Effectiveness of EDTA Dips on the Shelf-life of Oil Sardine *(Sardinella longiceps)*, Mackerel *(Rastrelliger kanagurta)* and Prawn *(Metapenaeus dobsoni)* in Iced Storage*

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Fresh oil sardine, mackerel and prawn were dipped in 0.1% and 1% solutions of Na_2EDTA, and stored in ice. Their storage-life was assessed by bacteriological, chemical and sensory methods. Eventhough EDTA treatment controlled the increase in bacterial counts and reduced TMA and TVBN production in oil sardine and mackerel, the consequent beneficial effect was not realised because of the deterioration of fat in these fishes, leading to rancidity. But, for prawn stored in ice, a dip in 1% solution of Na_2EDTA enhanced the shelf-life by at least 8 days over the untreated control. EDTA absorbed by the muscle of fish and prawn during dip in Na_2EDTA solution is not completely removed during their iced storage for 25 days.

Earlier works on chemical preservation of fish, particularly from the temperate waters, are well documented by Tarr (1961). Velankar & Kamasastri (1958), Surendran & Iyer (1971 & 1973) and Anand & Setty (1981 a) studied the effect of antibiotics on the bacteria causing spoilage of tropical fish. Possibility of the use of parabens and various other chemicals to preserve fish has been reported by Anand & Shetty (1981 b, c;) and Surendran & Gopakumar (1982 a).

Various sodium salts of ethylene diamine tetra acetic acid (EDTA) have been shown to be effective as dipping solutions for extending the storage life of raw fish (Levin, 1967). Boyd & Southcott (1968) tried Na_2EDTA and $Na_2CaEDTA$ for preservation of coho-salmon, leman sole and Pacific cod during refrigerated storage and found that Na_2Ca-EDTA was not effective, while Na_2EDTA was. Power *et al* (1968) found that a 1% solution of Na_4EDTA used as a dip for haddock could extend the storage life in ice for 11 days over the control. However, no work has so far been reported on the application of EDTA to preserve tropical fish, except the studies on the selection of bacterial flora in EDTA treated oil sardine, mackerel and prawn (Surendran&Gopakumar 1982 b). The results of our experiments on the use of EDTA dips to preserve oil sardine, mackerel and prawn in iced storage are presented in this paper.

Materials and Methods

Fresh oil sardine, mackerel and prawn were procured from fishing craft operating off Cochin and brought to the laboratory within 2 to 4 h, after catch.

Disodium ethylene diamine tetra acetate (Na_2EDTA) solutions (aqueous) of 1000 p.p.m. (0.1% w/v) and 10,000 p.p.m. (1% w/v) strength were used as dip solutions. The fish/prawn were dipped in the Na_2EDTA solutions for 10 min., drained well and packed in ordinary crushed ice, in the fish/prawn to ice ratio of 1:1 and stored in thermocole insulated ice boxes. In all cases, untreated prawn/fish stored in ordinary ice served as the control. The samples were stored for 20-30 days and ice loss was made up by addition of crushed ice, usually on alternate days.

Shelf-life of the fish and prawn was evaluated on the bais of total plate count (TPC), trimethylamine nitrogen content (TMAN), total volatile base nitrogen content (TVBN), volatile acid number (VAN), peroxide value (PV) and sensory qualities. The samples were analysed immediately on the beginning

*Formed part of the Ph. D. thesis of the first author (University of Kerala, Trivandrum) of the study and after intervals of 3 to 5 days during storage. TPC was determined using seawater agar (SWA) as described by Surendran (1980). TMAN and TVBN were determined by the Conway microdiffusion method (Conway, 1947) and VAN, PV, moisture and fat by the methods of A.O.A.C. (1960). Sensory evaluation of the samples were made in the raw and cooked state by a taste panel, as detailed by Surendran (1980). The EDTA content of the muscle was determined by the method of Sinclair & Power (1968).

Results and Discussion

(a) Shelf-life of EDTA treated fish and prawn during iced storage

(1) *Oil sardine*

Tables 1 and 2 present typical results of the storage study of Na_2EDTA treated oil sardines. It is evident from Table 1 that the bacterial counts of the control samples increased quite rapidly during storage, while the EDTA treated samples registered lower counts. Of the two concentrations, 0.1 and 1% (w/v), of Na_2EDTA the sample dipped in 1% Na_2EDTA had lower counts during the storage. The organoleptic scores of the cooked muscle of the samples showed trends similar to changes in bacterial counts.

The chemical indices like TMAN and TVBN (table 2) showed great variations among the control and treated samples. While the TMAN values of the control sample increased very much during storage, the same in the Na_2EDTA treated samples remained low, in spite of the fact that such great differences were not reflected in their bacterial populations. However, the changes in VAN and PV values were similar to the bacterial counts as well as organoleptic scores.

(2) *Mackerel*

Tables 3 and 4 show the effect of Na_2EDTA treatment on bacteriological, chemical and organoleptic indices of mackerel held in ordinary ice storage. Only a dip in 1% Na_2EDTA solution had been tried.

The changes in total bacterial count, TMAN, TVBN, VAN, PV and overall organoleptic scores of the cooked muscle, were more or less similar to those in oil sardines. The TMAN values of the Na_2 EDTA treated fish were appreciably low compared with the control.

Table 1. *Shelf life of Na_2EDTA treated oil sardines stored in ice*

		Concentration of Na_2EDTA in dip solutions		
Shelf life index	Days of storage	Control (Na_2EDTA)	0.1% (1000 p.p.m)	1% (10000 p.p.m)
	0 (before treatment)	4.08×10^3	4.08×10^3	4.08×10^3
Total plate count/g muscle (TPC)	4	7.25×10^4	1.68×10^3	2.06×10^3
	8	2.71×10^6	5.91×10^4	3.91×10^3
	12	5.11×10^7	2.04×10^5	1.86×10^4
	16	3.47×10^8	2.23×10^6	9.14×10^4
	24	2.08×10^9	1.81×10^8	3.32×10^6
Overall organoleptic score of cooked muscle	0	20	20	20
	4	10	12	12
	8	6	8	10
	12	0	2	4
	16	—	0	0
	24	—	—	—

Table 2. *Shelf life of Na_2EDTA treated oil sardines stored in ice*

Shelf life index	Days of storage	Concentration of Na_2EDTA in dip solutions: Control (No EDTA) TMAN	Control (No EDTA) TVBN	0.1% (1000 p.p.m) TMAN	0.1% (1000 p.p.m) TVBN	1% (10000 p.p.m) TMAN	1% (10000 p.p.m) TVBN
TMAN-mg % and TVBN-mg %	0 (before treatment)	1.45	6.40	1.45	6.40	1.45	6.40
	4	3.80	14.65	1.45	8.28	1.38	7.25
	8	4.70	18.10	1.60	8.16	1.49	7.68
	12	9.20	24.85	1.75	12.62	1.82	10.55
	16	14.15	29.25	2.20	16.66	1.96	10.76
	24	22.20	36.70	3.86	21.25	2.14	15.28
		VAN	PV	VAN	PV	VAN	PV
VAN and PV	0 (before treatment)	6.72	7.65	6.72	7.65	6.72	7.65
	4	9.86	18.50	7.79	11.28	6.68	6.52
	8	12.75	26.26	8.92	14.65	7.11	10.66
	12	15.08	29.11	11.65	18.22	9.92	12.08
	16	18.65	36.79	14.38	21.67	12.65	18.65
	24	24.28	42.34	21.97	28.08	18.78	24.34

Table 3. *Shelf life of Na_2EDTA treated mackerel during iced storage*

Shelf life index	Days of storage in ice	Control (No EDTA)		Dipped in 1% Na_2EDTA and stored in ice	
Total plate count/muscle	0 (before treatment)	2.176×10^4		2.176×10^4	
	4	3.390×10^4		3.112×10^4	
	7	5.62×10^5		3.826×10^4	
	12	1.903×10^7		6.071×10^4	
	17	2.271×10^8		4.795×10^6	
	24	8.02×10^9		7.210×10^7	
		TMAN	TVBN	TMAN	TVBN
TMAN-mg/100 g. and TVBN-mg/100 g muscle	0	2.52	7.90	2.52	7.90
	4	3.75	11.65	2.28	6.85
	7	5.60	19.28	2.70	9.42
	12	10.38	25.20	3.04	12.75
	17	15.25	28.05	4.60	11.92
	24	19.70	40.80	6.75	15.90

(3) *Prawn*

Results of a typical storage study of Na_2-EDTA treated prawns are presented in Tables 5 and 6. Na_2EDTA solutions of 0.1% and 1% strength have been used for dip treatments.

The total bacterial population of the untreated prawns was always greater compared

Table 4. *Shelf life of Na_2EDTA treated mackerel during iced storage*

Shelf life index	Days of storage in ice	Control (No EDTA)		Dipped in 1% Na_2EDTA and stored in ice	
		VAN	PV	VAN	PV
VAN and PV	0	5.40	6.60	5.40	6.60
	4	8.98	11.85	6.20	6.78
	7	14.25	19.70	8.45	9.25
	12	20.82	26.25	9.70	14.80
	17	26.65	31.75	14.24	16.22
	24	30.20	38.24	18.90	24.85
Overall organoleptic score of the cooked muscle	0		20	20	
	4		14	14	
	7		8	12	
	12		4	8	
	17		0	2	
	24		0	0	

Table 5. *Shelf life of Na_2EDTA treated prawn stored in ice*

Shelf life index	Days of storage	Concentration of Na_2EDTA in dip solutions		
		Control (No EDTA)	0.1% (1000 p.p.m)	1% (10000 p.p.m)
Total plate count/g muscle	0 (before treatment)	2.143×10^4	2.143×10^4	2.143×10^4
	3	6.741×10^3	7.198×10^3	6.674×10^3
	7	4.834×10^4	3.027×10^3	1.116×10^3
	12	2.771×10^6	1.716×10^5	5.815×10^4
	18	1.518×10^7	1.920×10^6	6.291×10^6
	25	1.936×10^9	6.813×10^8	3.926×10^8

with the Na_2EDTA treated samples. Between the two Na_2EDTA treated samples, much difference in the bacterial counts was not noticed during the entire iced storage.

The differences in the TMAN values among the control and EDTA treated samples were very significant. While a TMAN value of 22.86 mg/100g muscle of the untreated prawns was reached after 25 days in ice, the corresponding values in the 0.1% and 1% Na_2EDTA treated prawns were 6.95 mg% and 4.92 mg% respectively.

The TVBN and VAN values exhibited the same trend as that of bacterial counts.

Organoleptically, the Na_2EDTA treated samples were much superior to the control. The control showed the symptoms of spoilage in 10 to 12 days of iced storage. But the Na_2EDTA treated samples remained in acceptable conditions even after 16 to 18 days in ice-storage. Even after 18 days of iced storage, the samples dipped in 1% Na_2 EDTA solution did not develop foul smell indicative of spoilage, eventhough the bacteial count exceeded 6 millions per gram muscle.

It can be noted from the results on oil sardine, mackerel and prawn (Tables 1 to 6) that, eventhough the bacterial counts of

Table 6. *Shelf life of Na_2EDTA treated prawns stored in ice*

Shelf life index	Days of storage	Control (No EDTA) TMAN	Control (No EDTA) TVBN	0.1% (1000 p.p.m) TMAN	0.1% (1000 p.p.m) TVBN	1% (10000 p.p.m) TMAN	1% (10000 p.p.m) TVBN
	0 (Before treatment)	3.26	12.88	3.26	12.88	3.26	12.88
TMAN mg% and TVBN mg%	3	5.80	16.20	3.14	14.25	3.40	12.74
	7	7.66	17.74	3.70	16.06	3.40	15.80
	12	8.15	19.63	5.21	16.28	4.14	15.45
	18	15.20	24.14	5.26	17.15	4.75	17.60
	25	22.86	30.80	6.95	21.60	4.92	19.85
		VAN	O.S.	VAN	O.S.	VAN	O.S.
	0 (Before treatment)	11.60	20	11.60	20	11.60	20
VAN and overall organoleptic score of cooked muscle (O.S.)	3	14.85	16	11.85	16	11.25	16
	7	18.08	12	12.08	14	11.73	14
	12	17.60	8	15.26	14	14.22	14
	18	19.25	2	17.88	8	16.75	10
	25	24.15	0	22.05	4	19.88	4

the Na_2EDTA treated samples were almost one log cycle less than the counts of the respective control samples for the same period of storage in ice, the rate of increase in counts did not markedly differ between the untreated and Na_2EDTA treated samples.

The improvement in shelf-life, as evidenced from TMAN and TVBN values was not reflected in the bacterial counts. Especially the observation that the TMAN values were significantly low in Na_2EDTA treated fish and prawns in comparison with untreated controls implied that EDTA might preferentially affect TMA producing group of bacteria. Similar observations have been made by Pelroy & Seman (1969) in the case of EDTA treated petrale sole and ocean perch fillets. These authors have reported that a dip in 1% solution of EDTA extended the shelf-life at 0.5°C of petrale sole and ocean perch fillets by repressing growth of *Pseudomonas* spoilage organisms. They found that Na_2 EDTA and Na_4EDTA were the most effective, extending the shelf-life of ocean perch fillets by 7–10 days whereas $Na_2CaEDTA$ gave a 4 day extension of shelf-life over the control fillets.

Power *et al* (1968) have found that a 1% solution of Na_4EDTA used as a dip could extend the storage life in ice, of haddock *(Melanogrammus aeglefinus)* fillets by 11 days over that of the untreated controls. They also noticed that TMA production was considerably reduced, but the growth of bacteria was not at all affected by EDTA treatment. Levin (1967), and Boyd & Southcott (1968) have also found that the rate of increase of total bacterial population on haddock and salmon fillets was not affected by Na_4EDTA.

The fact that uncharacteristic, although not unpleasant tastes were noted in later stages of strorage of EDTA treated fish and prawn suggested that the normal spoilage pattern was altered by EDTA treatment. This idea is further reinforced by the fact (as pointed out earlier) that there was a definite repression of TMA values in the treated samples in comparison with untreated controls, but at the same time, there was no significant difference in total counts. It is possible that EDTA has a selective inhibitory action on the growth of TMA producing bacteria, thus allowing competitive strains to grow at a faster rate (Power *et al.* 1968).

Even though the data presented in Tables 1 to 4 show that EDTA treatments controlled the increase in bacterial counts and reduced the TMAN and TVBN production in oil sardines and mackerel, the consequent beneficial effect was not realised because of the deterioration of fat in these fishes, leading to rancidity, as evident from the rapid increase in VAN and PV values in control and treated samples. Thus EDTA treatment is not useful in extending the shelf-life of fatty fishes. But, for prawns stored in ice, a dip in 1% solution of EDTA enhanced the shelf-life by at least 8 days over the untreated control.

(b) Changes in the EDTA content of the muscle of EDTA treated fish and prawn during ice storage

Concentration of EDTA absorbed by the muscle of oil sardines and prawns during the dip in Na_2EDTA solutions and the changes in EDTA contents during subsequent storage in ordinary ice are given in Tables 7 and 8.

The quantity of EDTA absorbed by the muscle of both oil sardines and prawns, was directly dependent on the concentration of EDTA in the dip solutions; higher the concentration of EDTA in dip solutions, the greater the amount absorbed.

Table 7. *EDTA content of the muscle of oil sardines dipped in Na_2 EDTA solutions and subsequently stored in ice*

Days of storage	EDTA concentration micrograms/g muscle: Dipped in 0.1% Na_2EDTA solution	Dipped in 1% Na_2EDTA solution
Just after dip	110	326
4	102	290
12	95	246
24	80	212

Table 8. *EDTA content of the muscle of prawn dipped in Na_2EDTA solutions and subsequently stored in ice*

Days of storage	EDTA concentration micrograms/g muscle: Dipped in 0.1% Na_2 EDTA solution	Dipped in 1% Na_2EDTA solution
Just after dip	196	432
3	191	426
12	165	384
25	157	358

The EDTA content of the muscle of oil sardines, immediately after dip was 110 p.p.m in the case of dipping in 0.1% Na_2EDTA solution and 326 p.p.m in the case of dipping in 1% Na_2EDTA solution, the dip time being 10 min. On storage in ice, the EDTA content decreased, but not appreciably. After 24 days of iced storage, the EDTA content of the muscle was 80 p.p.m and 212 p.p.m, for oil sardines dipped in 0.1% Na_2EDTA solution and 1% Na_2EDTA solution respectively.

The amount of EDTA absorbed by prawn muscle during dip in Na_2EDTA solution was greater compared with that absorbed by fish. The EDTA content of prawns dipped in 0.1% Na_2EDTA solution for 10 min. was 196 p.p.m, which on storage in ice for 25 days, was depleted to 157 p.p.m. Whereas, 432 p.p.m of EDTA was absorbed by prawn muscle dipped in 1% Na_2 EDTA solution for 10 min. and on storage in ice for 25 days, it decreased to 358 p.p.m.

The fact that only a small percentage of EDTA absorbed by the muscle, was washed away during icedstorage might indicate that EDTA was bound or absorbed to the muscle of oil sardine and prawn. That higher amount of EDTA was absorbed by prawn muscle might be due to the fact that, in the case of prawn, the muscle was more exposed and that, owing to the small size of prawns compared with oil sardines, relatively larger areas were exposed to EDTA solution.

Pelroy & Seman (1969) found that the mean EDTA content of ocean perch fillets dipped in 1% solution was 305 p.p.m for the Na_4EDTA treated fish, 396 p.p.m for the Na_2EDTA treated fish and 411 p.p.m for the Na_2CaEDTA treated fish. The residual EDTA contents of haddock fillets treated with 1% Na_4EDTA solution were 187 to 333 p.p.m while in haddock fillets treated with 1% Na_2CaEDTA solution, the residual EDTA contents varied between 240 and 370 p.p.m. (Power *et al* 1968). They also found that soft fillets had higher residual EDTA contents than firm fillets of the same size. Boyd & Southcott (1968) have reported that the concentration of Na_2Ca EDTA in cod samples after immersion for one min. in 0.5% and 1% Na_2CaEDTA solution was 600 p.p.m and 750–810 p.p.m respectively. The concentration of EDTA in treated samples would undoubtedly be less if fish samples were larger in size. However, the EDTA absorbed by the muscle of oil sardines and prawns, in this study was found to be comparatively less than those reported by the above workers.

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