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STUDIES ON THE PROLONGATION OF KEEPING QUALITY OF SHRIMP IN ICE

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Twenty dip solutions were screened for their ability to prevent black spot formation (melanosis) and quality loss during the storage of shrimp in melting ice. The criteria used besides organoleptic judgement were the bacterial count, the content of total valatile bases (TVB), trimethylamine nitrogen (TMA-N), and α - amino nitrogen and pH of the shrimp. A treatment with oxytetracycline (30 ppm) and sodium metabisulphite (800 ppm) was found to offer a practical means of preventing melanosis and improving public health and sanitary quality aspects of the distribution and marketing of shrimp.

INTRODUCTION

The shrimp industry generally faces two major problems. The first is the inhibition or prevention of the growth of microorganisms during the storage of fresh shrimp prior to processing, in particular freezing. The second is the control of the development of the enzymatic defect known as black spot (melanosis). Black spot is the most obvious defect to develop in dead raw shrimp and it can be a serious problem in shrimp intended for export.

As a solution of the first problem much interest has been shown in the use of various substances [1–8] including antibiotics as preservative for food by scientists throughout the world, they have been used to extend frozen storage life, refrigerated storage life or ice storage life. From various experiments it has become apparent that, of the antibiotics investigated to date, only the tetracycline group and to some extent, chloramphenicol hold promise as commercially practical preservatives for use in the fishing industry.

For solving the second problem sodium metabisulphite (SMB) is added to raw shrimp after capture [9]. Due to the indiscriminate use of SMB high levels of free sulphur dioxide (FSD) are generally found in shrimp meant for export. This results in the rejection of the product by the importer since high sulphur dioxide impart off-flavour to the cooked shrimp. Without the use of SMB it is difficult to control black spot effectively and its indiscriminate use make it difficult to maintain low residual sulphur dioxide (FSD) levels. In Australia a permitted limit of 10 mg/kg or less has to be maintained in shrimp for export purposes [10].

A study of the effectiveness of additives as aids in the preservation of shrimp began sometime ago in this laboratory. An extensive survey study of the effectiveness of a wide variety of substances at ice temperature has been carried out and is still in progress. The present paper reports some of the results obtained to date dealing in particular with:

(1) the effect of a number of additives on the preservation of shrimp under conditions that might be used aboard ship, and
(2) the effect of sodium metabishulphite on the flavour of fried shrimp.

Organoleptic examination alone and in combination with chemical and microbiological test for freshness of shrimps were used in the evaluation of the condition of shrimp.

MATERIAL AND METHODS

Freshly caught shrimp (4–6 hr old) were collected from Ibrahim Hyderi, packed in ice and transported to the Laboratory. The shrimps used in this study were *Peneaus merguiensis* which forms about 80% of the total catch locally known as 'Jiara', measuring 10–22 cm and weighing 20 – 25 g.

The various additives singly or in combination as specified in Table 1 were tried. For the determination of shrimp quality the following parameters were used:

(1) Organoleptic analysis, (2) Total bacterial count (TBC), (3) α -Amino nitrogen (α- NH_2–N), (4) Trimethylamine nitrogen, (TMA–N), (5) Total volatile bases (TVB), and (6) pH.

Method of Preservation. Before any treatment, a portion of the collected shrimp was examined organoleptically, analyzed for the various parameters and this was taken as zero-time sample. The remaining shrimp were dipped in chilled additive solutions overnight or for 10 min.

at room temperature. After dipping they were divided into two portions, one was deheaded and the other left intact. Treated shrimp were packed in perforated polyethylene bags placed in crushed ice and stored in refrigerator maintained at $5 \pm 1^{\circ}$ for 14 days. Melted ice water was drained off every day and ice was continually replenished. At the end of storage the shrimps were tested organoleptically, bacteriologically and chemically.

Organoleptic Analysis. The method used was based on scoring difference test described by Larmond [11]. A scale was worked out between 11 (excellent) and 1 (very poor) for each of odour, texture and colour. The score of each parameter was calculated in terms of average score points awarded by a pannel of judges to each sample.

Bacteriological Analysis. Total count was made on serial decimal dilutions by spread plate technique on nutrient agar by placing 0.1 ml of appropriate dilution in peptone water. The plates were incubated in duplicates at 37° for 48 hr.

Chemical Analysis. All chemical analyses were performed on a 5% trichloroacetic acid (TCA) extract of shrimp tail by blending for 2 min in a sample to extractant ratio of 1:3.

Amino acid nitrogen (AAN) was determined by the method of Spies and Chamber [12] modified by Cobb, *et al.* [13].

Total volatile bases (TVB) were determined according to Cobb *et al.* [13] and trimethylamine nitrogen (TMA-N) was estimated according to the modified picrate method of Dyer [14].

pH of flesh was determined after blending with distilled water (1:1) for 1 min.

Measurement of Sulphite. The free sulphur dioxide (FSD) of shrimp samples was measured by a modified method of Shipton [17]. Shrimp with FSD concentration ranging from 20 to 98 mg/kg were presented to tasters.

A multiple comparison test was conducted to determine how much SMB could be added to shrimp without tasters detecting a difference in flavour. The shrimp tested contained no FSD, 28 62 and 98 mg/kg of FSD. Each panelist was presented a reference sample R that contained no FSD and three coded samples, 12 panelists were asked to evaluate these sample according to the score sheet described by Larmond [11]. The ratings were given numerical values 1 – 9 for analyzing the results 'no difference equalling 5', 'extremely better than R' equalling 1, and 'extremely inferior to R' equalling 9. The analysis of variance was calculated.

RESULTS AND DISCUSSION

Comparison of the Effect of Various Preservatives. This experiment was designed to assess the merits of these treatments to improve keeping quality and to prevent black spot formation in ice-stored shrimp. An overnight dip in chilled solution or a dip for 10 min at room temperature gave similar results. Results obtained using an overnight dip (Table 1) show some treatments were more effective than the others. Oxytetracycline (OTC, 30 ppm), OTC (30 ppm) + NaCl 10%, OTC (30 ppm) + sodium metabisulphite (800 ppm), chloramphenicol (30 ppm)+sodium metabisulphite (800 ppm), ascorbic acid (0.5%) + OTC (30 ppm), ascorbic acid (0.5%) + chloramphenicol (30 ppm)proved to be more effective than the others as evidenced by a lower bacterial count, higher organoleptic score, and visual assessment of black spot formation. Some treatments were found to be more effective for lowering the bacterial count but had little effect on the development of black spot. For instance, no black spot developed when the shrimp were treated with sodium metabisulphite (SMB) (treatment 15) but this treatment had very little effect on the increase of bacterial number (407.5×10^6/g) and the count was as high as the water treated control (426.8×10^6/g). On the other hand when shrimp were treated with OTC treatment 16), the bacterial multiplication was checked but the shrimp developed black spots.

This experiment, in which 20 treatments were employed, and in which organoleptic and bacterial assessments were made, bring out the following facts. All antibiotic treatments gave protection against bacterial growth and thus gave a better organoleptic quality score in terms of colour, odour and texture at 14 days storage but the development of black spot was not effectively controlled. All the treatments in which SMB was employed were found to be effective in the prevention of black spot but had very little or no effect on bacterial growth. Some of the treatments were neither effective against bacterial growth nor black spot formation. On the basis of these results eight treatments (treatments No. 4, 7, 11, 16, 17, 20, 21, 22, Table 1) were selected for further study. The criteria for the selection were low bacterial count, high organoleptic score and prevention of black spot formation. In this study the shrimp were dipped for 10 min at room temperature. Four chemical tests were used as spoilage indices for the shrimp to supplement organoleptic judgement and bacterial count. This was done because these measure the end results of spoilage, that is, the complex degradation of the shrimp tissue by autolytic, and microbial actions. The results are presented in Table 2.

The chemical results show the same general pattern as the organoleptic and microbial judgements. In the case of untreated controls the TVB and TMA-N content and pH were much higher than most of the treated samples. The results similarly demonstrated an improvement in the

Table 1. Effect of various treatments on organoleptic score and growth of bacteria of shrimp in ice.

S.No.	Treatment	Organoleptic score				Melanosis*	Bacteria X 10^6
		Odour	Texture	Colour	Mean		
1	0 hr (no treatment)	9	9	9	9	–	0.12
2	Control	3.3	5.3	5.6	4.9	+++	426.8
3	Ascorbic acid (0.5%)	6.0	5.3	4.6	5.3	++	226.6
4	Ascorbic acid (0.5%) + Citric acid (0.5%)	6.3	6.3	6.4	6.3	++	138.7
5	citric acid (0.5%)	4.6	6.3	6	5.6	++	123.6
6	Disodium EDTA (1)%	5	5	5	5	++	491.7
7	Tetrasodium EDTA (1%)	5	7	7	6.3	+	368.6
8	EDTA (1%)	4	7	6	5	+++	193.2
9	Glutamic acid (p. 5%)	4	7	6	5.6	+++	136.5
10	Glucose (0.25%) + sodium chloride (0.4%)	5	7	5	5.6	+++	203.4
11	Tartaric acid (0.5%)	6	7	7	6.6	++	312.6
12	Sorbic acid (0.5%) + sodium chloride (10%)	5	5	6	5.3	+++	38.4
13	Salt brine (20%) + benzoic acid (0.5%)	4.5	5.6	5.6	6.25	+++	49.6
14	Salt brine (10%) + citric acid (0.5%)	4	6	6	5.3	+++	198.4
15	sodium metabisulphite (800 ppm)	5	6	7	6	–	407.5
16	Oxytetracycline (30 ppm)	7	7	7	6	–	3.2
17	Oxytetracycline (30 ppm) + sodium metabisulphite (800 ppm)	7	7	5	6.3	+++	3.1
18	Oxytetracycline (30 ppm) + sodium chloride (10%)	7	7	7	7	–	2.9
19	Chloramphenicol (30 ppm) + sodium metabisulphite (800 ppm)	7	7	6	6.3	++	4.3
20	Ascorbic acid (0.5%) + oxytetracycline (30 ppm)	6	6	6	6.3	+±	6.2
21	Ascorbic acid (0.5%) + chloramphenicol (30 ppm)	6	7	6	6.3	+±	5.9
22	Ascorbic acid (0.5%) + + citric acid (0.5%) + sodium metabisulphite (800 ppm)	6	6	7	6.3	±	128.6

– No black spots. ± Slightly pinkish black tail. +± Black tail with few black spots near the appendages only. ++ Black tail with few black spots on the body. + Black tail only. +++ Black tail with black spots on the whole body.

quality as compared to the untreated control as noted in the organoleptic judgement. The data presented in Table 2 further illustrate the usefulness of various criteria used to evaluate the condition of the samples. Total volatile nitrogen (TVB), trimethylamine nitrogen (TMA-N) and pH showed greater correlation than α-amino nitrogen with organoleptic and microbial judgements. The treatment with OTC (30 ppm) + SMB (800 ppm) was found to be strikingly better than other treatments in retarding bacterial growth, in preventing the black spot formation and aids in giving a product of high organoleptic quality after 14-days storage. This treatment was also effective in minimizing the subjective changes during storage. This is clearly indicated in Fig. 1.

In another experiment the relative effectiveness of some of the treatments on whole and deheaded shrimp was determined. The results are presented in Table 3. It may be seen that all the treatments gave slightly better result with deheaded shrimp. However, no satistically significant difference was noted between the results of whole and deheaded shrimps indicating that the treatments may be effectively employed in either case depending on the convenience of the fishermen.

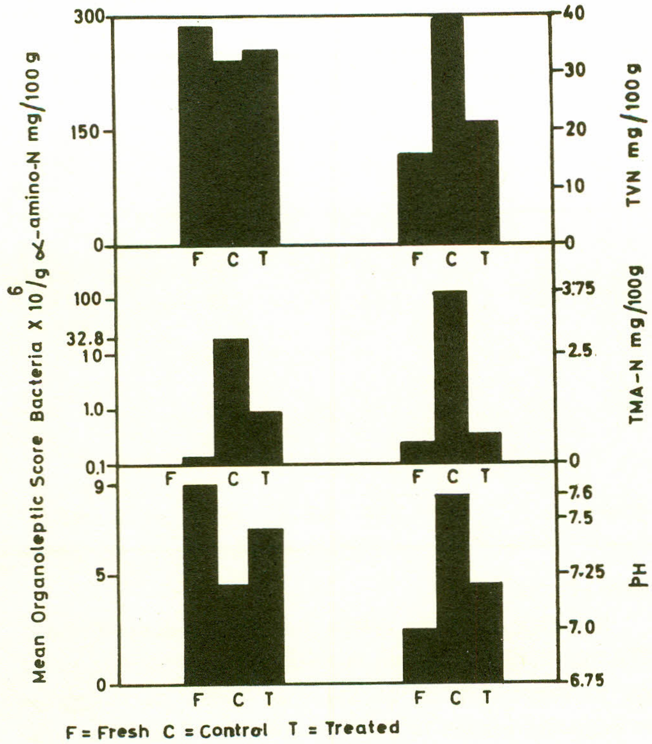

Fig. 1 A comparison of the parameters of fresh, treated and control and samples.

Overall results of these trials definitely demonstrated the superiority of OTC (30 ppm) and SMB (800 ppm) treatments when these are applied in shrimp dipping prior to ice-storage. It is clear that this method which can extend the storage-life of shrimp and check the black spot formation, will be very useful for the shrimp industry.

Residual FSD and OTC in Treated Shrimp. As SMB is widely used in the industry, it is important to know what effect it has on the eating quality of shrimp. A sensory experiment was, therefore, carried out to determine the minimum FSD level that cause a flavour change in cooked shrimp that could be detected by a panel and the nature of the flavour change due to the presence of FSD. For this purpose the peeled shrimp were soaked overnight in SMB solution of predetermined concentration. Panel testing was held using shrimp containing three levels of FSD between 28 and 98 mg/kg prepared as shown in Table 4, and a reference containing no FSD.

The data were analysed statistically. The panelists found no difference at any level between the treated samples containing different levels of FSD and the control having no FSD. The analysis of variance chart is given in Table 5.

It may be concluded that FSD up to 98 mg/kg in raw shrimp does not impart any unpleasent different flavour to shrimp.

Table 2. Effect of treatments on organoleptic, and chemical qualities of shrimp stored in ice for 14 days.

S. No.	Treatments	Organoleptic score			pH	Total volatile nitrogen (mg/100/g)	Trimethylamine nitrogen (mg/100 g).	Amino nitrogen (mg/100 g)	Bacteria $X 10^6/g$
		O	T	C					
1	0 hr (no treatment)	9	9	9	7.0	16.0	0.45	295.0	0.042
2	Control	3.3	6	5.6	7.6	42.0	3.75	241.2	32.8
3	Ascorbic acid (0.5%) + citric acid (0.5%)	6	7	6	7.55	38.0	3.25	265.0	39.2
4	Tetrasodium EDTA (0.5%)	5	7	7	7.6	33.5	3.2	245.6	29.8
5	Tartaric acid (0.5%)	6	7	7	7.65	40.0	3.1	260.0	27.6
6	Oxytetracyline (30 ppm)	7	7	5	7.15	16.5	0.75	247.5	0.73
7	Oxytetracyline (30 ppm) + sodium metabisulphite (800 ppm)	7	7	7	7.1	18.0	0.55	255.0	0.94
8	Ascorbic acid (0.5%) + oxytetracyline (30 ppm)	7	6	6	7.3	21.07	1.0	248.0	6.1
9	Chloramphenicol (30 ppm) + ascorbic acid (0.5%)	7	5	6	7.35	25.5	1.1	233.0	2.8
10	Ascorbic acid (0.5%) + citric acid (0.5%) + sodium metabishulphite 800 ppm.	6	6	7	7.6	33.0	3.0	255.0	43.2

Table 3. Effect of treatments on organoleptic, chemical and microbiological qualities of shrimp stored in ice for 14 days.

S. No.	Treatments	Organoleptic mean score		pH		Total volatile nitrogen (mg/ 100 g)		Trimethylamine nitrogen, (mg/ 100 g)		Amino nitrogen, mg/100 g)		Bacteria X 10^6 g	
		with head (O T C)	with-out head (O T C)	with head	with-out head	with head	with-out head	with head	with-out head	with head	with-out head	with head	with-out head
1	0 hr (no treatment)	9 9 9	9 9 9	7.0	7.0	16.0	16.0	0.45	0.45	295.0	295.0	0.16	0.16
2	Control	2 6 4	3.3 6 5.6	7.65	7.6	42	40	4.1	3.75	252	241.2	718.1	826.4
3	Oxytetracycline (30 ppm) + sodium metabisulphite (800 ppm)	7 7 6	7 7 7	7.25	7.2	20.8	20.8	0.85	0.65	253	247	6.2	7.8
4	Chloramphenicol (20 ppm) + sodium metabisulphite (800 ppm)	6 6 6	7 6 7	7.2	7.35	26.8	20.8	1.08	1.04	265	260	8.6	9.8
5	Sodium metabisul-phite (800 ppm)	5 6 6	5 6 7	7.5	7.5	41.0	36.3	2.9	2.68	266	264.5	411.3	529.2

Table 4. Free sulphur dioxide (FSD) content of soaking solution and of shrimp tails used in trial testing.

FSD content of soaking solutions (ppm)	FSD content of shrimps tail (mg/kg)
1600	98
800	62
400	28

Table 5. The analysis of variance chart of sensory test.

Variance source	df	SS	MS	F
Sample	3	0.75	0.25	0.25*
Panelists	11	6.42	0.58	0.58*
Error	33	32.76	0.99	
Total	47	39.92		

*Not significant at either 1 or 5% level.

In various treatments when the shrimps were dipped in SMB solution (800 ppm) for 10 min at room temperature or overnight in chilled solution the residual FSD content ranged between 41–62 mg/kg of shrimp tissue. No significant difference was found whether the shrimp were whole or peeled. The data just presented clearly demonstrated that FSD levels expected on shrimp after SMB treatment in these experimental conditions will have no effect on the flavour of the treated shrimp. The shrimp were not analyzed for residual OTC, since it has been demonstrated that tetracycline is destroyed by boiling or frying the fish tissue [15, 16].

It is clear from the results that immersion of shrimp in OTC and SMB for 10 min at room temperature is as effective in prevention of quality loss during ice storage as overnight immersion in a chilled solution. Long dips, however, will be impractical in shrimp fisheries where large quantities have to be treated quickly. This treatment offers a practical means to prevent black coloration and bacterial multiplication in the distribution and marketing of shrimp.

It is apparent that after this treatment a better grade shrimp may be delivered to the harbour in a given period of time and a longer trip may be made with maintenance of good quality in the shrimp. Better quality shrimp thus can be run through the freezing step. A combination of OTC and SMB can thus contribute significantly to the upgrading and quality control of frozen shrimp.

One of the dangers attached to the continual absorption of very small amounts of antibiotic is that a person may become sensitized. It must be emphasized that tetracycline should be used only under special conditions for those foods in which ptoection is desired up to the point of cooking. Because tetracycline is heat labile, cooking would destroy the antibiotic, so negating the theoretical possibilities of sensitization of the consumer to the antibiotic.

It is, therefore, necessary that the quantities of antibiotic used in a particular food must be regulated by law, as is the case with other preservatives. Use of CTC and OTC for fish continues to be permitted in England and Wales. Use of CTC for raw fish, as well as on peeled shrimp and shucked scallop is permitted in Canada.

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