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Sodium Acetate and Vacuum Packaging to Improve Shelf Life of Refrigerated *Lethrinus lentjan* Fillets

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The shelf life of vacuum packed fresh *Lethrinus lentjan* fillets, untreated or treated with various levels of sodium acetate, was evaluated. The quality was assessed periodically by selected biochemical, microbiological and sensory characteristics. The shelf life of product packed without vacuum and untreated vacuum-packed fillets was around 7-8 days whereas sodium acetate treated fillets had shelf life of 2-3 weeks. Reduced rate of bacterial growth, decreased rate of production of TMA-N and TVB-N and delayed development of off odours were observed in sodium acetate treated, vacuum packed fillets.

Key words: Shelf life, vacuum packaging, *Lethrinus lentjan*, sodium acetate, refrigerated storage, quality changes

Fish, being a quickly perishable food, has relatively short shelf life. There is currently considerable interest in extending the shelf life of chilled, packed and fresh fish for supermarket/retail market (Meekin *et al.*, 1982). To promote the marketing of fresh fishery products at retail level, novel methods of packaging and storage are required. One such method of current interest is vacuum packaging. This method of packaging can be a supplement to ice or refrigeration to delay spoilage, extend the shelf life, maintain a high quality, assure the safety and reduce economic loss of fish and fishery products. Psychrotrophic bacteria are the major group of micro-organisms responsible for spoilage of fresh seafoods (Adams *et al.*, 1964). Anaerobic bacterial growth is associated with spoilage of vacuum packed seafoods (Ahuang *et al.*, 1996). One way to retard microbial growth is through antimicrobial agents. Kim & Hearnsbergber (1994) have stated that sodium acetate inhibits the growth of gram-negative bacteria on refrigerated catfish fillets. Kim *et al.* (1995a) investigated the use of sodium acetate to increase the shelf life of catfish fillets. However, information on various quality aspects of vacuum-packed fish of tropical region under refrigerated storage is scarce.

This paper reports the effects of vacuum packaging with and without sodium acetate on the shelf life of refrigerated *Lethrinus lentjan* fillets as assessed by sensory, biochemical and microbiological methods.

Materials and Methods

Fresh *L. lentjan* procured from Tuticorin fish landing centre was brought to the laboratory immediately. The fish was washed in tap water, scaled, gutted and again washed with 2 ppm chlorine water and then filleted manually. Fillets (50±5 g) were divided into three lots. Lot I was packed without vacuum(CAP) and lot II, under vacuum (CVP), to serve as controls. Lot III fillets were further divided into three sublots and dip-treated in sodium acetate solution of concentrations of 1.0%, 1.5% and 2.0%, separately for 30 min (fish: treatment solution (w/v)-1:1.5) and vacuum packed (SAVP). Four fillets were placed in each multilayer nylon barrier film pack of 25 x 35 cm size and 100 μ thickness with oxygen transmission rate of 33.2 to 60 cc/m^2/day at

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23°C and at 80% relative humidity. The lot I (air pack) was sealed using a heat sealer while remaining lots (Lot II & III) were vacuum packed at -1 bar pressure using ALFA LAVAL, Kramer - Grebe (Quick 2000A, Germany) vacuum sealing machine. The packs were stored in a refrigerator (4±1°C). Samples of control air-packs, control vacuum packs and sodium acetate treated packs were analysed for sensory, biochemical and microbiological characterisitics at intervals of 3 days.

The pH was measured using Systronics digital pH meter. 10 g of sample was homogenised with 50 ml of distilled water and pH of the homogenate was measured. Trimethyl amine nitrogen (TMA-N) and total volatile base-nitrogen (TVB-N) contents were estimated by Conway microdifussion method (Beaty & Gibbons, 1937). Free fatty acids were estimated by titrimetric method (Ke *et al.*, 1976)

Enumeration of total plate count (TPC), psychrophiles, total anaerobes, *Lactobacillus* and *Staphylococcus* were done as per the procedure of APHA (1976). 25 g of meat was taken aseptically and homogenised with 225 ml of sterile saline (0.85% NaCl). The homogenised meat was serially diluted using 9 ml saline and plated (0.1 ml) in duplicate in soybean casein digest agar and incubated at 37°C for 48 h to enumerate TPC and incubated at 5°C for 5 days to determine psychrophiles. Total anaerobes was determined by 3 tube MPN technique using fluid thioglycollate incubating at 37°C for 48 hours. *Lactobacillus* and *Staphylococcus* were enumerated on MRS agar and Baird Parker agar respectively.

Various sensory characteristics such as skin appearance, skin discolouration, gaping, texture and odour were evaluated by five panelists. Five point scale suggested by Brenner (1985) was modified and used for the present study. Scores for each attribute were pooled and the mean panel scores are presented. The data obtained were analysed using ANOVA technique (Snedecor & Cochran, 1962) to find out whether significant differences existed among samples.

Results and Discussion

Changes in the biochemical parameters during storage of the experimental samples are presented in Table 1. The initial pH of the fillet was 6.33 and on storage it increased significantly ($p<0.01$) in control and treatment packs. The increase in pH may be attributed to the production of volatile base compounds by bacterial activity (Cann *et al*, 1983). Reddy *et al* (1997) reported that increase in surface pH of 100% air-packaged tilapia fillets stored at 4°C, 8°C and 16°C may be partly due to the production of volatile basic compounds such as ammonia by bacterial action. During the end of storage period, there was a decline in pH of the treated samples. Similar results were also reported by Zhuang *et al.* (1996) in shrimps treated with sodium acetate (2% w/w, dip for 30 min) and stored at 4°C.

TMA is often used as an index to assess the keeping quality and shelf life of seafood products (Hebard *et al.*, 1982) and the TMA-N contents of fish fillets showed an increasing trend in control packs ($p<0.05$) and also in sodium acetate treated packs (SAVP). In the initial sampling, TMA-N was found to be absent. Its presence was noted on the third day of sampling in control packs and on sixth day in SAVP 1.0% and SAVP 1.5 % and on ninth day in SAVP 2.0%. The delay in the formation of TMA-N may be due to lower bacterial load in the samples. Inhibitory effect of sodium acetate over the growth of bacteria may be the reason for the delayed formation of TMA-N. Dalgaard (1983) reported that TMA-N appears to be a useful index for assessing microbial spoilage of vacuum packed and modified atmosphere packed cod fillets stored at 0°C and a concentration of 30 mg % was the upper limit of acceptability.

TVB-N comprises of mainly TMA and ammonia (NH_3) that are produced by both

Table 1. Biochemical changes in fillets of *Lethrinus lentjan* during refrigerated storage.

Parameters	Storage days	Samples				
		CAP	CVP	SAVP 1.0%	SAVP 1.5%	SAVP 2.0%
pH	0	6.33 ± 0.06	6.33 ± 0.06	6.33 ± 0.06	6.33 ± 0.06	6.33 ± 0.06
	3	6.38 ± 0.01	6.39 ± 0.08	6.37 ± 0.08	6.35 ± 0.04	6.32 ± 0.09
	6	6.69 ± 0.04	6.65 ± 0.03	6.54 ± 0.04	6.54 ± 0.04	6.52 ± 0.11
	9	6.88 ± 0.10	6.80 ± 0.07	6.60 ± 0.07	6.58 ± 0.17	6.52 ± 0.07
	12	ND	ND	6.79 ± 0.12	6.78 ± 0.12	6.65 ± 0.11
	15	ND	ND	6.86 ± 0.16	6.84 ± 0.14	6.74 ± 0.14
	18	ND	ND	6.83 ± 0.09	6.80 ± 0.09	6.72 ± 0.08
	21	ND	ND	ND	ND	6.73 ± 0.13
TMA-IN (mg %)	0	0.00	0.00	0.00	0.00	0.00
	3	9.50 ± 0.01	7.28 ± 0.07	0.00	0.00	0.00
	6	25.20 ± 0.01	19.60 ± 0.11	11.20 ± 0.03	7.28 ± 0.14	0.00
	9	45.92 ± 0.15	35.28 ± 0.25	16.80 ± 0.21	12.88 ± 0.08	6.44 ± 0.04
	12	ND	ND	19.60 ± 0.19	16.80 ± 0.06	12.58 ± 0.08
	15	ND	ND	24.64 ± 0.08	19.36 ± 0.21	15.24 ± 0.06
	18	ND	ND	38.92 ± 0.10	27.36 ± 0.10	19.08 ± 0.12
	21	ND	ND	ND	ND	23.12 ± 0.10
TVB-IN (mg %)	0	13.72 ± 0.14	13.72 ± 0.14	13.72 ± 0.14	13.72 ± 0.14	13.72 ± 0.14
	3	22.40 ± 0.12	30.80 ± 0.09	22.40 ± 0.06	14.60 ± 0.18	14.06 ± 0.12
	6	50.42 ± 0.09	68.88 ± 0.12	24.28 ± 0.06	15.68 ± 0.13	14.68 ± 0.11
	9	91.00 ± 0.10	77.84 ± 0.20	40.04 ± 0.08	33.32 ± 0.09	25.22 ± 0.09
	12	ND	ND	49.20 ± 0.13	43.40 ± 0.10	29.46 ± 0.21
	15	ND	ND	65.80 ± 0.02	59.90 ± 0.06	36.28 ± 0.16
	18	ND	ND	99.68 ± 0.06	82.88 ± 0.12	45.40 ± 0.12
	21	ND	ND	ND	ND	60.28 ± 0.04
FFA (% of oleic acid)	0	0.150 ± 0.02	0.150 ± 0.02	0.150 ± 0.02	0.150 ± 0.02	0.150 ± 0.02
	3	0.885 ± 0.02	0.543 ± 0.03	0.098 ± 0.00	0.148 ± 0.01	0.279 ± 0.03
	6	1.230 ± 0.02	0.791 ± 0.00	0.214 ± 0.01	0.225 ± 0.02	0.330 ± 0.06
	9	3.800 ± 0.10	1.003 ± 0.01	0.584 ± 0.03	0.769 ± 0.03	0.462 ± 0.05
	12	ND	ND	0.940 ± 0.07	0.728 ± 0.04	1.611 ± 0.04
	15	ND	ND	1.462 ± 0.01	1.446 ± 0.06	1.660 ± 0.01
	18	ND	ND	2.400 ± 0.06	2.540 ± 0.04	2.030 ± 0.03
	21	ND	ND	ND	ND	2.050 ± 0.02

Note : Values indicate mean of three estimations
ND : Not Done

bacterial and endogenous enzymes (Lannelongue, 1980). The TVB-N values were found to increase with storage period in control packs ($p<0.05$) and in treated packs ($p<0.01$). The TVB-N content increased to 19.0 mg % in CAP and 77.84 mg % in CVP on the day of rejection which were much higher than that of treatment packs. According to Banks *et al.* (1980) low levels of TVB-N in treated samples were a consequence of either a reduced bacterial population and/or a decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds.

Oxidation and hydrolysis of lipid in fishes during storage cause quality deterioration. Lipid hydrolysis results in the formation of free fatty acids (FFA) (Huss, 1971). In the present study, the FFA value increased significantly ($p<0.01$) with storage period and the increase in CAP was found to be more than that in CVP and SAVP samples. Husang *et al.* (1991) observed that

Table 2. Microbiological changes in fish fillets of *Lethrinus lentjan* during refrigerated storage.

Parameters	Storage days	Samples				
		CAP	CVP	SAVP 1.0%	SAVP 1.5%	SAVP 2.0%
TPC (CFU/g)	0	4.00×10^3	3.00×10^3	3.20×10^3	3.40×10^3	3.00×10^3
	3	4.80×10^6	8.50×10^5	3.60×10^4	9.50×10^4	1.28×10^4
	6	1.63×10^6	2.10×10^6	3.50×10^5	9.80×10^5	3.00×10^5
	9	4.60×10^7	9.50×10^6	1.38×10^6	1.11×10^6	1.02×10^6
	12	ND	ND	6.75×10^6	5.15×10^6	3.56×10^6
	15	ND	ND	6.90×10^6	6.55×10^6	4.00×10^6
	18	ND	ND	7.80×10^7	4.30×10^7	1.48×10^7
	21	ND	ND	ND	ND	1.60×10^7
Psychrophilic count (CFU/g)	0	7.70×10^3	7.90×10^3	6.80×10^3	5.70×10^3	5.40×10^3
	3	5.60×10^5	7.40×10^4	7.20×10^4	1.00×10^5	6.32×10^4
	6	1.63×10^7	9.10×10^6	6.70×10^5	9.80×10^5	1.34×10^5
	9	4.50×10^8	1.31×10^8	2.00×10^7	5.30×10^6	3.20×10^6
	12	ND	ND	9.40×10^7	3.24×10^7	1.01×10^7
	15	ND	ND	1.43×10^8	1.60×10^8	1.94×10^7
	18	ND	ND	4.20×10^8	2.10×10^8	1.04×10^8
	21	ND	ND	ND	ND	1.46×10^8
LAB count (CFU/g)	0	7.80×10^2	9.50×10^2	6.50×10^2	1.10×10^3	9.84×10^2
	3	1.76×10^4	2.42×10^4	6.40×10^3	5.00×10^3	3.21×10^3
	6	5.40×10^5	1.00×10^5	6.70×10^4	5.40×10^4	9.94×10^3
	9	5.90×10^5	6.00×10^5	1.94×10^5	1.90×10^5	7.50×10^4
	12	ND	ND	7.90×10^6	5.94×10^6	1.08×10^6
	15	ND	ND	3.00×10^6	9.70×10^6	2.42×10^6
	18	ND	ND	8.00×10^6	7.00×10^6	2.81×10^6
	21	ND	ND	ND	ND	5.00×10^6
Staphylococcal count (CFU/g)	0	6.00×10^2	8.20×10^2	2.90×10^2	2.70×10^2	2.71×10^2
	3	5.00×10^3	1.31×10^3	6.10×10^3	2.60×10^3	2.40×10^3
	6	1.00×10^4	1.21×10^4	2.00×10^4	5.00×10^4	3.00×10^4
	9	6.00×10^6	2.65×10^5	2.40×10^5	2.00×10^5	8.90×10^4
	12	ND	ND	5.60×10^5	4.00×10^5	2.10×10^5
	15	ND	ND	6.00×10^5	5.62×10^5	3.90×10^5
	18	ND	ND	6.58×10^5	6.10×10^5	4.54×10^4
	21	ND	ND	ND	ND	4.80×10^5
Anaerobic bacterial count (MPN/g)	0	2.40×10^2	2.40×10^2	2.40×10^2	2.40×10^2	2.40×10^2
	3	2.40×10^3	2.40×10^3	2.40×10^3	2.40×10^3	2.40×10^3
	6	2.40×10^4	2.40×10^4	2.40×10^4	2.40×10^4	2.40×10^3
	9	2.40×10^4	2.40×10^5	2.40×10^5	2.40×10^7	2.40×10^5
	12	ND	ND	2.40×10^7	2.40×10^7	2.40×10^7
	15	ND	ND	2.40×10^7	2.40×10^7	2.40×10^7
	18	ND	ND	2.40×10^7	2.40×10^7	2.40×10^7
	21	ND	ND	ND	ND	2.40×10^7

ND : Not Done

vacuum packaging did slow down the lipid hydrolysis in farm raised channel cat fish during storage in ice. However, concentration of FFA may not be considered as a reliable index of spoilage in vacuum packed refrigerated fish fillets, treated with sodium acetate.

Microbial growth in fresh seafood is the main factor associated with quality deterio-

ration, spoilage and economic loss (Zhuang *et al.*, 1996). The total plate count was found to increase significantly ($p<0.01$) with storage period Table 2. The lower count in SAVP samples might be due to the preservative action of sodium acetate. This result is in agreement with that of Kim & Hearnsberger (1994) who reported lower counts in catfish fillets treated with sodium acetate under refrigeration temperature (4°C). Psychrotrophic bacteria are the major group of micro-organisms responsible for spoilage of fresh seafoods (Adams *et al.*, 1964). The psychrophilic count increased significantly ($p<0.01$) with storage period in all the samples. By the time the samples have become organolepetically unacceptable, the counts in the treatment packs (SAVP) were found to be significantly lower compared to control samples. This again might be due to the inhibition of psychrophiles by sodium acetate. Zhuang *et al.* (1996) showed that treatment of catfish fillets with 2% sodium acetate significantly controlled the growth of psychrotrophic bacteria.

Schroder *et al.* (1980) and Meekin *et al.* (1982) reported the development of *Lactobacillus* in chill stored fish. In this study there was an increase ($p<0.01$) in their counts in all the samples during storage. In the treated samples the counts were at the level of 10^6 on the twelfth day, after which the counts remained constant until the day of rejection, probably due to the suppression of its growth by other nutritionally competing organisms. The presence of *Staphylococcus* in fish is an indication of post harvest contamination. The Staphylococcal counts increased significantly in control and treated samples ($p<0.01$). Spoilage of vacuum-packed seafood is associated with anaerobic bacterial growth (Zhuang *et al.*, 1996). There was a significant increase of anaerobic bacteria ($p<0.05$) in control samples and ($p<0.01$) treated samples. From the twelfth day onwards the anaerobic counts remained almost constant. Reddy *et al.* (1997) reported that in cat fish fillets packed under modified atmosphere and stored at 4°C, the level of anaerobic microbes was the highest during the first 13-15 days of storage and thereafter remained constant until the samples got spoiled.

The fillets are considered to be acceptable for human consumption until the sensory score reached 3 (Table 3). The shelf life of sodium acetate treated samples was higher than that of controls showing increasing shelf life with increase in sodium acetate concentration. SAVP 2% samples had highest shelf life of 19-20 days. Kim & Hearnsberger (1994) reported that the combination of sodium acetate and lactic culture could extend storage life of refrigerated catfish

Table 3. Sensory score for refrigerated *Lethrinus lentjan* fillets

Senory Score	5	4	3	2	1
Skin Appearance	Characteristic Bright	Bright	Slight loss of bright colour	Moderately dull	Faded, dull, slimy
Texture	Firm	Slightly soft	Moderately soft	Soft	Very soft
Gaping	Absent	Very slight	Slight	Moderate	Severe
Odor	Characteristic, seaweedy, sealike	Loss of characteristic odour, condensed milk	Curdy, fruity (tart)	Curdy, Sour, acidic	Putrid, sulphide
Discoloration	Absent	Very slight red tinge	Red coloration in a portion of fillet	Spreading of red coloration in major portion of fillet	Complete reddening of fillet

fillets. Combination of sodium acetate and monopostassium phosphate prolonged the shelf life of catfish up to 12 days at 4°C (Kim *et al.* 1995b). In the present study it was found that the combination of sodium acetate and vacuum packaging prolonged the shelf life of fillets.

Table 4. Changes in overall sensory score of fish fillets during refrigerated storage

Storage days	Sample				
	CAP	CVP	SAVP 1.0 %	SAVP 1.5 %	SAVP 2.0 %
0	4.80	4.80	4.80	4.80	4.80
3	4.10	4.30	4.50	4.55	4.65
6	2.30	3.80	4.35	4.50	4.60
9	2.40	2.60	4.10	4.25	4.45
12	ND	ND	3.75	4.20	4.30
15	ND	ND	2.65	3.40	3.60
18	ND	ND	ND	2.70	3.25
21	ND	ND	ND	ND	2.80

It was also observed that vacuum packaging of fish fillets alone, without preservative treatment, would not be of much use, under the said experimental conditions. Several authors have demonstrated successfully the multiple barrier technology or hurdles concept i.e. combination of several superimposing factors at sub-inhibitory concentrations that can effectively control micro-organisms in refrigerated seafood (Leistner & Gorris, 1995 and Scott, 1989). In this regard, results of this study clearly suggested that the combination of different factors viz., vacuum packaging, treatment with preservative (sodium acetate) and storage at refrigerated temperature could be used to prolong the shelf life of fish fillets to a great extent.

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