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The Effect of Chlortetracycline on Shrimp Spoilage at Various Temperatures

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AS FOR OTHER FISHERIES PRODUCTS, quality is of prime importance in the shrimp industry. In this report two aspects of the quality problem are discussed, namely, the objective assessment of the quality of shrimp and the prolongation of the quality during handling and storage.

A number of reports have been published on the assessment of the quality of shrimp, among which may be mentioned those by Campbell and Williams (1952), Duggan and Strasburger (1946), Fieger and Friloux (1953), and

Lane and Whittaker (1953). None of the commonly used tests have proved to be of general applicability for evaluating early stages of spoilage of shrimp, where even the organoleptic judgments also vary with personal opinion and other physiological and psychological factors. In this report experiments are cited on the applicability of the content of volatile reducing substances (VRS), total volatile nitrogen (TVN) and of trimethylamine nitrogen (TMN) as measures of the state of preservation of shrimp. The VRS method has been shown to be a useful objective method to supplement and extend the value of organoleptic judgments. The content of TVN and TMN proved to have a more limited significance for the evaluation of the early stages of shrimp spoilage.

Refrigeration by ice or by cold brine has been shown by Dassow (1953), by Higman and Idyll (1952), and by Higman, Idyll and Thompson (1953) to prolong the storage life of fresh raw shrimp. Ice containing antibiotics, such as chlortetracycline (CTC), sodium chloride brine or seawater containing CTC used as a dip for shrimp, have been shown by Higman, Idyll and Thompson (1953) and by Camber (1955) to prolong significantly the storage life of raw shrimp under certain conditions beyond that of control lots. In this study the action of CTC in sodium chloride brine as a dip is reported under such categories as the effect of concentration of CTC, of dipping time and of peeling the beheaded raw shrimp.

The shrimp available for this study were mainly frozen Mexican white or brown shrimp from Lower California, with some frozen Gulf of Mexico white shrimp from Texas. These were obtained as 5 lb. glazed blocks of beheaded whole or unpeeled shrimp. They were thawed at ambient temperature for use. A stock 0.1 per cent or 1000 p.p.m. solution of CTC in distilled water was diluted with 5 per cent sodium chloride solution to give the desired final concentration of antibiotic. The shrimp were immersed in the dilute antibiotic or in plain salt solution for a specified time, separated by decanting and draining, and stored in a suitable container, such as a beaker. To minimize dehydration effects, the shrimp were covered with moist cheesecloth. Periodically each batch was examined organoleptically from which shrimp were removed for analysis. The tests used included the determination of VRS, TVN and TMN (described by Farber and Ferro, 1956) and of total aerobic bacterial numbers (described by Lerke and Farber, 1956). The content of VRS is expressed as microequivalents of reduction per 5 ml. of press juice; that of TVN and TMN as milligrams of nitrogen per 100 ml. of press juice; and the total aerobic bacterial numbers as logarithms of the number of organisms per ml. of press juice. Photographs of a single unit and of a multi-unit VRS apparatus are shown in Figures 1 and 2.

The results obtained are presented in Tables 1 to 6. The course of spoilage at 42°F. (in a household mechanical refrigerator) and at 30°F. of raw beheaded unpeeled shrimp is shown in Table 1, where the contents of VRS, TVN and TMN were measured after increasing storage intervals. Even though the TVN and TMN values showed some increase in the early stages of spoilage, the VRS values showed greater and more definite increases at the same spoilage levels. A sharper differentiation of early incipient spoilage is possible with the VRS determination than with the TVN method, which was better than the TMN determination. Shrimp spoilage at two higher temperatures, 76°F. (24.4°C.) and 82°F. (27.7°C.), is exemplified by the data of Tables 2 and 3. Here the values for VRS and log of bacterial numbers are shown after

TABLE 1

CHEMICAL CHANGES IN BEHEADED RAW SHRIMP DURING SPOILAGE AT VARIOUS TEMPERATURES

Days Stored	Storage Temperature °F					
	30			42		
	VRS	TVN	TMN	VRS	TVN	TMN
0	7.1	1.1	0	7.1	1.1	0
2	5.1	1.5	0	3.5	1.5	0
3	5.7	1.1	0	7.5	1.5	0
4	-	-	0	4.9	2.7	0
5	4.3	1.1	0	8.0	3.8	0
6	7.5	1.1	0	18.7[1]	8.3	0.7
7	9.8	3.0	0	35.9[2]	7.5	2.7
8	8.0	1.9	0			
10	16.1[1]	3.7	0.65			
11	23.0[2]	4.5	0.65			
12	35.0	6.8	1.5			
13	43.0	11.4	2.7			

VRS—Volatile Reducing Substances, as microequivalents per 5ml. press juice
TVN—Total Volatile Nitrogen } as milligrams nitrogen per 100ml. press juice
TMN—Trimethylamine Nitrogen }
1. Judged organoleptically to be at incipient stage of spoilage
2. Judged organoleptically to be definitely spoiled

TABLE 2

CHANGES IN VRS AND BACTERIAL NUMBERS IN RAW BEHEADED SHRIMP STORED AT 76°F. (24.4°C.) AFTER A 15 MINUTE DIP IN 5% SODIUM CHLORIDE SOLUTION WITH AND WITHOUT 15 P.P.M. CHLORTETRACYCLINE

Hours Stored	V. R. S.		Log Bacterial Numbers	
	Control	Treated	Control	Treated
0	6.5	6.5	4.65	4.65
18	9.5	1.0	6.80	5.80
22	13.0	6.5	7.85	6.30
26	44.0[1]	14.5	8.60	7.65
30		38.5[1]		8.50

1. Judged organoleptically to be definitely spoiled

increasing storage intervals. The progressive increase in VRS and bacterial numbers is evident. In addition, the definite though short-lived prolongation of the keeping quality by the CTC dip at the relatively high temperature is also noteworthy, as shown by the VRS and bacterial contents, as well as by the organoleptic judgment. A spoilage picture for peeled and unpeeled raw beheaded shrimp at another relatively high ambient temperature is shown by the data of Table 3. Here again the progressive increase in VRS values and bacterial numbers with storage time is clearly evident. In addition, the effect of peeling the shrimp on their keeping quality is also to be noted, as well as that of the CTC on the peeled and unpeeled shrimp. This prolongation of the storage time by the CTC and by peeling becomes much more marked

TABLE 3

CHANGES IN VRS AND BACTERIAL NUMBERS IN RAW BEHEADED SHRIMP STORED AT 82°F. (27.7°C), AFTER A 15 MINUTE DIP IN 5% SODIUM CHLORIDE SOLUTION WITH AND WITHOUT 15 P.P.M. CHLORTETRACYCLINE

Hours	V.R.S.				Log. Bacterial Numbers			
	Control		Treated		Control		Treated	
Stored	Whole	Peeled	Whole	Peeled	Whole	Peeled	Whole	Peeled
0	6.0	6.0	6.0	6.0	6.05	6.05	6.05	6.05
9	9.0	2.8	3.8	1.0	8.15	6.95	6.85	5.80
12	17.3[1]	10.3	9.5	4.5	8.20	7.65	7.30	6.60
13	21.0[1]	—	—	—	8.25	—	—	—
14	35.0[2]	14.5	18.5[1]	6.0	8.35	7.90	8.05	6.95
16	—	20.5[1]	33.5[2]	5.5	—	7.95	8.10	7.15
18	—	42.0[2]	—	7.0	—	8.00	—	7.65
19.5	—	—	—	14.0	—	—	—	7.82
21	—	—	—	35.0[2]	—	—	—	8.05

1. Judged organoleptically to be at incipient spoilage state
2. Judged organoleptically to be definitely spoiled

as the temperature is lowered, as shown in subsequent tables.

From the data presented above and from many other similar ones, it became evident that, under our experimental conditions, shrimp with a VRS content of 15 microequivalents per 5 ml. of press juice still could be considered passable for use, but were approaching the zone of early spoilage sometimes called incipient spoilage. At this stage the really fresh shrimp odors were weaker or even hardly noticeable and were accompanied to varying degrees by other odor components associated with deteriorative changes. Accordingly, in order to establish some objective criterion of the condition of shrimp treated in various ways, a VRS value of 15 microequivalents per 5 ml. of press juice was arbitrarily adopted. It was realized that this value represented a minimum value, since shrimp were still considered edible at this time and still had some storage life before any detectable off or abnormal or undesirable odors could be expected to develop. Nevertheless, it was felt that this was a reasonable

TABLE 4

EFFECT OF CHLORTETRACYCLINE (CTC) CONCENTRATION ON THE STORAGE LIFE OF BEHEADED RAW SHRIMP AT 42°F. (5.5°C.)

CTC Conc.	Days to attain a VRS Value of 15 microeq'ts per 5 ml. juice	
P. P. M.	Whole	Peeled
0	3.8	4.1
2	—	4.5
5	5.1	5.6
15	5.8	13.4
25	5.5	12.2
50	6.8	
100	7.3	

Shrimp dipped 5 minutes at each concentration dissolved in 5% NaCl solution. Averages of 3 to 5 experiments

objective criterion for use in judging the effectiveness of the diverse treatments which the shrimp were given.

Using this criterion of the condition of shrimp, the effectiveness of a number of CTC concentrations was studied with the results shown in Table 4. It is evident that beyond 15 p.p.m. no marked or significant increase in keeping time was obtained, particularly for the peeled shrimp. From practical considerations as well, including cost and uptake, it was felt that a concentration of 15 p.p.m. of CTC was a useful level with which to continue further experimentation.

Some data on the influence of length of dipping time in a 15 p.p.m. CTC in 5 per cent sodium chloride solution are shown in Table 5. A 5-minute dip

TABLE 5

EFFECT OF DIPPING TIME IN 15 P.P.M. CHLORTETRACYCLINE SOLUTION IN 5% NaCl SOLUTION ON STORAGE LIFE OF BEHEADED SHRIMP AT 42°F (5.5°C.)

Dipping Time Minutes	Days to Attain 15 Microeq'ts VRS
5	6.1*
15	6.0*
60	5.7*

*average of 3 experiments

was apparently adequate and no enhancement in effect was found with longer immersion. In fact, longer exposures apparently were less effective than the shorter times. This effect has also been noted by others, such as Camber (1955).

Peeled and unpeeled raw beheaded shrimp were dipped for five minutes in a 15 p.p.m. CTC solution in 5 per cent sodium chloride and then stored until spoiled at various temperatures. The results are given in Table 6. The increase in effectiveness of the CTC with a lowering of the temperature is quite marked. This is particularly evident for the treated samples, and even more outstanding for the peeled shrimp. To obtain the greatest benefit from a CTC treatment, temperatures around 40°F. or lower should be used.

From the data presented above a number of observations can be made. The

TABLE 6

EFFECT OF A 5 MINUTE 15 P.P.M. CHLORTETRACYCLINE IN 5% NaCl SOLUTION DIP ON THE STORAGE LIFE OF RAW BEHEADED SHRIMP AT VARIOUS TEMPERATURES

Storage Temp. °F.	Storage Temp. °C.	Days to Attain a VRS Value of 15 microeq'ts per 5 ml. juice			
		Untreated		Treated	
		Whole	Peeled	Whole	Peeled
82	27.7	0.46	0.56	0.59	0.82
76	24.4	0.94	—	1.09	—
58	14.4	1.64	1.77	1.85	2.60
53	11.7	2.0	—	2.8	—
48	8.6	2.1	—	3.8	—
42	5.5	3.8	4.1	6.1	13.4
30	1.2	9.8	10.0	16.0	37.4

VRS procedure offers a useful means to evaluate the condition of shrimp and closely parallels the organoleptic judgment. Using the VRS technic an arbitrary quality standard for shrimp held under the present experimental conditions has been adopted, based on the VRS value of 15 microequivalents per 5 ml. of press juice. Using this VRS value, the effect of various treatments could be quantitatively evaluated. The usefulness of the total volatile and trimethylamine nitrogen contents is very much less than that of the VRS content as an index of the condition of shrimp. This has also been pointed out by Fieger (1953).

A five minute dipping or immersion time and a CTC concentration of 15 p.p.m. appear to be the most useful and practical conditions to obtain a significant prolongation of the keeping quality of either unpeeled or peeled shrimp. Peeling the shrimp markedly enhances the efficacy of the CTC treatment in extending the storage time of the shrimp.

Summarizing the data presented in this report, the following points can be made:

1. The volatile reducing substances (VRS) procedure offers a useful means for the chemical evaluation of the state of freshness of shrimp.
2. A dipping or immersion time of five minutes appears to be adequate.
3. A concentration of chlortetracycline of 15 p.p.m. is adequate to obtain a significant prolongation of the keeping time of shrimp.
4. The efficacy of chlortetracycline to prolong the storage life of shrimp is influenced by the storage temperature. For significant effects storage temperatures of 40°F. or lower should be used.
5. Peeled raw beheaded shrimp are benefited by the chlortetracycline immersion to a greater extent than the unpeeled shrimp. The keeping quality has been increased more than twice for the peeled compared to that for the unpeeled raw beheaded shrimp.

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The Effect of the Antibiotic Chlortetracycline On Shrimp Freshness

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ABSTRACT

Control of bacterial spoilage in fish fillets, eviscerated, or round fish by the use of the antibiotic chlortetracycline has been demonstrated by Tarr and others. An investigation at The Marine Laboratory of the University of Miami was concerned with development of methods of application of CTC under commercial conditions to retard bacterial spoilage in pink shrimp, *Penaeus duorarum*. Results from these tests show that at levels of no less than 30 ppm (applied as a dip for five minutes) and five ppm in ice, the period preceding the rapid rise in bacterial growth can be extended by as much as seven days. Organoleptic tests showed that odor scores were higher for the treated samples than for the non-treated samples. Flavor was improved by all concentrations of CTC tested when applied as a dip. No differences in flavor were detected by the panel when shrimp were stored in CTC ices. The formation of black spot in shrimp seems to be accelerated in the presence of calcium and other bivalent ions and it was necessary to use a monovalent ion, such as potassium, in the preparation of ices containing the antibiotic. Cooked shrimp showed a considerable reduction in the residual content of CTC compared with uncooked samples, and generally less than one microgram (30 ppm CTC dip) after the ninth to tenth day of iced storage. Shrimp stored in CTC ices generally showed less than 0.15 micrograms of residual CTC during the ninth to twelfth storage day.