EXPERIMENTAL PRESERVATION OF FISH IN AUREOMYCIN ICE

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Exploratory work carried out in Canada during the last decade on the comparative value of several chemicals and antibiotics in delaying fish spoilage has indicated the possibility of increasing the storage life of iced fish by incorporating trace quantities of antibiotics in the ice (Tarr *et al.*, 1950; Boyd *et al.*, 1953; Gillespie *et al.*, 1955). Among the antibiotics tried so far aureomycin gave most encouraging results, according to the Canadian workers, who employed mainly the bacterial microscopic count for objective evaluation of the quality of fish in storage. These workers reported aureomycin to be effective at levels of 1 to 2 p.p.m. Velankar (1957) who examined the effect of aureomycin on the growth of a number of bacterial species isolated from marine sources, *i.e.*, sea-water, marine mud, plankton, fresh and spoiling sea-fish, found that at the level of 2 p.p.m. aureomycin delayed growth in 40%, while at the level of 5 p.p.m. growth was delayed in the case of 70%, of the bacteria. Work reported from the Torry Research Station, Aberdeen, based on bacteriological, chemical and organoleptic examination of the fish also indicates that a concentration of 5 p.p.m. of aureomycin in the ice is necessary for increasing the storage life significantly (Ingram *et al.*, 1956). The use of different species of fish, and also different criteria for assessing the state of preservation of the fish in storage, in the investigations of the Canadian and British workers probably explain the different findings.

The effect of emplying ice containing 5 p.p.m. of aureomycin as compared with ice containing no aureomycin, on the trimethylamine and total volatile nitrogen content as well as the bacterial population of the muscle of fish kept in ice was examined in a series of experiments in this laboratory. The increase in the capacity of the muscle to combine with iodine which has been recently suggested as a useful index of spoilage (Truttwin, 1954) was also followed. These investigations were essentially exploratory and hence relate to different species of fish and different tests for freshness. The observations are reported and discussed in this paper.

Materials and Methods

Fish in very fresh condition were obtained from shore-seine landings at Dhanushkodi and Rameswaram Road (on the Rameswaram Island) and

transported in crushed ice to the laboratory. The fish were iced immediately upon landing, unless otherwise stated (see following). The fish were kept, in the round condition, in ice containing 5 p.p.m. of aureomycin and in ordinary ice respectively. "Acronize B", a commercial preparation of aureomycin meant for incorporating in ice, was used in these investigations. The antibiotic ice was prepared freshly just before use. Trimethylamine and total volatile nitrogen contents and the bacterial count were determined according to procedures described previously (Velankar, 1952). The iodine titre was determined according to Truttwin (1954). In the first set of experiments batches of six fish were kept in ice storage and one fish at a time was removed for sampling. In a second set of experiments batches consisting of three or two fish were sampled after storage in ice for definite periods. In some of the experiments bacterial microscopic count, after prior incubation at higher temperatures, was also determined, since this procedure is stated to give a reliable indication of the potential keeping quality of fish stored at refrigeration temperatures (Tarr, 1943).

Results

The results of the first set of experiments are shown in Tables I to V.

Table I

Experiment 1. *Perch* (Lethrinus *sp.*) *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. fish muscle		Iodine titre (ml. of 0·08% iodine per g. of fish muscle)	Bacterial count (per g. of fish muscle)	Growth in peptone broth (at 0° C.) inoculated with fish muscle
		T.M.A.	T.V.N.			
4	Ordinary	2·73	21·4	7·5	5,500	–
	Aureomycin	1·39	24·31	7·2	3,800	–
7	Ordinary	3·45	27·62	8·2	10,100	+
	Aureomycin	2·10	14·00	7·95	17,500	–
14	Ordinary	10·31	27·40	9·6	24,300	+
	Aureomycin	9·14	24·40	9·3	Spreaders	–
21	Ordinary	21·10	30·40	14·63	27,900	+
	Aureomycin	16·60	29·70	12·75	9,800	+

TABLE II

Experiment 2. *Horse mackerel* (Caranx *sp.*) *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. fish muscle		Iodine titre (ml. of 0·08% iodine per g. of fish muscle)	Bacterial count (per g. of fish muscle)	Growth in peptone broth (at 0° C.) inoculated with fish muscle
		T.M.A.	T.V.N.			
5	Ordinary	1·40	20·30	7·5	..	+
	Aureomycin	Nil	16·10	9·0	..	—
10	Ordinary	5·49	32·86	9·0	<100	+
	Aureomycin	0·69	24·46	8·1	do.	—
27	Ordinary	7·54	40·19	13·5	172,000	+
	Aureomycin	5·80	32·11	12·0	12,000	+

TABLE III

Experiment 3. Lactarius *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. fish muscle		Iodine titre (ml. of 0·08% iodine per g. of fish muscle)	Bacterial count (per g. of fish muscle)	Growth in peptone broth (at 0° C.) inoculated with fish muscle
		T.M.A.	T.V.N.			
7	Ordinary	3·50	31·53	8·25	3,200	—
	Aureomycin	3·23	29·11	7·50	2,000	—
16	Ordinary	8·29	45·50	7·50	5,320,000	+
	Aureomycin	5·54	32·50	6·75	<100	—
26	Ordinary	9·00	41·06	10·87	Plates crowded	+
	Aureomycin	6·11	33·00	7·50	720,000	—

TABLE IV

Experiment 4. *Perch* (Lethrinus *sp.*) *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. fish muscle		Iodine titre (ml. of 0·08% iodine per g. of fish muscle)	Bacterial count (per g. of fish muscle)	Growth in peptone broth (at 0° C.) inoculated with fish muscle
		T.M.A.	T.V.N.			
8	Ordinary	2·01	23·37	9·00	<100	+
	Aureomycin	1·90	21·90	9·00	<100	—
19	Ordinary	5·90	35·97	10·50	3,000	+
	Aureomycin	5·00	30·50	9·75	1,000	—
25	Ordinary	No sample	..	..	..	..
	Aureomycin	6·40	32·04	Not estimated	1,000	—

In experiment 1 (Table I) significant difference in the T.M.A. values and plate count of the fish in the aureomycin and control series respectively is seen only after three weeks storage. T.V.N. and the iodine titre show little or no difference in the two treatments.

In experiment 2 (Table II) the T.M.A. and T.V.N. values indicate clearly the beneficial effect of aureomycin; the fish in aureomycin ice after 27 days in storage are comparable to fish kept in ordinary ice for 10 days.

In experiment 3 (Table III) T.M.A. and the bacterial plate count indicate that fish in aureomycin ice after 16 days storage were on the borderline of acceptance as "fresh" while after a corresponding period in ordinary ice proteolysis had commenced, as seen by the high T.V.N. content.

In experiment 4 (Table IV) the T.M.A. content and the idoine uitre of the fish is lower in aureomycin ice in the 19th day sample.

In experiment 5 (Table V) there appears to be no difference in the state of preservation of the mackerels in the two treatments during three weeks storage, as judged from the T.M.A., T.V.N. values, the iodine titre and the bacterial count. The iodine titre is high from the first week itself compared

TABLE V

Experiment 5 (a). Mackerel (Rastrelliger *sp.*) *iced immediately upon landing*

Days in storage	Type of ice used	Mg. N/100 g. fish muscle		Iodine titre (ml. of 0·08% iodine per g. of fish muscle)	Bacterial count (per g. of fish muscle)	Growth in peptone broth (at 0° C.) inoculated with fish muscle
		T.M.A.	T.V.N.			
7	Ordinary	3·21	24·67	11·40	< 100	+
	Aureomycin	3·44	24·06	7·96	< 100	−
16	Ordinary	1·62	20·06	10·50	< 100	+
	Aureomycin	3·46	27·67	11·20	< 100	−
20	Ordinary	13·46	23·56	11·50	305,000	+
	Aureomycin	12·25	27·33	11·20	275,000	+
	Experiment 5 (b). Mackerel (Rastrelliger *sp.*) *iced 5 hours after landing*					
3	Ordinary	6·20	36·80	Not estimated	300,000	Not tested
	Aureomycin	6·7	33·30	,,	200,000	
7	Ordinary	15·20	27·90	,,	300,500	
	Aureomycin	8·70	31·50	,,	250,000	

with other species of fish in the other experiments; also, unlike the other fish, the titre in mackerels does not show a progressive increase during storage in ice. In the case of mackerels which were iced after a lapse of 4 hours after landing the T.M.A., the T.V.N. and bacterial count indicate the onset of spoilage on the 3rd day of storage in both the treatments.

The appearance of growth at 0° C. in peptone broth inoculated with the muscle of fish kept in ordinary ice and the absence of such growth in the case of aureomycin-ice samples in the early days of storage, in these experiments is noteworthy.

The results of the second set of experiments are shown in Tables VI to IX.

In Table VI the T.M.A. values and the microscopic counts of the fish in the first batch (8 days storage) indicate an advantage with the antibiotic ice. In the second batch (16 days storage) the T.M.A. values show a scattering, but the plate count as well as the microscopic count are lower for the fish in aureomycin ice.

TABLE VI

Experiment 6. *Milk-fish* (Chanos) *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. of fish muscle		Bacterial plate count (per g. of muscle)	Bacterial microscopic count (per g. of muscle) after incubation for 5 hrs. at room temp. (27° C.
		T.M.A.	T.V.N.		
8	Ordinary ice:				
	Fish No. 1	3·8	8·1	Not determined	179×10^6
	Fish No. 2	2·9	10·5	,,	155×10^6
	Aureomycin Ice:				
	Fish No. 1	nil	5·8	,,	$33\cdot17\times10^6$
	Fish No. 2	nil	7·9	,,	$22\cdot05\times10^6$
16	Ordinary Ice:				
	Fish No. 1	8·7	20·3	52,600 (Sea-water agar)	320×10^6
	Fish No. 2	nil	13·05	Not determined	410×10^6
	Fish No. 3	4·1	15·38	,,	300×10^6
	Aureomycin ice:				
	Fish No. 1	5·6	11·00	28,100 (Sea-water agar)	32×10^6
	Fish No. 2	0·48	9·65	Not determined	130×10^6
	Fish No. 3	nil	10·35	,,	125×10^6

In experiment 7 (Table VII) the microscopic count is distinctly lower for the fish in aureomycin ice; the iodine titre as well as the T.M.A., T.V.N. values indicate no difference in the two treatments.

In experiment 8 (Table VIII) the T.M.A. values and the microscopic as well as plate counts indicate clearly the superiority of aureomycin ice over ice containing no aureomycin.

The indications in experiment 9 (Table IX) are similar to those in experiment 8 (above). In both the experiments 8 and 9, the fish kept in aureomycin ice were still in fresh condition, unlike the fish stored in ordinary ice, after 23 days storage.

From the results of the second set of experiments the keeping quality of horsemackerel and perch appear to be superior to that of milkfish or morral.

Organoleptically the fish kept in aureomycin ice remained in a better condition than the fish in ordinary ice; the flesh was usually more firm and showed no bleaching unlike in the ordinary ice. Markedly offensive odours rarely developed either in the control or experimental ice, even after 3 to 4 weeks storage.

TABLE VII

Experiment 7. Morral (Hemirhamphus) *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. of fish muscle)		Iodine titre (ml. of 0·08% iodine per g. of muscle	Bacterial microscopic count (per g. of muscle) after incubation for 5 hrs. at room temp. (27° C.)
		T.M.A.	T.V.N.		
10	Ordinary ice:				
	Fish No. 1	8·28	19·74	4·12	
	Fish No. 2	6·99	20·98	4·81	$124·8\times10^6$
	Aureomycin ice:				
	Fish No. 1	8·19	23·88	4·47	
	Fish No. 2	7·06	18·8	4·12	$58·65\times10^6$
16	Ordinary ice:				
	Fish No. 1	Not determined		5·4	172×10^6
	Fish No. 2	,,		6·1	135×10^6
	Fish No. 3	,,		5·4	$344·5\times10^6$
	Aureomycin ice:	,,		5·27	$79·6\times10^6$
	Fish No. 1				
	Fish No. 2	,,		5·1	$87·0\times10^6$
	Fish No. 3	2		5·2	$72·0\times10^6$

TABLE VIII

Experiment 8. *Perch* (Lethrinus) *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. of fish muscle)		Bacterial plate count (per g. of muscle	Bacterial microscopic count (per g. of muscle) after incubation at 40° F. for 24 hrs.
		T.M.A.	T.V.N.		
10	Ordinary ice:				
	Fish No. 1	1·0	12·5	Not determined	$23·3\times10^6$
	Fish No. 2	1·02	10·73	,,	$27·4\times10^6$
	Fish No. 3	1·04	10·93	,,	$19·5\times10^6$
	Aureomycin ice:				
	Fish No. 1	nil	11·9	,,	Less than 1 million
	Fish No. 2	0·54	11·39	,,	,,
	Fish No. 3	0·53	11·09	,,	,,
23	Ordinary ice:				
	Fish No. 1	7·1	22·5		$232·4\times10^6$
	Fish No. 2	6·9	21·5		150×10^6
	Fish No. 3	6·8	20·4	2,030,000	155×10^6
	Aureomycin ice:				
	Fish No. 1	2·0	18·3		Less than 1 million
	Fish No. 2	1·5	19·0		,,
	Fish No. 3	1·8	17·5	9,500	,,

A number of bacteria were isolated during these studies and among these gram-negative achromic rods preponderated; pink yeasts and some *Nocardia* spp. were so isolated, mainly from fish kept in aureomycin ice. Most of the bacterial isolates were identified with types described in a previous paper by the authors (Velankar and Kamasastri, 1956). Denitrifying bacteria were present in most of the samples of iced fish.

DISCUSSION

During the first week of storage there appears to be no advantage with aureomycin ice but its beneficial effect becomes perceptible in the subsequent period of storage. The results further indicate that fish when properly iced can remain in good condition up to 7 or 8 days, without the aid of chemical or antibiotic preservatives. The bacterial count and the T.M.A. content of the fish muscle usually indicate the superiority of ice containing 5 p.p.m. of aureomycin over ordinary ice. The result of muscle inoculation into peptone broth and subsequent incubation at 0° C. (Tables I to V) show

TABLE IX

Experiment 9. Horse mackerel (Caranx *sp.*) *kept in ice*

Days in storage	Type of ice used	Mg. N/100 g. of muscle		Iodine titre (ml. of 0·08% iodine per g. of muscle)	Bacterial plate count (per g. of muscle)	Bacterial microscopic count (per g. of muscle) after incubation for 24 hrs. at 40° F.)
		T.M.A.	T.V.N.			
10	Ordinary ice:					
	Fish No. 1			Not determined	> 100	$8{\cdot}0\times10^6$
	Fish No. 2			,,	,,	$10{\cdot}0\times10^6$
	Aureomycin ice:			,,	nil	Less than 1 million
	Fish No. 1			,,		
	Fish No. 2			,,	,,	,,
15	Ordinary ice:					
	Fish No. 1	2·23	14·85	4·92	> 100	Not determined
	Fish No. 2	1·5	11·34	4·12	,,	,,
	Fish No. 3	2·1	12·5	4·12	,,	,,
	Aureomycin ice:					
	Fish No. 1	nil	6·85	3·4	nil	,,
	Fish No. 2	,,	4·85	,,	,,	,,
	Fish No. 3	,,	8·65	,,	,,	,,
23	Ordinary ice:					
	Fish No. 1	3·26	16·31	Not determined	{125,000 (Sea-water agar) { 33,600 (Fresh-water agar)	120×10^6
	Fish No. 2	6·70	20·09	,,	Not determined	148×10^6
	Aureomycin ice:					
	Fish No. 1	0·96	7·66	,,	{11,000 (Sea-water agar) { 3,000 (Fresh-water agar)	37×10^6 37×10^6
	Fish No. 2	1·20	9·03	,,	Not determined	$43{\cdot}0\times10^6$

a paucity of psycrophiles in fish preserved in aureomycin ice as compared with fish kept in ordinary ice. This observation is significant since spoilage in iced fish can be caused only by the psycrophilic bacteria, the mesophiles having little or no influence (Castell *et al.*, 1948).

Truttwin recorded values of 13 to 14 ml. for the iodine titre, at the stage when the fish were considered spoiled, in the case of cod, haddock, plaice and whiting, the corresponding duration in ice storage being 12 to 15 days (Truttwin, 1955). In our investigations this value for the iodine titre was rarely reached after two weeks in ordinary ice and the usefulness of this test for assessing freshness, at least in relation to Indian fish, needs further examination. Evidence indicating that the volatile base content of the fish muscle can be relatively high without the bacterial count increasing significantly has been forthcoming recently (Horie *et al.*, 1956). On the other hand (Tarr, 1953) found that bacterial spoilage often occurred without any significant increase in the T.M.A. content, in the case of the Pacific fishes which he examined. Our observations, in the case of Indian fishes, suggest that the determination of trimethylamine as well as the bacterial count may be necessary for ascertaining the state of preservation of fish in storage (Velankar, 1952 and 1956). The results of experiment 5 *b* stress the necessity of icing the fish immediately upon landing.

The experimental studies indicate that the use of ice containing aureomycin may prove advantageous if the storage period is likely to exceed about 8 days.

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