Fishery Technology
2007, Vol. 44(2) pp : 205-212

Efficacy of heat treatment of salt contaminated with *Salinicoccus roseus* on the shelf life of cured ribbonfish (*Trichiurus lepturus*) Linnaeus 1758

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In the present study, the quality and shelf life of salt cured ribbonfish (*Trichiurus lepturus*) using heat-treated salt artificially contaminated with known density of *Salinicoccus roseus* was carried out. To determine whether crystal size of the salt had any effect on the quality of cured fish, semi-ground salt was used along with crystalline (natural) salt. *Salinicoccus roseus*, a dominant halophilic bacteria among red halophiles isolated from the commercial salt-cured fish showing red discoloration, was used to contaminate the sterile crystalline and semi-ground salts. The salts were further heat treated to test the efficacy of heat treatment of the salt on the bacteria. Shelf life studies were carried till signs of spoilage were evident. During the storage period, total bacterial count, moisture, total volatile nitrogen (TVN), peroxide value (PV) and free fatty acids (FFA) were monitored at three months intervals for one year. During three months of storage, TVN in ribbon fish cured with crystalline salt (control) reached 416.7 mg N% from an initial value of 25.33 mg N%. On the contrary, ribbon fish cured with heat treated crystalline salt (CS) and semi-ground (SS) salt had TVN 204.82 and 168.48 mg N% after 6 and 12 months storage periods respectively. The treatment effect in terms of TVN was 0.62 and 0.65 for CS and SS cured ribbon fish. The peroxide values (PV) increased in crystalline salt cured (control) samples from an initial value of 31.46 to 235 milliequivalents of O_2 per Kg of fat at the end of three month storage period. However, these values were 288.46 and 127 milliequivalents of O_2 per Kg of fat for heat treated CS and SS cured ribbon fish after 6 and 12 months storage period, respectively. The treatment effect in relation to PV was 0.60 and 0.90 for CS and SS cured ribbon fish. The present study revealed that heat treatment of solar salt used for curing of fish enhanced the shelf life of cured fish from 6 to 12 months when compared to 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.

Key words : Heat Treatment, Salt, Halophilic bacteria, shelf life, Ribbon fish

Salt curing of fish is one the most popular methods of preservation. However, bacterial spoilage, mainly red discoloration, limits the shelf life of cured fish. In tropical countries, annual losses of cured fish due to bacterial spoilage amount to 2 to 3 million tons (Clucas & Ward, 1996). The solar salt used for fish curing is an important source of contamination (Tindall, 1992; Prasad *et al.*, 1999). Several attempts have been made to control the red halophilic spoilage of salt cured dry fish by way of heat sterilization of the salt used for curing (Lamprecht & Riley, 1990; Sachindra & Sripathy, 1992). Earlier studies also revealed that heating of salt at 80ºC for 30 min destroyed red halophilic cocci (Prasad & Rao (1995).

The present study comprises the quality and shelf life of ribbonfish (*Trichiurus lepturus*) cured with heat-treated salt contaminated with known levels of *Salinicoccus*

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roseus was carried out. Apart from employing crystalline salt(natural), semi-ground salt was used to find out whether crystalline size of the salt had any effect on the quality of cured fish.

Materials and Methods

The most common varieties of fish ribbon fishes (*T. lepturus*) in fresh condition were selected for this study. Crude solar salt collected from manufacturing premises in East Godavari district of Andhra Pradesh (India) was used for curing the fish. Half of the lot was partially ground with a mechanical grinder and sieved (5 mm) to get semi-ground salt. Both crystalline and semi ground salts were sterilized by dry heat at 160°C for 2 h and wet heat at 126.5°C (20 psi) for 30 min. The dry and wet sterilization methods were employed to ensure total destruction of all types of bacteria harbored in the salt samples.

Salinicoccus roseus which is dominant among red halophiles, isolated from one of the commercial salt cured fish infested with red discoloration in the previous study (Prasad & Rao, 1995) was maintained in the laboratory and was used in the present study to contaminate the salt. The cells were cultivated by spreading a thick suspension of freshly grown *S. roseus* culture in 20% Nacl onto surface of the agar plates (Ventosa *et al*, 1993) at the rate of 2 ml/plate. After two months growth on the plates, cells were harvested by scraping the colonies with a sterile loop into sterile 20% NaCl solution. The *S. roseus* cells were centrifuged at 5000 rpm using refrigerated advanced high speed centrifuge at 5°C (type K-24 A, REMI-INDIA) for 10 min and the pellet was washed twice with the same diluent. The pellet was resuspended in sterile NaCl solution (20%) to give a final cell density of approximately 7log cfu/ml and was added at the rate of 60 ml / Kg (NaCl) salt to yield a uniform count of approximately 6 log cfu/ g of salt. Subsequent to the addition of the saline suspension of S. *roseus*, the salt was air dried under aseptic conditions and uniform distribution of the organisms achieved through mixing. The population of viable *S. roseus* in fresh fish was estimated by surface plating method (Ventosa *et al*, 1993) and the same was monitored in contaminated fish at regular intervels. Both artificially contaminated crystalline and the semi-ground salts were heat treated at 80°C for 30 minutes (Prasad & Rao, 1995).

Ribbonfish (*T. lepturus*) collected from the trawl catch of mechanized vessels were used in this study. In order to ensure uniformity of source, time of catch and quality, the fish from the same trawl catch were used. The fish were carried in iceboxes to the laboratory.

Dry salting, the most common practice in the area of study, was adopted. The fish after washing thoroughly was slit open ventrally, eviscerated, dressed in butterfly style and subjected to a second washing. A total of 24 Kg. of fish divided into two batches of 3 kg lots were used for curing with crystalline and semi ground salts that are contaminated and heat treated. The dressed fish were salted in 1:5 (salt: fish) ratio as per procedure of Sen *et al*, (1961). After holding the fish in salt for 24 h., the fish were quickly rinsed in running water and were spread out in the sun on raised horizontal cemented platform with flesh side up. Prior to drying of fish the platforms were cleaned with running water followed by chlorinated (150-ppm) water. During the three day drying the wind velocity was 17 KMPH and the relative humidity was 69%. After each day of drying the fish samples of each batch of fish were collected into polythene bags (200 gauge) and were kept

away. For drying different batches, separate platforms were provided and the space alotted for each batch was fixed thereby keeping the rate of drying uniform. The fish were dried for 3 days consequetly at 32+5°C with a total exposure of 21 hours. The fish samples cured with artificially contaminated salts with out any heat treatment served as control.

The moisture, total protein, fat, ash, peroxide value (PV), free fatty acid (FFA) and salt content in fish samples were estimated following AOAC (1995) methods. Calcium and iron content of both varieties of fish were estimated from the ash (AOAC 1965, 1970). Phosphorous was determined from the ash content by the Fiske & Subbarao method as described by Prasad *et al.* (1994). The total volatile nitrogen (TVN) in the fresh and cured fish samples was estimated by the method of Conway (1947).

The effectiveness of treatment is measured in terms of treatment effect (TE) against each biochemical parameter tested, assuming one as the case where no effect (nil) is indicated between treated and control. For example, in the case of total volatile nitrogen produced during the storage TE is expressed as,

$$\frac{\text{TVN of control} - \text{TVN of treated sample}}{\text{TVN of control}}$$

If we consider that the treated sample did not produce any total volatile nitrogen during the storage and the value is same as control, the treatment effect is taken as one. Depending on the total volatile nitrogen produced during storage, the treatment effect decreases. Accordingly the treatment effect (TE) was calculated as,

$$TE = \frac{\text{value (control)} - \text{value (treated sample)}}{\text{value control}}$$

The count of *S. roseus* was estimated by the method of proposed by Ventosa *et al.* (1993). The composition of the medium used for the growth of *Salinicoccus roseus* was g/l: 100 NaCl, 7.0 $MgCl_2$, 9.6 Mg SO_4, 0.36 $CaCl_2$, 2.0 KCl, 0.06 $NaHCO_3$, 0.026 NaBr, 5.0 proteose peptone no.3 (Difco Laboratories, Detroit, Mich.), 10.0 yeast extract (Difco), and 1.0 glucose and Agar 20.0. The pH 7.5 of the medium was adjusted with KOH. Sterile 10% NaCl solution was used as diluent throughout the study for estimation of *S. roseus* counts.

The bacteriological analysis of the fresh and cured fish samples included total bacterial count, *Escherichia coli*, coagulase positive staphylococci, salmonella and *Vibrio cholera* were carried out following standard methods (USFDA, 1984). Cured fish was cut into small pieces and 30 g cut pieces were mascerated with pestle and mortar and made up to 270 ml with saline. The dehydrated media and other chemicals used in this study were from Hi Media (Bombay).

For convenience, spoilage was assessed by noting the appearance of red discoloration on fish and its spread on the fish body. Based on the degree of discolouration on fish, spoilage was graded as four categories viz., small spots (SS), moderate red discoloration (MRD), high red discoloration (HRD) and complete red discoloration (CRD) when the fish totally spoiled.

Results and Discussion

Proximate chemical composition and test parameters associated with spoilage were recorded prior to salt curing (Table 1) to ensure the quality of fish used. The non-fatty nature and ribbon like bodies make ribbon fishes suitable for rapid preservation by sun drying. Thus, during the times of glut, large quantities of the fish are cured.

Table 1. Proximate chemical composition and microbiological quality of ribbon fish prior to salt curing

	Ribbon fish
Physical quality parameters	
Length of the fish	67+1.72 cm[1]
Chemical characteristics	
Moisture (%)	78.1+ 8.64
Protein (%)	18 + 0.19
Fat (%)	0.57 + 0.01
Ash (%)	1.4 + 0.02
Calcium (mg/100g)	169.64 + 1.16
Iron (mg/100g)	1.14 + 0.02
Inorganic phosphate (mg/100g)	650.8 + 1.03
Microbiological quality [2]	
Total bacterial count (log CFU/g)	6.32
Escherichia coli/g.	< 10
Salmonella/25g.	ND
Vibrio cholera/25g.	ND
Coagulase positive staphylococci/g.	< 10

1 Average of 35 fishes.
2. Bacteriological counts expressed in log CFU per g sample.The sampling was done in triplicates
ND - not detected

Table 2. Biochemical and bacteriological quality of ribbon fish immediately after curing with crystalline (CS) and semi-ground salts (SS)

Proximate chemical composition	CS cured	SS cured
Total volatile nitrogen (TVN) mg%	25.33+0.02	21.15+0.03
Peroxide value (milliequivalent of O_2 per Kg.fat)	31.46+0.04	28.13+0.05
Free fatty acid (percentage of total lipid as oleic acid)	5.49+0.12	4.53+0.08
Sodium chloride (%)	19.52+0.03	20.76+0.05
Moisture (%)	32.05+0.04	33.18+0.04
Bacteriological quality		
Mesophilic aerobic bacteria*	6.31	5.63

Counts expressed in log CFU per g fish sample.The sampling for all the parameters was done in triplicate

The ribbon fish samples in their fresh condition showed a total plate count (TPC) of 6 log cfu/g with *Ecoli* and coagulase positive Staphylococci < 10/g. They were free from *Salmonella* and *Vibrio cholera* (Table 1) and the samples were considered as good. The red halophilic bacteria *Salinicoccus roseus* was also not detected.

The physical, chemical and microbiological quality of the fish immediately after curing is shown in Table 2. The sodium chloride content in crystalline/ semi-ground salt cured ribbonfish varied from 19.52 to 20.76. The use of salt in 1:5 ratio (salt: fish) in curing the fish results in good quality end product with longer shelf life (Sen *et al.*, 1961).

The changes in total plate count (TPC) during the storage of ribbonfish cured with crystalline and semi-ground salt is shown in Table 3. Control samples were discarded at the end of 3 months. Heat treated samples cured with CS was discarded after 6 months while samples cured with SS was found to retain the quality even after one year. During one year storage of ribbon fish cured with SS, TPC decreased from 3.72 log cfu /g to 1.85 log cfu/g of the sample. However the microbial loads in all the samples were lower than the initial value. A decrease in TPC of about six to three log cycles was observed in the first 3 months and during the subsequent storage the decrease was found to be gradual. Sachindra & Sripathy, (1992) and Shetty *et al* (1996) made similar observations. Fish flesh containing 8 log cycles per g is considered to be spoiled and unfit for human consumption (Almas, 1981). As per Indian Standards (IS14950: 2001), a TPC of 10^5/g is proposed for dried/cured fish in domestic trade. In the present study all the heat-treated samples harbored TPC which was far below these levels. The decrease in TPC during the storage may be due to lowered water activity/moisture content in the cured fish.

Table 3. Changes in heat-treated crystalline salt (CS) and semi-ground salt (SS) cured ribbon fish during storage

Type of treatment	Salt used	Storage period (mo)			
		3	6	9	12
TPC (log CFU/g)					
Control	CS	3.45	D	D	D
	SS	3.15	D	D	D
Heat treatment	CS	3.75	3.62	D	D
	SS	3.72	3.48	2.08	1.85
Moisture (%)					
Control	CS	37.4 ± 0.02	D	D	D
	SS	36.72 ± 0.04	D	D	D
Heat treatment	CS	30.43 ± 0.04	30.33 ± 0.02	D	D
	SS	14.83 ± 0.04	15.38 ± 0.04	15.63 ± 0.03	17.15 ± 0.03
TVN (mg N%)					
Control	CS	416.70 ± 0.26	D	D	D
	SS	383.34 ± 0.05	D	D	D
Heat treatment	CS	146.32 ± 0.03	204.82 ± 0.04	D	D
	SS	58.18 ± 0.05	98.92 ± 0.03	133.83 ± 0.02	168.48 ± 0.04
PV milliequivalent of O_2 Kg.fat					
Control	CS	235 ± 0.02	D	D	D
	SS	224.2 ± 0.47	D	D	D
Heat treatment	CS	173.9 ± 0.59	288.46 ± 0.03	D	D
	SS	37 ± 0.17	68 ± 0.02	99 ± 0.38	127 ± 0.03
FFA (% of total lipids as oleic acid)					
Control	CS	27.34 ± 0.02	D	D	D
	SS	27.55 ± 0.04	D	D	D
Heat treatment	CS	46 ± 0.05	94 ± 0.04	D	D
	SS	17.42 ± 0.05	59.07 ± 0.02	54.3 ± 0.06	78.37 ± 0.02

D: discarded, Mean ± standard deviation. Results are the average of triplicate sampling.

Changes in moisture content of fish during storage are presented in Table 3. The fish cured with heat-treated salts showed lower moisture content than the control. Also, an increase in moisture content of fish during storage is seen in the samples cured with heat-treated salt. The increase in moisture content of salt cured fish during storage has been observed by Wheaton & Lawson (1985) and attributed this to absorption of water by complex compounds formed by salt and proteins in the fish tissue. In general, fish treated with semi ground salt had lower moisture content compared to crystalline salt treated samples and a substantial fall in moisture content was observed in fish that were cured with heat treated semi-ground salt. Thus, the curing of fish with semi-ground salt is more efficient in removing free water from the fish tissue.

The TVN in fresh salt cured fish is shown in Table 2. In the present study the total volatile nitrogen values have increased during the course of storage (Table 3). High total volatile nitrogen values were reported with high bacterial activity (Vanderzant *et al*, 1973). The TVN value of 200-mg N% was suggested as the threshold value for spoilage of cured fish (Sreenivasan & Joseph, 1966;

Table 4. The red discoloration and *Salinicoccus roseus* count of treated crystalline salt (CS) and semi-ground salt (SS) cured ribbon fish during storage

Type of treatment	Type of salt	Storage period (mo)				Remarks
		3	6	9	12	
Control	CS	CRD(7.84)	D	D	D	RD appeared in 5 wks
	SS	CRD(7.45)	D	D	D	RD appeared in 5 wks
Heat treatment	CS	NC(< 1.0)	SS (2.94)	D	D	
	SS	NC(< 1.0)	NC(< 1.0)	NC(< 1.0)	NC(< 1.0)	Good even after 15 months

NC; no change, D: discarded, SS: small spots of red halophilic growth, MRD: moderate discoloration, HRD : high red discoloration CRD: complete red discoloration growth of red halophiles, Counts of *Salinicoccus roseus* are shown in parentheses log CFU/g of fish sample.

Prasad & Rao, 1994). In control samples, TVN reached beyond threshold value by 3 months of storage. The TVN values of ribbon fish cured with heat-treated CS was above permissible level by 6 months while fish cured semi ground salt did not reach the threshold levels even after storage for 1 year. The result may be due to the better and even spread of heat in semi- ground salt that increased efficiency of salt in preservation.

Peroxide values also showed a pattern similar to that of TVN. Fish cured with heat treated CS salt had a shelf life of 6 months while fish cured with SS salt had a shelf life of 12 months. The effectiveness of treatment (TE) calculated for ribbon fish cured with crystalline and semi ground salts is 0.62 and 0.65 at the end of 6 and 12 months respectively. In the cured fish samples, peroxide values reached from 31 (soon after curing) to 235 me O_2 / Kg fat and 224 me O_2/ Kg in control ribbonfish cured with crystalline and semiground salt (Table 2 and 3). In heat treated fish samples, the peroxide value had increased, but never reached the level of the control except on two occasions during the 12 months storage. The treatment effectiveness in terms of peroxide value in fish samples is 0.60 and 0.90 in heat-treated crystalline and semi-ground salt fish samples after 6 and 12 months respectively. It is clear that the treatment effectiveness is higher in semi-ground than crystalline salt treated fish. This confirms the advantage of using semi ground salt for curing of fish.

The free fatty acid values recorded during the course of the one year storage period in fish cured with heat treated / untreated crystalline and semi-ground salt are shown in Table 3. In all the samples, the FFA were higher in fish cured with crystalline salt. The FFA increased gradually during the course of storage. The formation of secondary oxidation products in fats of fish possibly play an important role in higher free fatty acid values (Udgata, 1989., Shetty *et al*, 1996).

The occurrence of red discoloration during the course of storage in control and heat-treated salt cured ribbon fish along with red halophilic counts are given in Table 4. The salt cured ribbon fish without any treatment (control) were discarded at the end of 3 months due to complete red-discoloration (CRD). In ribbonfish cured with heat-treated, crystalline-salt, the red discoloration appeared after 6 months of storage while fish cured with heat-treated, semi-ground salt were good even after 15 months of storage. The longer shelf life may be due to the even spread of heat in uniform sized semi ground salt crystal resulting in better elimination of red halophiles.

This study revealed that heat treatment of solar salt used for curing of fish had a positive effect on improving the shelf life of cured fish. The shelf life of 3months observed in the case of fish cured with ordinary, un heated (control) salt was increased to 6 months for crystalline salt cured fish and 12 months for heat treated semiground salt cured fish. The study also reveals that heat treated semi-ground salt is superior to crystalline salt for curing of fish.

First author wish to thank the Director, Central Institute of Fisheries technology, Kochi, India for study leave permission.

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