

Vacuum Packaging and Quality Analysis of Silver Pomfret (*Pampus argenteus*) Under Refrigerated Condition ($4\pm 1^{\circ}\text{C}$)

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ABSTRACT: In the present investigation, the effect of vacuum packing and sodium acetate treatment on the shelf life of Silver Pomfret (*Pampus argenteus*) fillets was investigated during refrigerated storage ($4\pm 1^{\circ}\text{C}$). On the basis of sensory analysis, the sodium acetate treated vacuum packed samples were found to be acceptable up to 21 days followed by 15 days for control vacuum packed and 9 days for control air packed samples. A prominent lag phase was observed for mesophilic and specific spoilage bacteria, particularly for the sodium acetate treated vacuum packed samples on the day of sensory rejection the total mesophilic counts did not reach $7.0 \log \text{cfu/g}$ for all the samples. *Lactobacillus* spp. was the dominant microflora in sodium acetate treated vacuum packed samples. In vacuum packed samples the growth of total enterobacteriaceae was inhibited. The biochemical parameters were analyzed in vacuum packed refrigerated storage condition and found to be within the acceptable limit during the storage period.

Key words: Vacuum, Sodium acetate, Polyester, Fillets

INTRODUCTION

Fish is highly nutritious and protein rich food. Health benefits associated with fish consumption have resulted in consumers favoring fish products; as a result of this the fish eaters of the world have doubled during the last 50 years. India's seafood industry is one of our biggest foreign exchange earners. While considering fish as a source of food the main emphasis has been put on the protein (Borgstrom, 1962). About 85-90% of fish protein is easily digestible and contains all essential amino acids (Stansby, 1962). Fish represents about 14% of all animal proteins and about 5% of total protein is eaten on a global basis. Worldwide fish consumption is on the rise due to its rich sources of high quality proteins, essential vitamins and healthful poly-unsaturated fatty acids. Technology up gradation for enhancing shelf life of fresh fish has become necessity in fish processing sector to successfully marketing them in the urban domestic markets. The increased demand for fresh fish has prompted the development of many new preservation techniques which can be adopted by the

fish processing industry without sacrificing safety, quality, shelf life and satisfying the consumer demand. The tropical country like ours favors the rapid growth of microorganism. Over the last two decades the demand for mild preserved seafood products is on the rise. This has promoted the growth of chilled and refrigerated seafood products. Various techniques like mild chemical treatment, vacuum packaging, modified atmosphere packaging with increased carbon dioxide concentration and active packaging are being practiced to extend the shelf life of chilled seafood products. Food packaging like any other packaging is an external means of preservation of food during storage, transportation and distribution and has to be provided at the manufacturing/ production center. Hence it forms an integral part of the product manufacture/production and has an important function in the distribution of foodstuffs (Ganguly, 2014).

Vacuum packaging represents a static form of hypobaric storage which is widely used in the food industry due to its effectiveness in reducing oxidative

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reactions in the product at relatively lower costs. In vacuum packaging, the product is contained in a package made of a material having low oxygen permeability and is sealed air tight after evacuating the air. Vacuum packaging has been shown to extend the shelf life for periods varying from six days upwards. However, even though the product could not develop rancidity in extended periods of storage, it could develop objectionable odours and flavours due to bacterial activity (Kumar and Ganguly, 2014).

Pomfrets are one of the most highly esteemed table fishes of India both from the point of view of consumer acceptability and nutritive value. Pomfret has extremely attractive flavor. So the present study was undertaken to find the effect of vacuum packaging on the self-life of Pomfret fillets in refrigerated condition.

MATERIALS AND METHODS

Fresh silver pomfret (*Pampus argenteus*) with an average weight of 60 grams were purchased from the fish market and were transported to the laboratory in an insulated box under iced condition.

Sample for Refrigerated Storage

Pomfret fillets were divided into three batch. Polyester laminated with low density polyethylene of thickness 42 μ was used as packaging pouches. The samples were tested for sensory, bacteriological and biochemical quality at periodical intervals.

In the laboratory fishes were washed in chilled potable water and kept in iced condition during processing. Then, these were beheaded, gutted and washed in chilled potable water. Dressed fish were filleted and then washed in chilled potable water. The fillets were divided into 3 batches. Batch 1 was air packed (control air pack-CAP) and batch 2 vacuum packed (control vacuum pack-CVP). Batch 3 was given a dip treatment in 2% (w/v) sodium acetate (1:2 fish: sodium acetate solution) solution at chilled condition (1-2°C) for 10 min., drain well and then vacuum packed (SATVP). Samples of all three batches were stored in refrigerator (4 \pm 1°C)

Microbiological Characteristics

Microbiological characteristics of stored pomfret fillets were carried out as per the Standard methods (APHA, 1998). Appropriate dilution of the homogenate was made in a physiological saline (0.85%) plated, in duplicate, on nutrient agar, by spread plate method. The plates were incubated at 37°C for 48 h and TPC was calculated accordingly.

Likewise SSO was done in Iron Agar for 48 hours, Enterobacteriaceae count was done on Mac Conkey agar for 48 h and Lactic acid bacteria count was done on MRS agar for 48-72 h and calculated accordingly.

Total Volatile Base-nitrogen (TVB-N)

The TVB-N was determined by Conway (1968) method. In this method ammonia and other aliphatic amines in the meat and products were estimated. For this, 100 g of sample was blended with 300 ml of 5% trichloroacetic acid (TCA) solution and filtered to obtain clear extract. Five ml of extract was heated with 5 ml of 2N NaOH. The distillate was collected in 15 ml of 0.01N HCl containing 0.1 ml of resolic acid indicator. After distillation, excess acid was titrated by using 0.01 N NaOH to a pale pink end point. One blank was also determined. The result is expressed as mg/100 g of sample.

Trimethylamine (TMA)

The TMA content of the sample was determined by the Conway micro-diffusion method described by Beatty and Gibbon (1937). The sample treated with 20% TCA a predetermined (1 ml) quantity was taken in the inner chamber of Conway micro diffusion unit. On the outer chamber formalin and concentrated potassium carbonate was laid. After 3-6 hrs of keeping inside a dark place, it was titrated with 0.002 N sulphuric acids and calculated for TMA content of the sample. The result is expressed as mg/100 g sample.

Free Fatty Acids (FFA)

The FFA content in sample was determined by the method as recommended by Jacobs (1958). A suitable quantity of the minced muscle was blended thoroughly with twice its weight of anhydrous Na₂SO₄ in a mortar. The blend was shaken in distilled chloroform for 5 to 10 minutes and filtered. Fat content of 10 ml of the extract was determined by evaporating the chloroform. Another 10 ml of extract was evaporated and to it 10 ml of neutral alcohol was added. It was titrated against 0.01N NaOH using phenolphthalein as indicator. The result is expressed as % as oleic acid.

Sensory Characteristics

The sensory evaluation of raw material was based on characterization and differentiation of the various sensory characters such as appearance, texture, odour and flavor. Sensory score was given based on 9-point hedonic scale (Manju *et al.*, 2007a). Trained taste panel

scoring of the fish was conducted after boiling the fillets in 1.5% brine for 10 minutes. Sensory score of 4 was taken as the borderline of acceptability. Initially, pomfret fillet sample had a bright characteristic appearance, color and odor which showed a declining trend with the storage period. The fresh fillet was firm in texture and taste was rated high for initial samples. Among the samples packed under different atmospheric conditions, significant differences ($P < 0.05$) between treated and untreated samples were observed towards the end of the storage period. The overall acceptability score was plotted using different attributes (appearance, color, odor, flavor, firmness and taste). The fillet samples were considered to be acceptable for human consumption until the sensory score reached 4, below which it was considered as unacceptable as described by Manju *et al.* (2007a).

Statistical Analysis

Statistical analysis was performed as per Snedecor and Cochran (1968). Correlation coefficient (r) was calculated for the chemical quality parameters for the raw material to observe their acceptance level. One way analysis of variance (ANOVA) and least significant test in the form of critical difference was performed to test the significant difference between samples and storage days ($P < 0.05$). 'P' is defined at 99% level in the present study.

RESULTS AND DISCUSSION

Total Plate Count

The results for TPC showed (Table 1) a gradual increase ($P > 0.05$) in all samples over the period of storage. The initial TPC of raw fillets was 4.63 log cfu/g. In the pomfret fillets packed in control air packs and control vacuum packs, the counts increased steadily and no lag phase was observed. However, the microbial counts of CVP samples were significantly ($p < 0.01$) lower than the CAP samples. The SATVP samples showed a lag phase of 5 days. The lag phase observed in the present study could be

attributed to the inhibitory effect of low oxygen concentration in the package and sodium acetate treatment, on the growth of bacteria.

Similar lag phase in the range of 6-10 days were reported for brown shrimp (Lannelongue *et al.* 1982a), sword fish (Lannelongue *et al.*, 1982b), fresh water crayfish (Wang and Brown, 1983) and seer fish (Yesudhasan, 2007) packed in modified atmosphere. For fresh quality fish, the microbiological limit for human consumption as proposed by ICMSF (1986) is 7 log cfu/gm. Lakshmanan (2000) reported that the TPC levels above 10^7 cfu/g results in visible spoilage of fish flesh. In the present study microbial counts did not exceed the acceptable limit, on day 9 in CAP, on day 15 in CVP and on day 21 in SATVP samples. The results of present study indicates that the vacuum packaging are effective in inhibiting the microbial spoilage to a great extent compared to 100% air packed sample by extending the lag phase and generation time (Parkin and Brown 1982, Genigeorgis 1985). Low temperature storage and sodium acetate treatment also played a significant role in further extending the shelf life, which suppressed the growth of aerobic spoilage bacteria similar to the results reported for other chemical treatment like sodium acetate, sodium citrate and sodium lactate (Zhuang *et al.*, 1996; Shalini *et al.*, 2000; Sallam, 2007 a,b).

Specific Spoilage Organisms (SSO) Count

The results for SSO showed (Table 2) a gradual increase in all samples over the period of storage. H_2S producing bacteria were the dominating micro flora in the present study. H_2S producing bacteria has been reported as specific spoilage organisms in fish from temperate and tropical water (Lima dos Santos 1981, Gram and Huss 1996) and fresh fish stored aerobically under refrigeration (Koutsoumanis and Nychas, 1999). The initial SSO count was 3.6 log cfu/gm. The SATVP samples showed a lag phase of 5 days. In the control air packs and control vacuum packs samples the counts increased steadily ($P < 0.01$) and no lag phase was observed.

Table 1
TPC of Silver pomfret fillets packet under different atmospheric condition during storage at $4 \pm 1^\circ C$

Storage days	CAP	CVP	SATVP
0	4.63±0.125	4.63±0.125	4.63±0.125
5	6.31±0.07	5.57±0.103	4.65±0.023
10	7.18±0.76	6.38±0.01	5.40±0.05
15	8.59±0.124	7.00±0.027	6.30±0.07
20	9.63±0.02	7.93±0.05	6.80±0.016
25	11.14±0.1	8.67±0.027	7.40±0.1

Values were expressed as Mean±S.D

Table 2
SSO of Silver pomfret fillets packet under different atmospheric condition during storage at $4 \pm 1^\circ C$

Storage days	CAP	CVP	SATVP
0	3.6±0.05	3.6±0.05	3.6±0.05
5	6.2±0.07	5.2±0.026	3.6±0.05
10	7.1±0.16	5.7±0.02	4.2±0.125
15	9.13±0.02	7±0.102	4.8±0.02
20	10.02±0.14	8.050.1	5.1±0.076
25	12.63±0.102	9.12±0.15	5.21±0.1

Values were expressed as Mean±S.D

The lag phase observed in the SATVP samples could be attributed to the inhibitory effect of low oxygen concentration in the package and sodium acetate treatment, on the growth of H₂S producing bacteria. On the day of sensory rejection the lesser counts of SSO for SATVP could be attributed to the low oxygen concentration and antimicrobial activity of sodium acetate caused by the undissociated acetic acid molecule and the reduced pH, below which the growth of many bacteria is inhibited (Zhuang *et al.*, 1996). Many H₂S producers including *S. putrefaciens* were unable to grow at low O₂ and high CO₂ concentration (>50%), due to its effect on the bacterial enzymes (Boskou and Debevere, 1998). Many authors have reported similar results for various fishes packed in 100% air (Gillespie 1981, Gram and Huss 1996; Koutsoumanis and Nychas 1999; Chytiri *et al.*, 2004), vacuum and MAP with high CO₂ concentrations, stored under chilled conditions (Lyhs *et al.*, 2001; Goulas *et al.*, 2005).

Lactobacillus spp. count

The initial LAB count was 4.3 log cfu/g (Table 3), which increased to maximum values of 5.7, 6.2, and 5.7 on day 10, 10 and 15 for CAP, CVP and SATVP samples respectively. After reaching the maximum counts, a declining trend during the remaining storage period was observed. In the SATVP samples the *Lactobacillus spp.* was the dominant bacteria.

Table 3
Lactobacillus spp. count (log cfu/g) of Silver pomfret fillets packet under different atmospheric condition during storage at 4±1°C

Storage days	CAP	CVP	SATVP
0	4.30±0.02	4.30±0.02	4.30±0.02
5	5.40±0.1	5.20±0.028	4.80±0.125
10	5.70±0.5	6.20±0.17	5.50±0.76
15	5.40±0.01	5.90±0.16	5.70±0.06
20	5.10±0.05	5.60±0.02	5.40±0.103
25	4.80±0.07	5.30±0.12	5.10±0.01

Values were expressed as Mean±S.D

Similar results were reported for the mullets stored under MAP (Pourniss *et al.*, 2005) and vacuum packed tropical fish (Pedersen and Snabe, 1995).

The reason behind this could be absence of oxygen in the package, which restricts the growth of *Pseudomonas* and H₂S producer, so that *Lactobacillus spp.* became the major component of the spoilage microflora (Korkeala and Bjorkroth 1997; Hansen and Bautista, 2000). Contrary to the present observations, very slow or no growth of *Lactobacillus spp.* was reported for seabream (Gopal *et al.*, 1996) and

pearlspot (Lalitha *et al.*, 2005) stored under 100% air and modified atmosphere packages, respectively. Under anaerobic conditions, *Lactobacillus spp.* could cause souring, slimy, swelling of the package (Blickstad and Molin, 1983) which was observed in the present study.

Total Enterobacteriaceae Count

In the present study, the initial total enterobacteriaceae count was 3.5 log cfu/g (Table 4). The presence of enterobacteriaceae in fish indicates the faecal contamination.

Table 4
Total Enterobacteriaceae count (log cfu/g) of Silver pomfret fillets packet under different atmospheric condition during storage at 4±1°C

Storage days	CAP	CVP	SATVP
0	3.50±0.01	3.50±0.01	3.50±0.01
5	3.70±0.103	3.65±0.05	3.40±0.028
10	4.10±0.06	2.55±0.02	2.40±0.16
15	4.37±0.14	2.1±0.17	1.90±0.125
20	4.57±0.07	1.63±0.124	1.55±0.76
25	4.97±0.1	1.1±0.01	1.0±0.07

Values were expressed as Mean±S.D

In CVP and SATVP samples the growth of enterobacteriaceae was gradually retarded, which could be due to protective effect of low oxygen and sodium acetate treatment which causes death of these organisms, as was also reported for seabass (Papadopoulos *et al.*, 2003; Taliadourou *et al.*, 2003). In CAP samples enterobacteriaceae showed slightly increasing trend (P<0.05) and reached a maximum of 4.1 log cfu/g on day 10.

Total Volatile Base Nitrogen (TVB-N)

The values of TVB-N showed (Table 5) a gradual increase (P>0.05) in all samples. The TVB-N content of fillets increased from an initial value of 4.57-41.12 mg % in CAP and 35.79 mg% in CVP at the end of storage periods, respectively. In sample SATVP, the TVB-N content rose to 27.01mg % on 25 days of storage which was significantly (P<0.01) lower than other treatments.

Table 5
TVB-N of Silver pomfret fillets packet under different atmospheric condition during storage at 4±1°C

Storage days	CAP	CVP	SATVP
0	4.57±0.05	4.57±0.05	4.57±0.05
5	10.29±0.17	7.75±0.028	7±0.3
10	19.42±0.1	14.13±0.16	11.16±0.015
15	26.27±0.01	24.42±0.021	17.61±0.011
20	31.8±0.02	29.32±0.14	23.54±0.01
25	41.12±0.05	35.79±0.07	27.01±0.1

Values were expressed as Mean±S.D

Lakshmanan (2000) recommended a level of 30-40 mg % TVB-N in sea foods as a limit of acceptability. Therefore, it is evident that CAP and CVP samples crossed the limit of acceptability for TVB-N registering a reading of 40% after 20 and 25 days respectively. On the other hand SATVP retained the acceptable limits till the end of study although the products were sensorily rejected on 21st day. Hence TVB-N in the present study did not serve as a good index of spoilage.

The TVB-N values of vacuum packed samples were lower than CAP samples. Similar results have been obtained by Ozogul et al. (2004) who observed lower TVB-N values for vacuum packed samples than air packed samples during storage of vacuum packed sardine at 4°C. The TVB-N values of SATVP samples were found lower than control packs. Similar results have been reported by Shalini *et al.* (2000) and by Rajesh *et al.* (2002). Banks *et al.* (1980) justified that the low levels of TVB-N in treated samples were due to either a reduced bacterial population or a decreased capacity of bacteria for oxidative deamination of non-protein nitrogenous compounds or both. The same reason could be the cause for lower TVB-N values in sodium acetate treated samples. Kim and Hearnberger (1994) and Kim *et al.* (1995) observed an inhibition of aerobic Gram -ve spoilage bacteria by sodium acetate which otherwise would have caused an increase in TVB-N values.

Trimethylamine

The value of TMA-N showed (Table 6) a gradual increase ($P > 0.05$) in all samples. The TMA-N content increased from an initial value of 0.85-16.6 mg % in CAP and 11.98 mg% in CVP after 25 days of storage respectively. In case of SATVP samples, the TMA-N content rose to 8.6 mg% on 25 day of storage, which was significantly ($P < 0.01$) lower than the CAP and CVP samples.

Table 6
TMA of Silver pomfret fillets packet under different atmospheric condition during storage at 4±1°C

Storage days	CAP	CVP	SATVP
0	0.85±0.1	0.85±0.1	0.85±0.1
5	5.04±0.16	4.8±0.05	3.65±0.012
10	7±0.02	6.5±0.125	4.7±0.026
15	10.4±50.02	8.4±0.103	6.5±0.05
20	14.52±0.025	11.33±0.14	7.3±0.01
25	16.6±0.12	11.98±0.07	8.6±0.01

Values were expressed as Mean±S.D

TMA is a product of bacterial spoilage and is often used as an index to assess the keeping quality and

shelf life of seafood products (Hebard *et al.* 1982). Many of the specific spoilage bacteria on fish use TMAO as electron acceptor in an anaerobic respiration. The reduced component formed is TMA which is one of the dominant components of spoiled fish responsible for foul smell once the values reach beyond the acceptable limit. Lakshmanan (2000) recommended an acceptable level of TMA in sea foods to be 10-15 mg%. In the present study TVB-N values of all the samples except CAP were well within the suggested limit throughout the storage period.

The TMA-N content of treated sample was lower than that of control vacuum pack samples. The delay in the TMA-N formation in the treated samples could be attributed to the inhibitory effect of sodium acetate over the growth of bacteria as suggested by several authors (Parkin and Brown, 1983; Reddy *et al.*, 1995; Jensen *et al.*, 1980). On the day of sensory rejection, the TMA content of sample was 6.7 mg % for CAP, 8.4 mg% for CVP and 7.5 mg% for SATVP with a high degree of negative correlation (-0.9948) between sensory and TMA-N content observed in case of CAP.

Free Fatty Acids

A significant ($P < 0.05$) change in FFA values were observed (Table 7) in all samples during the period of study. The FFA content increased from an initial value of 2.16-6.78 % of oleic acid in CAP and 4.27% of oleic acid in CVP till the end of storage period indicating a significantly ($P < 0.05$) higher value of FFA when compared to SATVP sample. In case of SATVP samples, the FFA content rose to 3.37% of oleic acid on 25th day of storage.

Table 7
FFA of Silver pomfret fillets packet under different atmospheric condition during storage at 4±1°C

Storage days	CAP	CVP	SATVP
0	2.16±0.1	2.16±0.1	2.16±0.1
5	3.15±0.12	3.23±0.01	3.21±0.014
10	4.0±0.027	3.28±0.16	3.26±0.05
15	4.74±0.023	3.35±0.025	3.32±0.1
20	5.56±0.01	3.90.01	3.34±0.028
25	6.78±0.07	4.27±0.14	3.37±0.021

Values were expressed as Mean±S.D

The rate of formation of FFA was very slow in vacuum packed sample. The reason behind this was absence of oxygen inside the vacuum package. So, lipid was not oxidized. Similar observations were reported by Huang *et al.* (1992) and Shalini *et al.* (2000).

Sensory Evaluation

The CAP samples showed a sharp decline (Table 8) and crossed the acceptable limit before 10 days of storage. This could be due to the fish spoilage, which leads to the development of volatile, ammoniacal, strong fishy, rancid and putrid odor. The CVP sample showed sensory score value 4 on 15th day of storage. The SATVP sample crossed the acceptable limit on 25th day of storage. Therefore it could be concluded that the CAP, CVP and SATVP samples crossed their acceptability limit on 9, 15 and 21 days of storage, respectively. So, compared to CAP the CVP extended the shelf life of pomfret fillet by 6 days and SATVP further extended the shelf life by 6 days compared to CVP.

Table 8
Overall sensory score of Silver pomfret fillets packet under different atmospheric condition during storage at 4±1°C

Storage days	CAP	CVP	SATVP
0	9±0.17	9±0.17	9±0.17
5	6±0.1	7.5±0.05	8.5±0.02
10	3.5±0.025	6±0.01	7.0±0.027
15	1±0.021	4±0.16	6±0.05
20	--	2.5±0.05	4.5±0.1
25	--	1±0.13	2.5±0.16

Values were expressed as Mean±S.D; -- Not detected

The use of 2% sodium acetate was found effective in extending the shelf life by 6 days compared to untreated samples stored under the same condition. Similar results have been reported by various authors. Meekin *et al.* (1982) reported that aerobically packed refrigerated sand flat head spoiled in 8-9 days. Reddy *et al.* (1994) reported that tilapia fillets packed under 100% air spoiled after 9 days. Slightly higher shelf life of 17-18 days was reported for sea breams (Alasalvar *et al.*, 2001) and 21 days for seer fish (Rajesh *et al.*, 2002) stored in 100% air package. Extension of shelf life was also reported for various fishes by using sodium acetate treatment alone (Zhuang *et al.*, 1996; Manju *et al.*, 2007a,b, 2008; Sallam, 2007a) or with vacuum packaging (Rajesh *et al.*, 2002; Manju *et al.*, 2007a,b, 2008), which is similar to the results obtained for CVP and SATVP samples in the present study.

CONCLUSION

The vacuum packaging was effective in inhibiting the microbial spoilage to a great extent as compared to air packed samples. It extended the shelf life of the stored pomfret fillets under refrigerated conditions. It is concluded that this ready to cook pomfret fillets will definitely match with present trend of consumer

demand in urban market.

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