus vannamei*) reared in inland saline water (ISRV) with those reared in natural brackish water (BWRV) during frozen storage. Freshly harvested *L. vannamei* shrimp cultured in inland saline water and brackish water were collected and biochemical, microbial and Sensory quality parameters were evaluated for 5 months during frozen storage with sampling interval of 30 days. From the biochemical quality analysis, it was observed that pH, TVB-N and TMA values increased in both BWRV and ISRV samples. The total viable count of both the samples decreased initially and in the later stage it increased and reached the level of 4.52 log CFU/g in BWRV sample and 4.48 log CFU/g in ISRV sample. However, these values were within the acceptable limit of TVC. Sensory scores were within the acceptable limit for BWRV and ISRV samples during frozen storage of 5 months.

Keywords: Inland saline water, *Litopenaeus vannamei*, Frozen storage, Shelf life

Introduction

Pacific white shrimp (*Litopenaus vannamei*) is an important shrimp aquaculture species traded all over the world representing more than 90 % of the shrimp production (Senapati et al., 2017). It has become a popular species throughout the world due to its high nutrient content, which includes amino

acids, peptides, polyunsaturated fatty acids and other useful substances (Zhang et al., 2015). The preference of this species during the past years is also due to its increased productivity, relatively disease-free nature, higher yield in processing, persistent demand and high prices in the market (MPEDA, 2020). *L. vannamei* has an ability to grow under saline environment, due to which it has become a candidate species for inland saline aquaculture. In India, inland saline aquaculture has been identified as a high-priority research area in order to utilize the available resources, which cannot be otherwise used for agriculture due to salinity (Singh et al., 2017).

Shrimps are mainly processed into frozen form in India, which are primarily exported to USA, Europe and South East Asia. The high content of free amino acids and other soluble non-nitrogenous substances partly contribute to the desirable sweet taste of shrimp. Freezing is an effective way of long term preservation, which helps in keeping the product stable up to a minimum of six months under ideal conditions without any distinguishing changes from fresh products with respect to color, taste and texture. Frozen shrimp is a product of high commercial value and has huge demand in the export market due to its competitive price and extended shelf life. After the death of shrimp, a series of biochemical reactions starts, which is of paramount importance for the quality and shelf life of products. These reactions depend on several different factors; the type of fish species, physiological condition of the fish as well as environmental influences (water temperature and salinity). In addition, catching and harvesting methods and killing procedures have a great effect on the biochemical reactions related to disintegration of the

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fish muscles (Oehlschlager & Rehbein, 2009). The most important quality changes occur during storage of frozen shrimp are colour fading, lipid oxidation, denaturation of protein, off-flavour development, rancidity, dehydration, weight loss, loss of juiciness, drip loss, textural changes and increase in volatile basic nitrogen.

A previous study indicated that meat quality of *L. vannamei* was not affected when they were cultured in inland saline water (Javith et al., 2020). In addition, *L. vannamei* reared in inland saline water, when stored in iced condition, was safe up to 12 days for consumption (Javith et al., 2021). However, in export point of view, it is important to know the quality of inland saline water reared *L. vannamei* during frozen storage. Therefore, frozen storage study for 5 months was carried out to compare the shelf life of BWRV and ISRV.

Materials and Methods

Freshly harvested Pacific white shrimp (*L. vannamei*) cultured in brackish water (BWRV) and inland saline water (ISRV) with the salinity of 24 ppt and 14 ppt were collected and immediately iced in a plastic polystyrene insulated container with a shrimp:ice ratio of 1:1 (w/w) and brought to the laboratory. The average count of both the shrimp samples were 40-50/kg. Upon arrival at the laboratory, shrimp was washed with chilled water to remove excess salt from the surface and the head was removed. The headless shrimp was frozen by plate freezer at -40 °C for 90 minutes. After freezing, the material was stored at -18 °C. Frozen storage study was carried out up to 5 months with sampling interval of one month. The samples were subjected to physical, biochemical, microbial and sensory quality evaluation.

Process yield parameters such as headless yield and PUD yield were calculated. The headless yield was calculated from the difference between the weight of whole shrimp and weight of headless shrimp and PUD yield was calculated from the difference between the weight of whole shrimp and weight of peeled undeveloped shrimp.

$$\text{Headless yield \%} = \frac{\text{Headless weight}}{\text{Whole shrimp weight}} \times 100$$

$$\text{PUD yield \%} = \frac{\text{Wt of PUD shrimp}}{\text{Whole shrimp weight}} \times 100$$

Shrimp muscle (10 g) was mixed with distilled water (50 ml) and the mixture was homogenised for 30 S by using a homogenizer (Polytron system PT 2100, Germany). The 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.

TVB-N and TMA-N were measured according to the method described by EU/EC, (2008). The levels of TVB-N and TMA-N were calculated and expressed in mg N/100 g of shrimp flesh sample. The Peroxide Value (PV) was determined as per the method of AOAC (2005). The PV was calculated and expressed as m Eq O₂/kg lipids. Thiobarbituric acid reactive substances (TBARS) content was measured spectrophotometrically using the method described by Tarladgis et al. (1960).

Total viable count (TVC) of the fresh and ice stored shrimp muscle was determined as per USFDA BAM (2004). About 10 g 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 0.1 ml of the sample from each dilution was spread over the sterile agar petri-plates and incubated at 35±2 °C for 24 hours. Bacterial colonies developed after incubation were enumerated manually and expressed as log CFU g⁻¹.

The sensory evaluation for overall acceptability of the shrimp sample was done by a non-trained panel, which included staff and students from post-harvest technology department, ICAR-CIFE, Mumbai, India (n=30) using 9 point hedonic scale with 1 being the lowest and 9 being the highest score.

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 ± standard deviation.

Results and Discussion

The process yields of *L. vannamei* reared in inland saline water and brackish water are given in Table 1. The headless and PUD yield were found significantly higher ($p < 0.05$) in ISRV (71.19 % and 59.96 %) than the BWRV (69.75 % and 57.41 %). The yield could be attributed to the rigor mortis period of the shrimp. During rigor, the muscle of shrimp

will get stiffened and well attached with the shell. So, complete removal of meat is not possible and thus gives a lower yield. Consequently, the higher yield of ISRV sample may be due to the fact that the shrimp passed the rigor stage as the transportation time was slightly higher in ISRV sample. Similarly, Bhat et al. (2017) observed highest yield (54.18 %) in shrimp (*L. vannamei*) in the post-rigor stage, followed by in-rigor stage (51.98 %) whereas the lowest yield (47.68 %) was observed in pre-rigor stage.

Table 1. Process yield of freshly harvested *Litopenaeus vannamei*

Sample	Headless yield (%)	PUD yield (%)
BWRV	69.75±1.81 ^a	57.41±1.61 ^a
ISRV	71.19±1.76 ^b	59.96±1.80 ^b

BWRV-Brackish water reared *vannamei*, ISRV-Inland saline reared *vannamei*

Data expressed as mean ± SD (n=3), the mean values in the same column with different superscripts are significantly different (p<0.05) between samples.

The changes in all the biochemical parameters are given in Table 2. pH is an important index for determining the quality of fish and shell fish. Fresh BWRV and ISRV had a pH of 6.14 and 6.65, respectively. As the storage time increased, the pH of the samples increased significantly (p<0.05) and

reached 6.94 in BWRV sample and 7.65 in ISRV sample at the end of the storage. The increase in pH values could be associated with the production of basic components induced by the growth of bacteria. pH increased with time and temperature due to biochemical reactions. Tsironi et al. (2009) reported that the initial pH of shrimp was 6.95, which increased to pH 7.93 after 39 days at -5 °C and 7.85 after 74 days at -8 °C, respectively. Keer (2015) reported that the pH of *Acetes sp.* increased gradually from 6.56 to 8.20 during frozen storage for up to 4 months.

TVB-N and TMA have often been documented as the biochemical quality indices to assess freshness and quality of seafood. The biochemical indices are 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 12.00 mg N/100 g. The TVB-N content of both the samples increased as the storage period progressed. The TVB-N content of BWRV significantly (p<0.05) increased from 11.43 to 29.33 mg N/100 g and ISRV from 12.00 to 29.12 mg N/100 g. The increase of TVB-N in initial days of storage is related to the activity of spoilage bacteria and endogenous enzymes. The increased activity of bacteria and proteolytic enzymes might have contributed to the increase in TVB-N content in stored fish (Subramanian, 2007). A limit of 30-35 mg N/ 100 g is set for TVB-N in marine seafood products and the

Table 2. Changes in the biochemical quality of *L. vannamei* during frozen storage

Particulars	Samples	0 th day	30 th day	60 th day	90 th day	120 th day	150 th day
pH	BWRV	6.14±0.02 ^{aA}	6.55±0.07 ^{bA}	6.66±0.06 ^{cA}	6.84±0.09 ^{dA}	6.92±0.01 ^{deA}	6.94±0.02 ^{eA}
	ISRV	6.65±0.04 ^{aB}	7.10±0.04 ^{aB}	7.44±0.02 ^{aB}	7.50±0.02 ^{bB}	7.57±0.04 ^{cB}	7.65±0.03 ^{dB}
TVB-N	BWRV	11.43±1.07 ^{aA}	25.06±0.50 ^{bA}	26.60±0.70 ^{bA}	28.93±1.62 ^{cA}	29.87±0.81 ^{cA}	29.93±1.62 ^{cA}
	ISRV	12.00±1.40 ^{aB}	22.40±0.28 ^{aB}	25.43±1.07 ^{bA}	28.75±1.71 ^{cA}	28.93±0.65 ^{cA}	29.12±0.56 ^{cA}
TMA	BWRV	ND	2.47±0.35 ^{bA}	3.97±0.35 ^{cA}	4.11±0.16 ^{cA}	9.33±1.07 ^{dA}	12.88±0.56 ^{eA}
	ISRV	5.60±0.70 ^{aB}	5.83±0.81 ^{aB}	6.11±0.99 ^{aB}	7.09±1.17 ^{aB}	9.30±0.81 ^{bA}	9.89±0.86 ^{bB}
PV	BWRV	0.12±0.03 ^{aA}	0.13±0.03 ^{aA}	0.25±0.05 ^{bA}	0.28±0.03 ^{bA}	0.35±0.05 ^{cA}	0.53±0.03 ^{dA}
	ISRV	0.12±0.02 ^{aA}	0.13±0.03 ^{aB}	0.18±0.03 ^{bB}	0.23±0.03 ^{cA}	0.29±0.04 ^{dA}	0.41±0.03 ^{eB}
TBARS	BWRV	0.08±0.01 ^{aA}	0.15±0.04 ^{bA}	0.16±0.02 ^{bA}	0.17±0.01 ^{bA}	0.18±0.01 ^{bA}	0.22±0.01 ^{cA}
	ISRV	0.05±0.00 ^{aB}	0.09±0.02 ^{bB}	0.15±0.03 ^{bA}	0.17±0.03 ^{bcA}	0.20±0.01 ^{cdB}	0.25±0.02 ^{dA}

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 (a-e) are significantly different (p<0.05) between storage period. The mean value in the same column with different superscripts (A-B) superscripts are significantly different (p<0.05) between samples.

samples with TVB-N values above this are usually regarded as spoiled (Farajzadeh et al., 2016; Bindu et al., 2013). Yamagata & Low (1995) reported that the TVB-N value of banana Shrimp (*Penaeus merguensis*) increased ($P<0.05$) after 2 months at -20°C and reached a maximum of 10.93 mg/100 g after 4 months. These results were comparatively lower than the present study. Tsironi et al. (2009) found that TVB-N value of frozen shrimp changed from 6.49 mg N/100 g to 25 mg N/100 g. This result was similar to the findings of the present study.

TMA increased significantly ($p<0.05$) in BWRV and ISRV samples during frozen storage for 5 months. In BWRV sample, TMA significantly ($p<0.05$) increased from 0 to 12.88 mg N/100 g and in ISRV sample, TMA increased from 5.60 to 9.89 mg N/100 g at the end of storage study. The absence of TMA in BWRV sample on the initial day of storage could be due to the less initial concentration of TMAO. The initial concentration of TMAO depends on the species, origin of species, season, fishing ground and depth of living (Etienne, 2005). Tsironi et al. (2009) noticed that the TMA value of frozen shrimp increased from 2.85 mg N/100 g to 14 mg N/100 g during storage. Acceptable limit of TMA is 5–15 mg N/100 g (Bindu et al., 2013). Annamalai et al. (2015) & Connell (1995) reported 10–15 mg TMA-N/100 g as the limit of TMA for human consumption.

The Peroxide value (PV) indicates the formation of peroxides and hydroperoxides, which are considered as the primary products of lipid oxidation. The PV of the BWRV and ISRV gradually increased ($p<0.05$) up to 0.53 meq O_2/kg and 0.41 meq O_2/kg of fat, respectively at the end of frozen storage. The increased PV was due to the presence of fatty acids in the shrimp muscles that had undergone oxidation during storage, which resulted in the formation of hydroperoxides and peroxides (Okpala et al., 2014). However, the present study showed only a slight increase in PV throughout the storage in both the samples. This could be directly correlated to the odour parameter in sensory analysis during frozen

storage. The results of the present investigation match with the previous observation of Keyvan et al. (2008), who found a slight increase of PV of Caspian Sea white fish from 0.2 to 0.8 meq of O_2/kg on the 4th month of storage at -18°C . According to Farajzadeh et al. (2016), when PV exceeds 10 meq O_2/kg of lipid, fish is generally considered as spoiled. In the present study, PV of both BWRV and ISRV samples were within the acceptable limit at the end of storage period.

The TBA value is a measure of secondary lipid oxidation products. It gives an idea about the formation of aldehyde compounds like malonaldehyde during oxidation of lipid and generally, it is produced after degradation of peroxides (Keer, 2015). There was a slight increase ($p<0.05$) in TBA value from 0.08 mg MDA/kg to 0.22 mg MDA/kg in BWRV sample and 0.05 mg MDA/kg to 0.25 mg MDA/kg in ISRV during frozen storage of 5 months. The present investigation is in agreement with the previous observation of Keer (2015), who reported a gradual increase in TBARS value of *Acetes sp.* from 0.03 ± 0.00 mg MDA/kg to 0.32 ± 0.02 mg MDA/kg during frozen storage up to 4 months. Zhang et al. (2015) observed that the TBARS value of peeled Pacific white shrimp was 0.32 mg malonaldehyde/kg and which increased up to 2.02 mg malonaldehyde/kg on 100th day of frozen storage. Benjakul & Sutthiphan (2009) found an increase in the thiobarbituric acid value of crab muscle during 12 weeks of frozen storage at -20°C . According to Connell (1995), TBA value of 1-2 mg MDA/kg in fish muscle is related with unpleasant taste and odour.

The changes in Total Viable Count of *L. vannamei* reared in brackish water and inland saline water during frozen storage are shown in Table 3. The initial total viable count of BWRV and ISRV samples were 4.62 log CFU/g and 4.51 log CFU/g, respectively. The total viable count of both the samples decreased when tested on 30th day of frozen storage (3.71 and 3.60 log CFU/g, respectively for BWRV

Table 3. Changes in the Total Viable Count of *L. vannamei* during frozen storage

Particulars	Samples	0 th day	30 th day	60 th day	90 th day	120 th day	150 th day
TVC (log	BWRV	4.62	3.71	4.15	4.41	4.51	4.52
CFU/g)	ISRV	4.51	3.60	4.05	4.21	4.36	4.48

BWRV-Brackish water reared *vannamei*, ISRV-Inland saline reared *vannamei*

and ISRV). Further, a slow increase was observed and reached the value of 4.52 log CFU/g in BWRV sample and 4.48 log CFU/g in ISRV sample on 150th day of frozen storage. The decrease of microflora in the earlier stage of storage might be due to the effect of low temperature (Nirmal & Benjakul, 2009; Zeng et al., 2005) and the later increase might be due to the tolerance of psychrophilic microorganisms up to a certain limit (Nirmal & Benjakul, 2009). Tsironi et al. (2009) found that thawed shrimp samples stored at -5 °C and -8 °C showed an initial total viable count of 5.7 log CFU/g. Then, the microbial load gradually increased and reached the maximum limit at the end of the storage period. Keer (2015) observed an increase in the microbial load in *Acetes sp.* from 1.42×10⁵ CFU/g to 3.71×10⁵ CFU/g during 120 days of frozen storage. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the acceptable limit of TVC of fresh and frozen shrimp is 7 log CFU/g. In the present study, the TVC of both BWRV and ISRV samples were within the acceptable limit at the end of 150 days of storage period.

Table 4 shows sensory quality parameters (Appearance, shell colour, meat colour, odour, texture, taste, flavour and overall acceptability) analysed for

BWRV and ISRV samples during frozen storage based on a 9-point hedonic scale. All the parameters were significantly affected by storage period (p<0.05). The score for all the sensory attributes on 0th day were excellent, but as time progressed, the scores for all the parameters reduced. The decrease of sensory scores indicated that the quality of both BWRV and ISRV samples deteriorated gradually due to the breakdown of major cellular components such as protein and lipids. The formation of objectionable odour may be due to volatile compounds produced during lipid oxidation. However, the sensory scores of both BWRV and ISRV samples were within the acceptable limit at the end of 150 days of storage. Fatima et al. (1988) found that shrimps (*Penaeus merguensis*) in frozen storage (-3 °C) retained the prime quality up to 16 days after which there was a loss of the characteristic sweet flavour associated with fresh shrimps. Keer (2015) and Tsironi et al. (2009) also reported declining trends in sensory score of *Acetes* and shrimp during frozen storage but the scores were within the acceptable limit.

The results of this study indicated that the pH, TVB-N and TMA values increased in both BWRV and ISRV samples during 150 days of frozen storage. The total viable count of both the samples decreased in

Table 4. Changes in the sensory score of *L. vannamei* during frozen storage

Parameters	Samples	0 th day	30 th day	60 th day	90 th day	120 th day	150 th day
Appearance	BWRV	9.00±0.00 ^{aA}	8.63±0.52 ^{abA}	8.38±0.52 ^{ba}	7.67±0.52 ^{cA}	7.50±0.53 ^{cA}	7.19±0.37 ^{cA}
	ISRV	9.00±0.00 ^{aA}	8.50±0.55 ^{ba}	8.25±0.27 ^{ba}	7.69±0.26 ^{cA}	7.43±0.45 ^{cdA}	7.06±0.32 ^{dA}
Colour	BWRV	8.83±0.41 ^{aA}	8.63±0.52 ^{aA}	8.25±0.46 ^{abA}	8.00±0.89 ^{bA}	7.75±0.46 ^{bcA}	7.31±0.37 ^{cA}
	ISRV	8.90±0.22 ^{aA}	8.50±0.55 ^{abA}	8.38±0.44 ^{ba}	8.13±0.23 ^{bcA}	7.86±0.38 ^{cdA}	7.50±0.27 ^{dA}
Odour	BWRV	9.00±0.00 ^{aA}	8.25±0.89 ^{abA}	8.13±0.64 ^{ba}	8.00±1.10 ^{bA}	7.75±0.71 ^{ba}	7.44±0.42 ^{ba}
	ISRV	9.00±0.00 ^{aA}	8.33±0.82 ^{ba}	8.25±0.46 ^{bcA}	7.94±0.32 ^{bcA}	7.71±0.49 ^{cdA}	7.38±0.35 ^{dA}
Texture	BWRV	9.00±0.00 ^{aA}	8.38±0.92 ^{abA}	8.00±0.76 ^{ba}	7.92±0.49 ^{bA}	7.50±0.53 ^{cA}	7.25±0.27 ^{cA}
	ISRV	9.00±0.00 ^{aA}	8.33±0.82 ^{ba}	8.13±0.35 ^{bcA}	8.00±0.27 ^{bcA}	7.71±0.49 ^{cdA}	7.31±0.26 ^{dA}
Taste	BWRV	9.00±0.00 ^{aA}	8.66±0.35 ^{abA}	8.38±0.33 ^{bcA}	8.25±0.42 ^{cA}	7.44±0.50 ^{cA}	7.13±0.58 ^{cA}
	ISRV	8.86±0.22 ^{aA}	8.17±0.41 ^{ba}	8.06±0.50 ^{ba}	7.88±0.35 ^{bA}	7.07±0.19 ^{cA}	6.63±0.35 ^{dA}
Flavour	BWRV	9.00±0.00 ^{aA}	8.69±0.35 ^{abA}	8.35±0.31 ^{ba}	8.25±0.76 ^{bA}	7.50±0.46 ^{cA}	7.31±0.26 ^{cA}
	ISRV	8.86±0.22 ^{aA}	8.17±0.41 ^{ba}	8.00±0.60 ^{ba}	7.94±0.42 ^{ba}	7.29±0.27 ^{cA}	6.94±0.32 ^{cb}
Overall acceptability	BWRV	9.00±0.00 ^{aA}	8.35±0.49 ^{ba}	8.31±0.46 ^{ba}	7.95±0.83 ^{bcA}	7.44±0.50 ^{cdA}	7.25±0.27 ^{dA}
	ISRV	9.00±0.00 ^{aA}	8.17±0.41 ^{ba}	8.06±0.42 ^{ba}	7.94±0.32 ^{ba}	7.29±0.27 ^{cA}	7.06±0.18 ^{cA}

BWRV-Brackish water reared *vannamei*, ISRV-Inland saline reared *vannamei*. Data expressed as mean ± SD (n=10), the mean value in the same row with different superscripts (a-e) are significantly different (p<0.05) between storage periods. The mean value in the same column with different capital letters (A-B) superscripts are significantly different (p<0.05) between samples.

the beginning and in the later stage it increased. But the values were within the acceptable limit. From the present study, it can be concluded that the *vannamei* reared in brackish water and inland saline water can be kept in good condition under frozen storage up to 5 months. It is also evident from the results that there is no negative impact on shelf life of shrimp reared in inland saline water on frozen storage. Therefore, rearing of shrimp in inland saline water can be recommended to the farmers having salt affected land for their income opportunities.

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