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Research Article

EFFECT OF VACUUM PACKAGING ON SHELF LIFE OF FROZEN SHRIMP

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ABSTRACT
Fresh Tiger Shrimp *(Penaeus monodon)* were considering important in export, the present study was undertaken to find the effect of packaging on the shelf life of frozen condition. Fresh shrimp were packed in CAP (Control atmospheric pack), TAP (Treated atmospheric pack), CVP (Control vacuum pack) and TVP (Treated vacuum pack) samples and record the shelf life. At the end of 165th day of storage TMA-N values of flesh were 5.48, 3.34, 3.1and 2.24 mg% in CAP, CVP, TAP and TVP samples respectively. The lowest TMA-N values were recorded in TVP flesh. The initial value of TVB-N was 14mg% in fresh shrimp flesh. In case of CAP flesh the TVB-N values gradually increased to 40.34 mg% on the 165th day of storage. In case of CVP flesh at the end of 165th day TVB-N value rose to 30.6 mg%. Drip loss was slightly higher in CAP samples (0.29 to 20.96) and CVP samples (0.29 to 17.53) as compared to that of TAP samples (0.29 to 16.87) and TVP samples (0.29 to 14.68) during 165 days of storage. The counts increased from 1.62 x 10^3 cfu/g in fresh shrimp to 2.40X10^5, 3.00 X$10^{4,}$ 1.43X10^4 and 2.36X10^4 in case of CAP, TAP, CVP and TVP samples respectively at the end of 90th day of storage. Compared to untreated samples, treated samples showed slow growth of microorganisms. At the end of 165th day of the storage TPC count goes on increases up to 2.12X10^6, 3.10 X$10^{5,}$ 1.76X10^5 and 1.36X10^5 in case of CAP, TAP, CVP and TVP samples respectively. On the basis of 10-scale a score of 5 taken as the acceptable limit for determining the shelf life of tiger shrimp flesh during frozen storage. Untreated air packed samples were acceptable up to 4months, whereas treated air pack found acceptable up to 5and half month and vacuum packed samples remained in good condition above six months.

INTRODUCTION
Seafood scenario the world over is witnessing vast changes. Over the last decades, the seafood sector has undergone massive change. As a result of considerable interest in its nutritional benefits, seafood today is perceived as a health food. India is committed to boost its marine product export, but the prime factor of concern is the assurance of quality and safety of our products in the export. The shrimp is important and high unit value commodity of Indian export. Frozen shrimp continued to be the major item contributing in value (53.88%) of total export of marine products from India (MPEDA 2006). Shrimp undergo rapid spoilage faster than finfish due to high free amino acid content and soft texture (Murugnathan *et al.*, 2006). Therefore, shrimps have to be handled with great care and be chilled as rapidly as possible to delay spoilage and be processed quickly to ensure quality. Presently ice and mechanical refrigeration are the most common means of retarding microbial and biochemical spoilage of freshly caught seafood during distribution and marketing.

Technology development over the past decade have contributed to the modernization and growth of the seafood sector with processing and packaging facilities of more efficient storage and distribution systems. A variety of seafood items in attractive packaging is a common sight today in the refrigerated shelves of the seafood counters in super markets. The seafood packaging industry, the world over is adopting innovative packaging styles according to consumer demand. The packages have been developed, keeping in mind the physical and chemical properties of food and its storage, distribution and acceptance among the end users. Packages for convenience food requires a container shape and size that makes easy opening, pouching, serving, carrying, re-closing and storage. Therefore, to promote the marketing of fresh fishery products at retail level, the sophisticated techniques of packaging and storage is vacuum packaging.

Research Article

Vacuum packaging has revolutionized the packaging industry and brought vendors, retailers and consumers together from east to west and north to south regardless of distance or mode of transportation. This method of packaging has managed to keep the product hygienic with a highly extended shelf life. Vacuum packaging is one of the natural preservative packaging methods which can greatly enhance the shelf life and overall quality of muscle foods for a long time (Sahoo, 2006). It is the most viable packaging method to get better shelf-life (Day, 2003). Vacuum packaging can be supplement to ice or refrigeration to delay spoilage, extend the shelf-life of fishery products (Shalini *et al.*, 2000). Vacuum packaging involves the removal of air from the packaging, and then extends the viable shelf-life of many cooked foods. It should be stressed that vacuum packaging must be used under strict conditions of hygiene and control. This could be applied to cook-chill food systems to increase food quality and microbiological assurance (Rajesh *et al.*, 2002)

Penaeid shrimps are economically important demersal resources and their demand in the domestic and export markets are on increase. *Penaeus monodon* locally known as 'Tiger kolambi' is one of the highly cherished seafood of India. In India *Penaeus monodon* is distributed along the east and west coast. Along with capture it is most important culturable species in east and west coast. The production along the India is about 1, 44, 347 MT (Fishing Chimes, 2008). Therefore, with considering their important in export, the present study was undertaken to find the effect of vacuum packaging on the shelf life of tiger shrimp in frozen temperature.

MATERIALS AND METHODS

Preparation of Sample

Fresh shrimp (*Penaeus manodon*) locally known as 'tiger kolambi' was used for the study. The material was procured from 'Zadgaon brackish water shrimp farm'. The shrimp used for this study had a weight and total length of 25±15 gm and 17± 5cm respectively. Sodium tripolyphosphate (Hi-media) was used as preservative for this study. Standup pouches (Trend packs) made up of nylon laminated polyethylene with a capacity of 200g each and a composition of l2 micron clear polyester/400 gauge polythene pouches (PE/P) of size 20.5 ><l0.5 >< 3.25 cm were used for packaging of tiger shrimp. Vacuum packaging machine (Dong Bang Machineries Pvt. Ltd., China) was used for packaging headless shrimps

The treatment solution i.e. Sodium tripolyphosphate 12.5 % w/v (Heen *et al.*, 1965) was prepared in potable water. The volume of the drip solution was 60% of the weight of the material and dipping time was two minutes. The drip treated materials were drained for three minutes (Mathen, 1970). Shrimps were placed in each laminated the packs used for frozen storage was stored in deep freezer at -18 ^{0}C temperature. In frozen storage samples were drawn at an interval of 3 days and 15 days respectively for analysis purpose. All the packs were analyzed for sensory, microbiological and biochemical parameters,

Biochemical Analysis

Proximate composition:

Proximate composition i.e. moisture, crude protein, crude fat and ash contents were determined according to AOAC (2005).

Determination of Trimethylamine Nitrogen (TMA-N):

TMA was determined by micro diffusion method of Conway (1962). 10 g sample was homogenized with 20 ml of 20% Trichloroacetic acid (TCA). The homogenate was filtered through Whatman No 1 filter paper to a 100 ml standard flask. The residue was triturated with 80 ml of 5% TCA and made up to the volume. The filtrate was then used for further analysis. Grease was applied on the edges of micro diffusion unit and l ml of 0.0lN standard sulphuric acid was taken in inner chamber, l ml 20% TCA extract, 0.5 ml neutralized formaldehyde and 0.5 ml saturated potassium carbonate was taken in outer chamber of the unit. The unit was sealed with glass lid and gently swirled. The unit was kept overnight undisturbed. The amount of un-reacted acid in the chamber was determined by titration against standard 0.01 NaOH using Tashiro's indicator. A blank was run simultaneously prepared with l ml of 20% TCA solution. TMA-N was calculated as mg/100g of muscle as follows

$$\text{TMA -N (mg \%)} = \frac{\text{(A — B) X0.14 X Volume of extract X100}}{\text{Volume of sample taken X Sample weight}}$$

Where A = volume of 0.0l N NaOH used up for titration of the blank and B = volume of ml of 0.01 NaOH used up for titration of the sample

Determination of Total Volatile Base Nitrogen (TVB-N):

TVB-N was determined by micro diffusion method of Conway (1962). 10 g minced sample was homogenized with 20 ml of 20% Trichloroacetic acid (TCA). The homogenate was filtered through Whatman No. l filter paper to a 100 ml standard flask. The residue was triturated with 80 ml of 5% TCA and made up to the volume. The filtrate was then used for further analysis. Grease was applied on the edges of micro diffusion unit and 1 ml of 0.0l N standard sulphuric acid was taken in inner chamber, l ml 20% TCA extract and 0.5 ml saturated potassium carbonate were taken in outer chamber of the unit. The unit was sealed with glass lid and gently swirled. The unit was kept overnight undisturbed. The amount of unreacted acid in the chamber was determined by titration against standard 0.01 NaOH using Tashiro's indicator. A blank was run simultaneously prepared with 1 ml of 20% TCA solution. TVB—N was calculated as mg/100g of muscle as follows

$$\text{TVB -N (mg \%)} = \frac{\text{(A — B) X 0.14 X Volume of extract X100}}{\text{Volume of sample taken X Sample weight}}$$

Where, A = volume of 0.0l N NaOH used up for titration of the blank B = volume of ml of 0.01 NaOH used up for titration of the sample

Alpha Amino Nitrogen (AAN):

10 g minced sample was homogenized with 20 ml of 20% Trichloroacctic acid (TCA). The homogenate was filtered through Whatman No. 1 filter paper to a 100 ml standard flask. The residue was triturated with 80 ml of 5% TCA and made up to the volume. The filtrate was then used for further analysis. 10 ml of TCA extract was taken into 50 ml volumetric flask. Few drops of thymopthalein were added and extract is made alkaline by adding Normal NaOH, till a distinct blue color appeared. 1 part by volume of CuCl2 solution was mixed with 2 parts by volume of tri-sodium phosphate and 2 parts by volume of borate buffer. This solution was mixed well and 30 ml of suspension was added to alkaline solution in standard flask, which contains 10 ml of TCA extract. The volume was made up to 50 ml with distilled water. After shaking, it was allowed to stand for 15 minutes and then filtered. 10 ml of the filtrate was pipetted out to a conical flask; 0.5ml of glacial acetic acid (CH_3COOH) is added followed by addition of 0.5 g of potassium iodide. The liberated iodine was titrated against N/500 sodium thiosulphate using starch as an indicator, when yellow solution of Iodine becomes faint yellow, few drops of starch solution was added and titration was continued till blue colour disappeared. AAN was calculated as mg/100gm of muscle as follows 1.0 ml of N/500 $Na2S_2O_3$=0.056mg of α-amino acid

Estimation of Drip Loss:

Drip loss was estimated by the method as per Hiremath and Dhananjaya (1979). After storage smaller pieces of 5 mm thickness of sample was weighed. Then the meat was placed between two filter papers without causing any pressure at room temperature and thawed it for 3-4 hours until the flesh was completely thawed. Then the filter paper, which had absorbed the exuded moisture, was removed. Then the sample was weighed. Drip loss of flesh was calculated as follows

$$\text{Drip loss (\%)} = \frac{A-B}{A} \times 100$$

Where, a = initial weight of sample b = final weight of sample

Microbiological Analysis

Determination of Anaerobic Count:

Total anaerobes were determined by 3 tube MPN technique as per USFDA Bacteriological Analytical Manual online (2006). 10 g of sample was aseptically weighed and diluted with 90 ml physiological saline solution. Samples weighed homogenized using mortar and pestle. 10 ml of the diluted sample was inoculated in to 3 tubes of 10 ml thioglycollate broth, 1 ml each to 3 tubes of thioglycollate broth and 0.1 ml of each to 3 tubes of thioglycollate broth. The test tubes were overlaid with sterile paraffin oil to prevent contact with air and incubated at 35 °C or in room temperature. The tubes were observed for growth after 48 hours. The probable number of anaerobic count can be calculated according to the various combinations of +ve and —ve reactions by using Mac crady table.

Sensory Evaluation:

Sensory evaluation was based on the various sensory characters i.e appearance, odour, texture and taste. A panel of five judges did sensory analysis of stored samples. The samples were evaluated by a 10 point scoring system. High score indicated good quality and vice versa. The border line of acceptability was five for shrimp.

Statistical Analysis

Recorded results were analyzed by using appropriate statistical method (Snedecor and Cochran, 1967). The significant results were stated as $P<0.05$.

RESULTS AND DISCUSSION

Proximate Composition

Proximate compositions were recorded in Tiger shrimp meats were summarized in Table 1.

Table 1: Proximate composition of shrimp

Sr. No.	Component	Values (%)
1	Moisture	82.45±1.55
2	Protein	38.50±1.50
3	Fat	2.89±1.75
4	Ash	1.33±1.45

Chemical and Bacteriological Quality of Fresh Tiger Shrimp:

The chemical and bacteriological qualities of fresh tiger shrimp meat were shown in Table 2.

Table 2: Sensory evaluation, biochemical chemical and microbiological Quality of Fresh Tiger Shrimp

Sr No	Parameters	Initial Value
1	pH	6.50
2	TVB-N	14.00 mg%
3	TMA-N	0.28 mg%
4	AAN	21.46 mg%
5	TPC	$1.62X10^3$
6	Psychotropic count	$1.30X10^3$
7	Anaerobic count	Nil
8	Overall acceptability	9.6 (Over 0-10 scale)

Drip Loss

There was increase in drip loss in all the samples during frozen storage Figure 1. However, increase in drip loss was slightly higher in CAP samples (0.29 to 20.96) and CVP samples (0.29 to 17.53) as
Compared to that of TAP samples (0.29 to 16.87) and TVP samples (0.29 to 14.68) during 165 days of storage.

The drip loss in TVP shrimp flesh during frozen storage had correlation coefficient 0.9855 with storage period of 165 days and it was significant (P<0.05). The drip loss of TVP samples were correlated well with the storage days. The estimated equation showed an increase of 0.0886% drip loss per day. The estimated equation was Y = 0.0886X - 0.5314 and is depicted in.

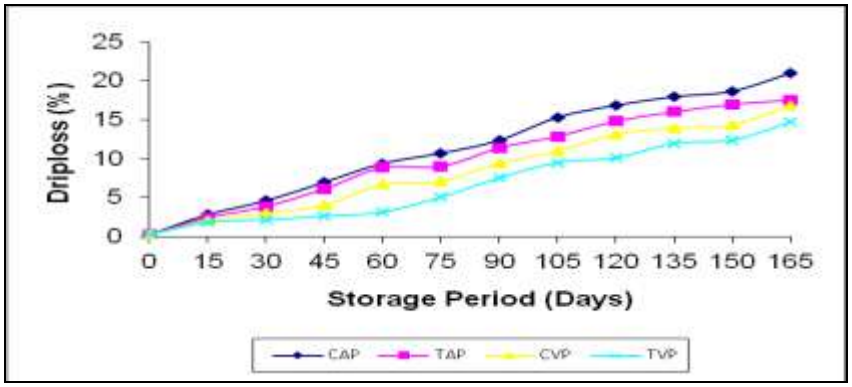

Figure 1: Drip loss during frozen storage

Alpha Amino Nitrogen (AA-N):

The changes in Alpha amino nitrogen content of tiger shrimp during frozen storage is shown in Table 1 and depicted in Figure 2. AAN increased gradually from 21.46 mg% in fresh shrimp to 54.34, 46.84, 41.29 and 31.28 mg % during storage in case of CAP, TAP, CVP and TVP samples respectively at the end of 135 days storage of frozen storage. The AAN value further increases to 48.24, 47.26 and 34.26 mg% at the end of 150 days storage in case of TAP, CVP and TVP samples respectively. Further the AA-N value increases to 48.14 and 36.84 in CVP and TVP. The lower AAN values were recorded in TVP after 165 days storage. Even after 165 days storage, the AA-N value was loss in the TVP sample, being 36.84 mg%.

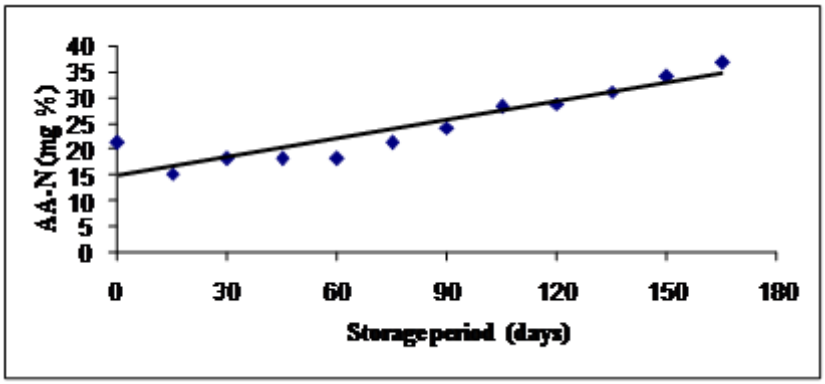

Figure 2: Alpha amino acid of frozen shrimp

The AAN of TVP shrimp during frozen storage had correlation coefficient of 0.9256 with storage period of 165 days and it was significant (P<0.05). Thus the AAN values of TVP shrimp flesh was correlated

well with the storage days. The estimated equation was y = 0.1201x + 14.873. The estimated equation showed a gain 0.1201% per day.

Trimethylamine Nitrogen (TMA-N):

The changes in TMA-N content of tiger shrimp flesh during frozen storage increased steadily for the entire sample Figure 3. In fresh shrimp flesh the initial value of TMA-N was 0.28 mg%, which has increased to 3.44, 2.32, 1.8, and 1.7 mg% in case of CAP, TAP, CVP and TVP samples respectively on 90th day of frozen storage. At the end of 165th day of storage TMA-N values of flesh were 5.48, 3.34, 3.1and 2.24 mg% in CAP, CVP, TAP and TVP samples respectively. The lowest TMA-N values were recorded in TVP flesh.

Correlation coefficient between TMA-N content of 12% sodium tripolyphosphate treated vacuum packed shrimp during frozen storage of 165 days was 0.6984 and it was significant (P<0.05). Thus the TMA-N content of TVP samples correlated well with the storage days. The estimated equation showed 0.0065X mg% gain of TMA-N per day. The estimated equation was Y = 0.0065X+ 1.1292.

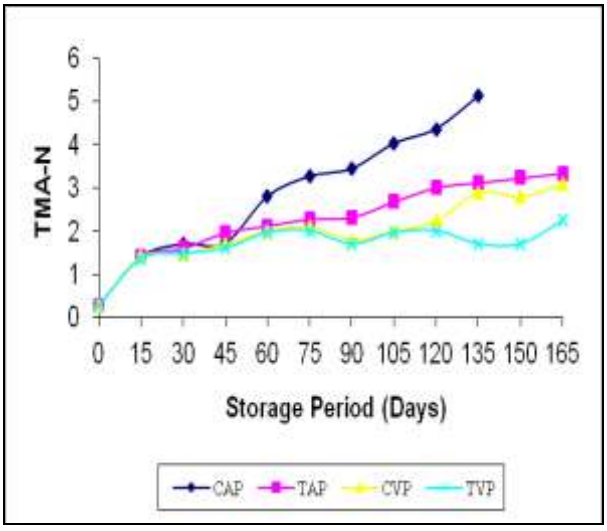

Figure 3: TMA-N during frozen storage

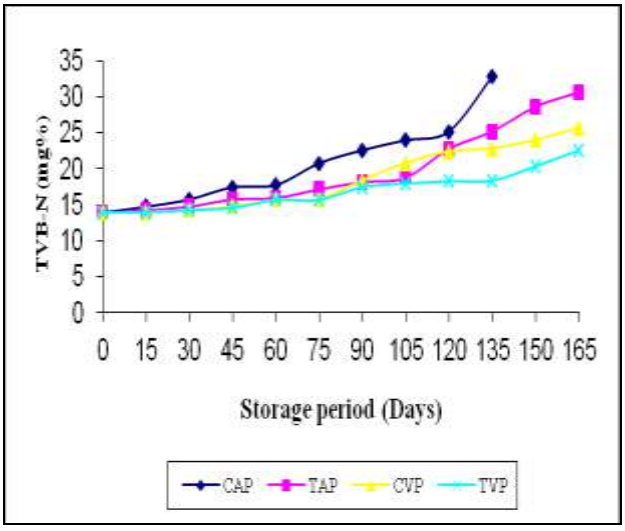

Figure 4: TVB-N during frozen storage

Total Volatile Bases (TVB-N):

TVB-N content of all the samples showed increasing trend throughout the storage period Figure 4. The initial value of TVB-N was 14mg% in fresh shrimp flesh. In case of CAP flesh the TVB-N values gradually increased to 40.34 mg% on the 165th day of storage. In case of CVP flesh at the end of 165th day TVB-N value rose to 30.6 mg%. The increase in TVB-N content of TAP and TVP samples was very gradual and reached a value of 25.6 mg% and 22.6 mg% respectively at the end of 165th day of frozen storage. The TVB-N values were recorded much lower in case of TVP.

Changes in Total Plate Count

The changes in TPC during frozen storage are shown in Table 3. In the present study, the total plate count was found to increase significantly with storage period. It is observed that TPC counts in the treatment pack were lower in comparison to control samples. The counts increased from 1.62 x 10^3 cfu/g in fresh shrimp to 2.40X10^5, 3.00X10^4, 1.43X10^4 and 2.36X10^4 in case of CAP, TAP, CVP and TVP samples respectively at the end of 90th day of storage. Compared to untreated samples, treated samples showed slow growth of microorganisms. At the end of 165th day of the storage TPC count goes on increases up to 2.12X10^6, 3.10 X10^5, 1.76X10^5 and 1.36X10^5 in case of CAP, TAP, CVP and TVP samples respectively.

Correlation coefficient was 0.84947 between TPC of 12% sodium tripolyphosphate treated vacuum packed shrimp flesh during storage of 165 days and it was significant (P<0.05). Thus the TPC in TVP

shrimp flesh directly correlated to storage days. The estimated equation showed an increase in the bacterial count at the rate of 0.0059 cfu/g. per day. The estimated equation was y = 0.0059x + 2.9334.

Table 3: TPC during frozen storage

No of Days	CAP	CVP	CVP	TVP
0	1.62×10^3	1.62×10^3	1.62×10^3	1.62×10^3
15	$1.79X10^3$	$1.63X10^3$	$1.48X10^3$	1.20×10^3
30	$2.10X10^3$	$1.94X10^3$	$1.21X10^3$	$1.00X10^3$
45	$1.76X10^3$	$1.89X10^3$	$1.10X10^3$	$1.63X10^3$
60	$1.96X10^4$	$1.38X10^4$	$3.21X10^3$	$2.20X10^3$
75	$3.04X10^4$	$2.10X10^4$	$1.31X10^4$	$1.24X10^4$
90	$2.40X10^5$	$3.00X10^4$	$1.43X10^4$	$2.36X10^4$
105	3.20X105	$3.56X10^4$	$1.60X10^4$	$2.49X10^4$
120	3.48X105	$1.36X10^5$	$2.10X10^4$	$3.15X10^4$
135	$1.34X10^6$	$2.42X10^5$	$1.30X10^5$	$3.82X10^4$
150	$1.86X10^6$	$2.81X10^5$	$1.46X10^5$	$1.21X10^5$
165	$2.12X10^6$	$3.10X10^5$	$1.76X10^5$	$1.36X10^5$

Changes in Psychrotrophic Counts

The changes in psychotropic count during frozen storage are shown in Table 4. In the present study, the psychotropic count was found to increase significantly with storage period. It is observed that psychotropic count in the treatment pack were lower in comparison to control samples. The counts increased from 1.10×10^3 cfu/g in fresh fish to 2.42×10^5, 7.50×10^4, 1.96×10^5 and 1.10×10^4 in case of CAP, TAP, CVP and TVP samples respectively at the end of 165^{th} day of storage. Compared to untreated samples, treated samples showed slow growth of microorganisms. Correlation coefficient between psychotropic count of TVP flesh was 0.991615 during storage of 165 days and it was significant ($P<0.05$). Thus the psychotropic count in TVP shrimp flesh correlated well with the storage days. The estimated equation showed an increase in the bacterial count at the rate of 0.0061cfu/g per day. The estimated equation was Y = 0.0061X + 3.0891.

Table 4: Psychrotrophic counts during frozen storage

No of Days	CAP	CVP	CVP	TVP
0	1.10×10^3	1.10×10^3	1.10×10^3	1.10×10^3
15	2.80×10^3	1.70×10^3	1.80×10^3	1.34×10^3
30	3.20×10^3	2.60×10^3	2.50×10^3	2.20×10^3
45	3.80×10^3	2.60×10^3	2.86×10^3	2.50×10^3
60	4.00×10^3	3.20×10^3	3.40×10^3	2.86×10^3
75	4.80×10^3	4.00×10^3	4.50×10^3	3.20×10^3
90	6.80×10^3	5.40×10^3	5.86×10^3	4.80×10^3
105	8.70×10^3	7.80×10^3	8.40×10^3	5.40×10^3
120	1.23×10^4	9.60×10^3	1.64×10^4	7.30×10^3
135	3.82×10^4	1.34×10^4	3.50×10^4	8.50×10^3
150	5.40×10^4	4.10×10^4	4.80×10^4	9.60×10^3
165	2.42×10^5	7.50×10^4	1.96×10^5	1.10×10^4

Changes in Anaerobic Count

The changes in anaerobic bacterial count during frozen storage are shown in Figure 6. In the present study, the anaerobic bacterial count was found to increase significantly with storage period. It is observed that anaerobic bacterial counts in the treatment pack were lower in comparison to control samples. The count in fresh shrimp flesh was absent and increased to 55, 45, 25 and 20 MPN/100 g in case of CAP, TAP, CVP, and TVP samples respectively at the end of 165th day of storage. Compared to untreated samples, treated samples showed significantly slower growth of microorganisms.

Correlation coefficient was 0.9756 between anaerobic bacterial count of TVP flesh during storage of 165 days and it was significant ($P<0.05$). Thus the anaerobic count in TVP shrimp flesh was correlated well with the storage days. The estimated equation showed an increase in the bacterial count at the rate of 0.1105 MPN/100g per day. The estimated equation was $Y = 0.1105X - 1.1154$.

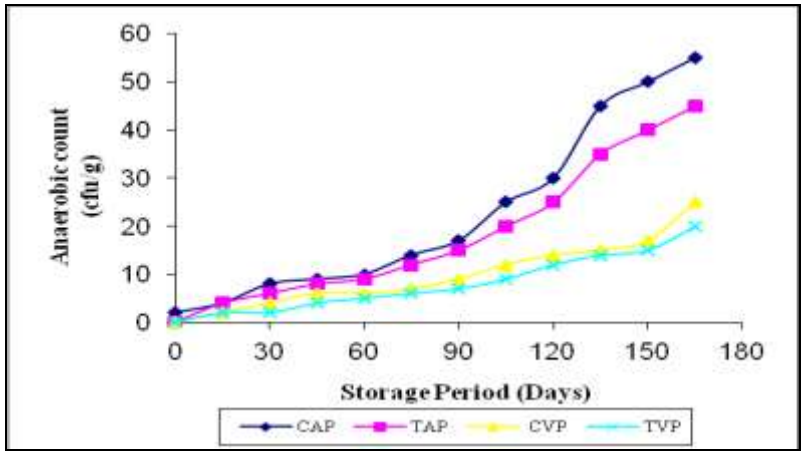

Figure 5: Aerobic count during frozen storage

Sensory Changes in Tiger Shrimp Flesh during Frozen Storage

Scores given by taste panelists for appearance, texture, color, odour, taste and overall acceptability are given in. There was a significant decline in sensory scores in all the control and treated packs with storage period. On the basis of 10-scale a score of 5 taken as the acceptable limit for determining the shelf life of tiger shrimp flesh during frozen storage. Untreated air packed samples were acceptable up to 4months, whereas treated air pack found acceptable up to 5and half month and vacuum packed samples remained in good condition above six months. It is observed that combination of vacuum packaging and treatment with sodium tripolyphosphate increased the shelf life of tiger shrimp flesh significantly.

Over all acceptability of CAP, TAP, CVP and TVP tiger shrimp flesh was compared by ANOVA. Results revealed no significant difference among all the treated samples during frozen storage ($P<0.05$). Thus the means of overall organoleptic scores of 4 months frozen flesh were tested by L.S.D. test.

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