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Quality of *Salinicoccus roseus* inoculated and preservatives amended cured sin croaker, *Johnius dussumieri* (Cuvier, 1830)

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Abstract

Salinicoccus roseus, which is responsible for spoilage of cured fish, was inoculated to sterile crystalline and semi-ground salts and was challenged with chemicals. These salts were employed for curing of sin croaker (*Johnius dussumieri*). The study revealed that the use of solar salt treated with chemical preservatives *viz*., sodium metabisulphite, sodium acid phosphate, sodium benzoate, sodium tripolyphosphate, sodium hexametaphosphate and brifisol extended the shelf life of cured *J. dussumieri* from 6 months to 9 months. Quality assessment of the product that includes total bacterial count (TBC), moisture, total volatile nitrogen (TVN), free fatty acids (FFA) and peroxide value (PV) was carried out for a period of one year at quarterly intervals. The red discolouration was monitored up to the point of spoilage. The TVN and PV in the semi-ground salt cured control samples increased from 23.56 to 392.46 mg N% and from 37.41 to 491.42 meq O_2 /kg during 3 months of storage period, respectively. In the crystalline salt cured samples, the TVN and PV increased more, from 28.24 to 425.22 mg N% from 43 to 640 meq O_2/kg at the end of first quarter of the storage period. The treatment effect was more in semi ground salt cured fish than in crystallized salt cured fish.

Keywords: *Salinicoccus roseus*, shelf life, salt cured, croaker, preservatives

Introduction

Cured fish is one of the rich sources of animal protein available at an affordable price to all sections of the society. Studies revealed that spoilage occurs in 55% of salt cured fish due to red discoloration (Prasad and Rao, 1994) thus limiting the shelf life (Prasad *et al.*, 2007). The shelf life of wet cured fish can be extended from its normal 2 weeks to 6 weeks by using calcium propionate as preservative (Valsan, 1985). Employment of sodium sorbate, sodium benzoate (SB), sodium propionate and sodium bisulphate reduces microbial deterioration in salt cured fish (Santoso and Quantick, 1992). The use of SB and sodium phosphate with common salt in curing mackerel and the use of sterile salt or adding preservatives to curing salt did not alter the microbial load of the fish at the salting stage (Sachindra and Sripathy, 1992). However, the importance of SB and sodium acid phosphate in controlling the growth of red halophiles, total bacterial count (TBC) and staphylococcal count during prolonged storage of salt cured dry fish was reported (Sachindra and Sripathy, 1992). Wet salted pink perch (*Nemipterus japonicus*), prepared using a curing mixture containing common salt, SB, potassium sorbate sodium dihydrogen phosphate and butylated hydroxy anisole reported to have better keeping quality and longer shelf life over those prepared using common salt alone (Khuntia *et al.*, 1993). The quality and shelf life of salted dried Indian mackerel (*Rastrelliger kanagurta* Cuvier) was extended by using film-forming gums such as sodium alginate, tamarind kennel powder, guar gum and agar agar (Shetty *et al.,* 1996).

Among the demersal catches landed by small trawlers at Kakinada, the sciaenids (croakers) rank

first in abundance among fishes and second in abundance to prawns. *Johnius dussumieri* and *J. carutta* were the two abundant species in the sciaenid catches off Kakinada (Sriramachandramurty, 1985). To improve the quality and shelf life of cured sciaenids the chemical preservatives treated salt were used for curing and the changes in the quality of cured fish during storage period were studied.

Materials and methods

Sterilization, inoculation and chemical treatment of solar salt: Crude solar salt collected from manufacturing premises in East Godavari district of Andhra Pradesh was used for curing the fish. Half of the lot was partially ground and sieved (5 mm) to get semi-ground salt. Both crystalline and semi ground salts were sterilized by dry (160^0C for 2 hours) and wet (20 psi for 30 minutes) heat.

Salinicoccus roseus isolated from one of the commercial salt cured fish, infested with red discolouration, was used to contaminate the salt. After two weeks of growth of *S. roseus* on the solid media plates (Ventosa *et al.*, 1993), the colonies were scraped with a sterile loop into sterile 10% NaCl solution (saline). *S. roseus* was centrifuged at 5,000 rpm using refrigerated advanced high-speed centrifuge (K-24 A, Remi-India) for 10 minutes and the pellet was washed twice with sterile saline. The temperature inside the centrifuge was 5^0C. The pellet was suspended in sterile saline (10%) and with the salts to yield a uniform count of 6 logs CFU/g of the salt.

The artificially contaminated crystalline and semi ground salts were treated with chemical preservatives *viz*., sodium metabisulphite (SM, 0.5%, Loba Chemie, India), sodium acid phosphate (SAP2%, Ranbaxy, India), sodium benzoate (SB2%, Qualigens-Bombay), sodium hexametaphosphate (SHMP 2%, Qualigens-Bombay) and sodium tripolyphosphate (STPP 2%, Amrut Industrial Products, Thane, Bombay) and brifisol (3%) a combination of sodium tripolyphosphate and sodium phosphate (dibasic) in a 3:1 ratio (Benckiser-Knapsack, Laderberg/Neckar, Germany].

Fish: The sin croaker, *Johnius dussumieri* collected from the trawl catch (average length: 203 mm) of mechanized vessels were used in this study. The fish were carried in the ice boxes and the same lots were used for all the experiments.

Curing: Dry salting, the most common practice in Andhra Pradesh, was adopted for the present study. The fish were cleaned well, slit open ventrally, and after evisceration dressed in butterfly style and washed again thoroughly. The samples consisting of 28 lots of 3 kg batch each were used for curing with crystalline and semi ground salts. Two batches were used for each treatment. The dressed fish were salted in 1:5 (salt: fish) ratio and after 24 hours of holding the fish in salt, the fish were quickly rinsed in running water and were spread out in the sun, flesh side up on a raised horizontal cemented platform. After each day of drying, fish samples of each batch were stored in polypropylene bags (200 gauze). The fish were dried for three days, and during this period, the wind velocity was 17 km/h. The fish samples cured with artificially contaminated salts without chemical treatment served as controls.

The moisture, total protein, fats, ash, peroxide value (PV), free fatty acid (FFA), Ca and Fe and salt contents in fish samples were estimated by standard methods (AOAC, 1995). Phosphorous was determined from the ash content by the Fiske and Subbarow method (1925). The total volatile nitrogen (TVN) in the fresh and cured fish samples was estimated by the method of Conway (1947).

The treatment effect (TE) was calculated using the following formula (Prasad, 1999),

$$TE = \frac{\text{TVN/PV of control} - \text{TVN/PV treated sample}}{\text{TVN/PV of control}}$$

The bacteriological analysis of the fresh and cured fish samples *viz*., the total bacterial count, *Escherichia coli*, coagulase positive staphylococci, salmonella and *Vibrio cholera* were carried out following standard methods (Bennet, 1984). The counts of *S. roseus* were estimated using the media described by Ventosa *et al*. (1993). The dehydrated media and other chemicals used in this study were manufactured by HiMedia, Bombay. Statistical analyses were carried by standard methods

(Visweswara Rao, 1996). Results are the average of triplicate determinations from a composite sample for each treatment.

Red discolouration (RD): The onset of red discolouration and its spread on the fish is divided into four categories *viz*., small spots (SS), moderate red discolouration (MRD), high red discolouration (HRD) and complete red discolouration (CRD) for the convenience of spoilage assessment (Prasad, 1999).

Results and Discussion

The proximate chemical composition of the fish samples prior to salting was as follows on dry weight basis: 76.2 ± 9.6 % moisture, 19.42 ± 0.22% protein, 1.38 ± 0.02% fat, 193.12 ± 1.93 mg/100g Ca, 4.81 ± 0.03mg/100g Fe and 524.65 ± 4.19mg/100g inorganic phosphate. The average TBC was 6.14 log CFU/g. The sodium chloride content in crystalline and semi-ground salt cured sciaenids was 20.31 and 21.93 %, respectively. This is the optimum concentration of sodium chloride for cured fish (FAO, 2005).

Total bacterial count (TBC): During one-year storage period, the TBC in treated and untreated salt cured fish varied between 1 and 5.45 log CFU/g (Figs. 1 and 2). The higher count was observed in untreated cured fish samples and the count was less in salt cured fish with different preservative treatments. Among the treatments, the SB samples maintained the lowest microbial load whereas, the SHMP treated crystalline salt cured samples had microbial load similar to untreated samples. However, the microbial load in all the samples was lower than the initially introduced microbial load.

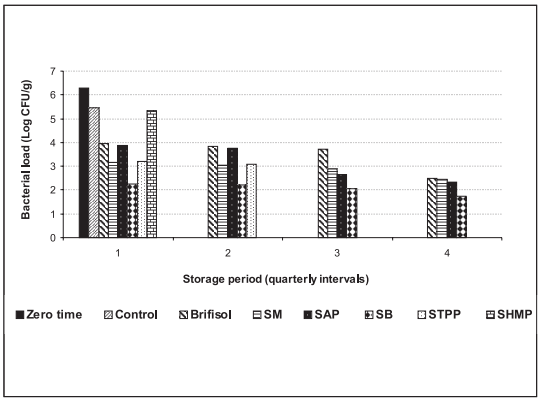

Fig. 1. Changes in bacterial load of treated crystalline salt cured sciaenids during storage (Control and STPP samples were discarded after 3 and 6 months of storage due to red discolouration); SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate

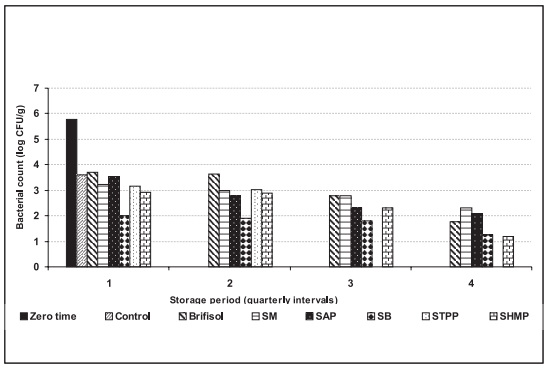

Fig. 2. Changes in total bacterial count of treated semi-ground salt (SS) cured sciaenids (S) during storage (Control and STPP samples were discarded after 3 and 6 months of storage due to red discolouration); SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate

It is clear that with few exceptions a decrease in TBC was observed from about six to three log cycles in the first three months and during the subsequent storage the decrease was gradual. Sachindra and Sripathy (1992) and Shetty *et al.* (1996) made similar observations. The decrease in TBC during the storage may be due to antibacterial action of the preservatives used or lower a_w/moisture content in the cured fish. Food preservative chemicals such as SB and potassium sorbate have been reported to retard microbial activity by inhibiting various enzymes of the microbial cell (Lueck, 1980). The TBC in the present study was low in the treated as well as control samples in comparison with commercial samples (Basu *et al.*, 1989; Prasad *et a1.,* 1994).

Moisture content: The Indian standard specifications (ISI, 1973, presently known as

Bureau of Indian Standards-BIS) for moisture content of sciaenids is 45 percent. It is clear from Figs. 3 and 4 that the moisture levels are below 45 per cent in all the treated samples. In general, fish treated with semi ground salt had lower moisture content when compared to crystalline salt treated samples. Thus, the curing of fish with semi-ground salt is more efficient in removing free water from fish tissue. The moisture of the fish samples treated with brifisol, SM, SAP, SB and STPP fluctuated marginally throughout the period of study. The variations in moisture content may be dependent on ambient temperature and RH during storage. Polypropylene bags may also be playing an important role by way of high water vapour transmission (Shetty *et al*., 1996). Wheaton and Lawson (1985) attributed the increase in moisture content of salt cured fish during storage to absorption of water by complex compounds formed by salt and proteins in fish tissue.

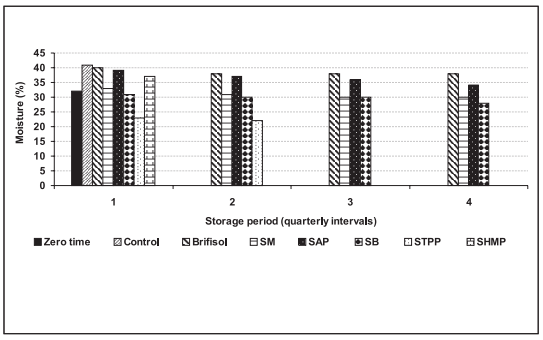

Fig. 3. Changes in per cent moisture of crystalline salt cured sciaenids during storage. Control and STPP samples were discarded after 3 and 6 months of storage due to red discolouration. SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate

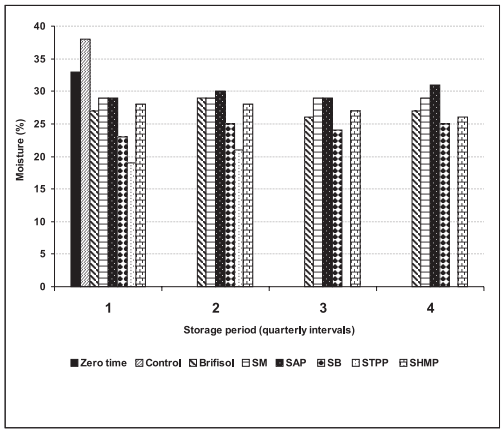

Fig. 4. Changes in per cent moisture of semi-ground salt cured sciaenids during storage. Control and STPP samples were discarded after 3 and 6 months of storage due to red discolouration. SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate

Total volatile nitrogen (TVN): The TVN increased during storage and the increase was more in crystalline salt cured fish (Table 1). This is in agreement with the reports of Adebona (1978) and Khuntia *et al*. (1993) in dry, wet, mixed salted fish and chemical preservative treated cured fish, respectively. However, the TVN in the present study are lower than the observations made by Basu *et al*. (1989) and Prasad *et al*. (1994). The threshold value of TVN as 200 mg N% was suggested to assess the quality of cured fish (Prasad and Rao, 1994). In the present study during the one-year storage in the control and in some treated samples the TVN exceeded the threshold levels. In the fish samples cured with SM amended salt, the TVN increased gradually and the rate of increase was slightly higher in crystalline salt cured fish. The TVN values of fish cured with SAP treated salt were well within the threshold levels up to six months of storage. All the STPP and SHMP treated samples showed higher TVN values and were discarded after six months of storage due to red discolouration.

The treatment effectiveness at the end of three month storage in terms of TVN in sciaenids cured with treated crystalline salt and amended with brifisol, SM, SAP, SB, STPP and SHMP was 0.88, 0.88, 0.80, 0.88, 0.39 and 0.37 and it was 0.89, 0.89, 0.89, 0.89, 0.51 and 0.81 for semi-ground salt cured samples. This corroborates the findings of Prasad *et al.* (2007).

Peroxide value (PV): The control samples were discarded after 3 months' storage due to red

Table 1. *Changes in total volatile nitrogen of treated crystalline salt (CS) and semi-ground salt (SS) cured sciaenids during storage (mg N%)*

Treatment type	Salt used	Storage period in months			
		3	6	9	12
Zero time*	CS	28.24 ± 0.01			
	SS	23.56 ± 0.01			
Control	CS	425.22 ± 0.21	D	D	D
	SS	392.46 ± 0.06	D	D	D
Brifisol (0.6%)	CS	51.27± 0.04	74.43± 0.02	156.84± 0.01	278.46 ± 0.07
	SS	42.31± 0.02	56.78± 0.03	90.47± 0.02	128.92± 0.04
SM (0.1%)	CS	50.08 ± 0.07	63.61 ± 0.02	185.26 ± 0.03	310.38 ± 0.03
	SS	41.37 ± 0.03	52.48 ± 0.04	164.17 ± 0.02	287.05 ± 0.02
SAP (0.4%)	CS	83.96 ± 0.04	112.84 ± 0.05	245.25 ± 0.02	367.93 ± 0.06
	SS	42.35 ± 0.05	100.66 ± 0.02	210.45 ± 0.03	302.87 ± 0.02
SB (0.4%)	CS	49.56 ± 0.04	79.63 ± 0.03	96.54 ± 0.03	239.79 ± 0.03
	SS	41.35 ± 0.09	48.17 ± 0.04	50.33 ± 0.01	152.41 ± 0.02
STPP (0.4%)	CS	259.72 ± 0.03	374.56 ± 0.08	D	D
	SS	190.92 ± 0.03	325.48 ± 0.05	D	D
SHMP (0.4%)	CS	269.73 ± 0.02	432.19 ± 0.04	D	D
	SS	74.17 ± 0.02	121.36 ± 0.03	194.55 ± 0.03	244.08 ± 0.01

SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate; D: discarded; Mean ± standard deviation; Zero time* : immediately after curing

discolouration. During this period the PV increased from 43 and 37 (soon after curing) to 640 and 491.42 m e q O_2/kg fat in crystalline and semi-ground salt cured sciaenids, respectively (Table 2). During the one year of storage, the PV value increased but never reached the level of the control except on two occasions. In general, the PV were lower in semi ground salt cured sciaenids but increased gradually with crystalline and semi ground salt treatments, except on a few occasions. Shetty *et al.* (1996) observed fluctuations in PV in the stored salt cured fish.

The treatment effectiveness in terms of PV at the end of the first quarter of storage year in cured samples treated with crystalline salt and amended with brifisol, SM, SAP, SB, STPP and SHMP was 0.88, 0.88, 0.80, 0.70, 0.59 and 0.41. However, semi-ground salt cured sciaenids showed better treatment effect and it was 0.92, 0.85, 0.90, 0.81, 0.75 and 0.64. Similar observations were made in the studies on *Salinicoccus roseus* inoculated, heat-treated salt cured ribbon fish (Prasad *et al*., 2007).

Free fatty acids (FFA): In all the samples, the FFA was higher in crystalline salt cured fish (Figs. 5 and 6). The FFA increased gradually during the course of storage with few exceptions. The increase in FFA content is possibly due to the formation of secondary oxidation products in the fats of fish (Shetty *et al.*, 1996). The FFA was detected during the processing and storage of Japanese jack mackerel (*Trachurus japonicus*) treated with antioxidants *viz*., butylated hydroxyanisole, butylated hydroxytoluene, tert-butylhydroquinone or tocopherol (Oshima *et al*., 1998).

Table 2. *Changes in peroxide value (milliequivalents of O_2/Kg fat) of treated crystalline salt (CS) and semi-ground salt (SS) cured sciaenids during storage*

Type of treatment	Salt used	Storage period (months)			
		3	6	9	12
Zero time*	CS	42.78 ± 0.04			
	SS	37.14 ± 0.06			
Control	CS	640 ± 0.53	D	D	D
	SS	491.42 ± 0.03	D	D	D
Brifisol (0.6%)	CS	80 ± 0.61	119 ± 0.06	142 ± 0.06	162 ± 0.09
	SS	37.14 + 0.03	88.93 ± 0.44	120.48 ± 0.04	110.34 ± 0.03
SM (0.1%)	CS	74.92 ± 0.04	252.26 ± 0.02	290.17 ± 0.02	380.23 ± 0.05
	SS	72.63 ± 0.06	162.54 ± 0.01	283.91 ± 0.06	306.23 ± 0.03
SAP (0.4%)	CS	127.79 ± 0.02	247.14 ± 0.02	238.46 ± 0.04	310.96 ± 0.04
	SS	50.37 ± 0.01	120.35 ± 0.02	194.18 ± 0.04	218.33 ± 0.02
SB (0.4%)	CS	189.63 ± 0.01	166.8 ± 0.03	292.28 ± 0.01	684.98 ± 0.04
	SS	93.62 ± 0.04	128.47 ± 0.04	267.54 ± 0.01	675 ± 0.06
STPP (0.4%)	CS	259.19 ± 0.04	583.95 ± 0.04	D	D
	SS	125 ± 0.05	353.84 ± 0.03	D	D
SHMP (0.4%)	CS	376.47 + 0.04	D	D	D
	SS	177 ± 0.12	266 ± 0.22	304 ± 0.04	428.19 ± 0.03

SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate; D: discarded; Mean ± standard deviation; Zero time*: immediately after curing

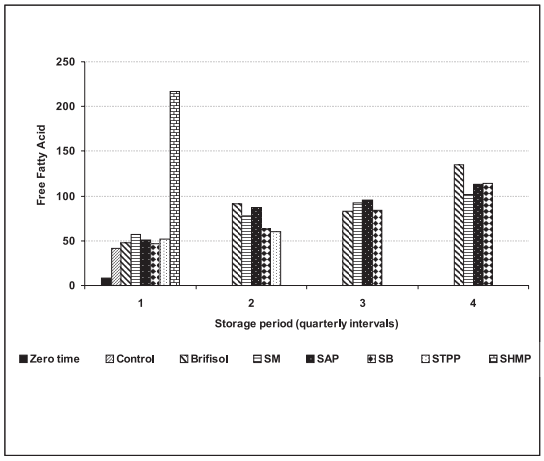

Fig. 5. Changes in free fatty acid (% of total lipids as oleic acid) contents of treated crystalline salt cured sciaenids during storage. Control and STPP samples were discarded after 3 and 6 months of storage due to red discolouration. SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate

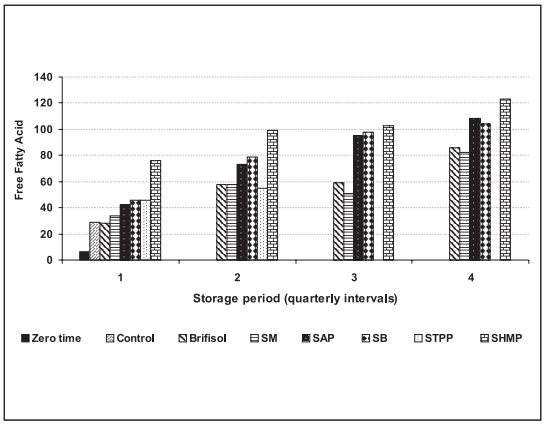

Fig. 6. Changes in free fatty acid (% of total lipids as oleic acid) contents of treated semi-ground salt (SS) cured sciaenids (S) during storage. Control and STPP samples were discarded after 3 and 6 months of storage due to red discolouration. SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium tripolyphosphate; SHMP: Sodium hexametaphosphate

Table 3. *The red discolouration and* Salinicoccus roseus *count of treated crystalline salt (CS) and semi-ground salt (SS) cured sciaenids during storage*

Treatment	Type of salt	Storage period in months				Remarks
		3	6	9	12	
Control	CS	CRD	D	D	D	RD appeared in 3 weeks
	SS	CRD	D	D	D	RD appeared in 4 weeks
Brifisol (0.6%)	CS	NC	NC	NC	ss (1.65)	CRD in 15 months
	SS	NC	NC	NC	ss (1.59)	CRD in 15 months
SM (0.1%)	CS	NC	ss (2.47)MRD (4.12)CRD (8.23)			
	SS	NC	ss (2.18)MRD (5.53)CRD (8.29)			
SAP (0.4%)	CS	ss (2.53)MRD (4.38)HRD (6.14)CRD (8.93)				
	SS	NC	ss (2.62)HRD (5.78)CRD (8.16)			
SB (0.4%)	CS	NC	NC	ss (2.87)	HRD (6.14)	CRD after 13 months
	SS	NC	NC	ss (3.52)	HRD (7.15)	CRD after 12 months
STPP (0.4%)	CS	ss (2.96)HRD (6.98)		D	D	
	SS	ss (2.95)HRD (6.07)		D	D	CRD after 8 months
SHMP (0.4%)	CS	ss (3.85)	D	D	D	
	SS	NC	NC	ss (3.25)	MRD (5.02)	

SM: Sodium metabisulphite; SAP: Sodium acid phosphate; SB: Sodium benzoate; STPP: Sodium triploy phosphate; SHMP: Sodium hexametaphosphate; NC: no change; D: discarded; ss: small spots of red halophilic growth; MRD: moderate discolouration; HRD : high red discoloration; CRD: complete red discolouration growth of red halophiles; FG: fungal growth. Counts of *Salinococcus roseus* are shown in parentheses log CFU/g of fish sample. All the sampling was done in triplicates

Red discoloration (RD)*:* Details of occurrence of red discolouration during the course of storage in control and treated sciaenids along with red halophilic counts are given in Table 3. The salt cured sciaenids without any treatment were discarded at the end of three months due to complete red-discolouration (CRD). Use of brifisol was very promising since all the brifisol treated samples were good during the one-year storage. With the use of other preservatives, the shelf life extended by three to six months as compared to that of the control. STPP and SB were successfully employed in slowing the red discoluration spoilage in salted fish (Anon, 2002).

Red halophilic counts (Salinicoccus): The small spots of red-discoluration appeared in twelve treatments at different stages and the count varied from 1.59 to 3.85 log CFU/g of the fish sample. The moderate red spoilage appeared in four treatments and the count varied from 4.12 to 5.53 log cycles. High red discolouration appeared in six treatments and the count ranged from 5.78 to 7.15 log cycles. The red spoilage was all over the fish (CRD) in six treatments and the counts varied from 8.16 to 8.93 log cycles per gram of the fish (Table 3). Prasad and Rao (1994) observed a similar trend, in which the cured fish had mean red halophilic counts of 4.32 and 7.99 log cycles when

the red discoloration was moderate and all over the fish, respectively.

This study reveals that the use of preservatives amended solar salt used for curing of fish resulted in extending the shelf life of cured fish from six to nine months over that of the control. The use of semi ground salt for curing of fish has the added advantage of superior quality, treatment effectiveness and longer shelf life than that of crystalline salt cured fishes.

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