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Effects of Some Antioxidants and EDTA on the Development of Rancidity in Spanish Mackerel *(Scomberomorus maculatus)* During Frozen Storage

ROBERT N. FARRAGUT

SEATTLE, WA
February 1972

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Effects of Some Antioxidants and EDTA on the Development of Rancidity in Spanish Mackerel (*Scomberomorus maculatus*) During Frozen Storage

ROBERT N. FARRAGUT

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Effects of Some Antioxidants and EDTA on the Development of Rancidity in Spanish Mackerel (*Scomeromorus maculatus*) During Frozen Storage

by

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ABSTRACT

Spanish mackerel (*Scomberomorus maculatus*) were treated with antioxidant solutions containing BHA and BHT (Tenox 4); BHA, BHT, PG, citric acid, and propylene glycol (Tenox 6); Tenox 4 plus EDTA; Tenox 6 plus EDTA; $Ca(Na)_2EDTA$; $(Na)_2EDTA$; $(CA)_2EDTA$; and $(Na)_4EDTA$ both by dipping and injecting methods. Samples analyzed at 3-month intervals showed fillets packed in vacuum and treated with EDTA remained in good condition over the 12-month storage period. However, samples treated with $(Na)_4EDTA$ remained superior to other samples throughout the storage period.

INTRODUCTION

Spanish mackerel can be found in abundance in waters from the Chesapeake Bay to Texas. Early records indicate major landings of Spanish mackerel from the Chesapeake Bay during the last part of the 19th century. Around the turn of the century, landings declined in the Chesapeake Bay area but increased in southern Florida waters, where the fishery stabilized and is presently located. The stocks are tremendous but annual landings have remained in the neighborhood of 8 million pounds for the past 18 years (Lyles, 1969).

At present the commercial processing of Spanish mackerel usually is limited to evisceration and freezing of whole fish. The fish are usually marketed in this manner with only a small percentage of the catch sold as boneless fillets or steaks. Mackerel treated in this manner begin to show signs of rancidity within as little time as a 3-month period and are usually rejected by taste panels between the sixth and ninth month of storage.

As good fish, Spanish mackerel have many assets. The delicate flavor, the good yield of edible boneless fillets, and their availability contribute to the demand for good quality Spanish mackerel. A major factor influencing increased production and wider markets is the rapid onset of rancidity that occurs prior to and during frozen storage.

The problem of rancidity in fish has been studied for many years. Reports of the effect of various antioxidants on the development of rancidity in red spring salmon, haddock fillets, herring, and other species show little or no increase in shelf life over the controls. Typical antioxidants used in these experiments included BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), propylene glycol (Voss and Munkmer, 1966), ethyl gallate, ascorbic acid, and α-tocopherol (vitamin E). Castell and Spears (1968) report the induction of rancidity by minute quantities of trace minerals

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in fish tissue homogenates of non-oily species. The presence of trace metals such as iron and copper in oils is known to increase the rate of development of rancidity. If this were the case in whole fish, treatment by a chelating agent such as EDTA (ethylenediaminetetraacetic acid) should retard rancidity.

The use of EDTA compounds for the preservation of haddock fillets was reported by Power et al. (1968). Power found that iced haddock fillets dipped in 1% tetrasodium EDTA will remain organoleptically acceptable 11 days longer than untreated controls.

Tarr (1947) investigated the potential value of antibiotics for the preservation of fish. The reduction of bacteria by antibiotics did enhance the keeping qualities of certain species but did not significantly retard rancidity.

This paper will be divided into two parts, The first part will describe an experiment which was designed to show the effect of some antioxidants and EDTA, as well as vacuum packaging, on the development of oxidative rancidity in frozen Spanish mackerel. The second part will describe the effects of various EDTA compounds on the development of rancidity in and on the texture of Spanish mackerel fillets.

Part I. THE EFFECT OF VARIOUS TREATMENTS UPON THE DEVELOPMENT OF RANCIDITY IN SPANISH MACKEREL

MATERIALS AND PROCEDURE

A lot of Spanish mackerel was obtained from a Marathon, Fla., dealer and processed less than 24 hr from time of capture. Samples were either dipped or injected with the various solutions. Preliminary experiments revealed an uptake of 50 ppm of the antioxidants for every 1.1 g of oil when the fish were dipped. These calculations were used to determine a dipping time of 20 min. Fish were injected with antioxidant solutions with a Hamilton syringe and a 16-gauge needle. Samples were given four injections of 1 ml each to achieve the 250 ppm level. The injections were placed along the lateral line of each fish at 2- to 3-inch intervals. The samples were placed in the freezer within 1 hr after injecting.

Antioxidants were obtained from Eastman Organic Chemical Co. as solutions. The solutions contained BHA and BHT (Tenox 4), BHA, BHT, PG, citric acid, and propylene glycol (Tenox 6). EDTA was added to these solutions as well as being used independently. The treated fillet samples were packed in Cryovac bags and a vacuum of 29 inch Hg pulled on the bag with a Cryovac vacuum machine. The vacuum-sealed samples were frozen immediately. Other fillets were frozen and glazed for comparison. Whole fish were treated, frozen, and glazed. All glazed samples were glazed twice to insure a heavy and complete covering of ice over the samples. All samples were stored at —10° F.

Samples were analyzed in duplicate every 3 months for peroxide content, free fatty acid content, and organoleptic characteristics. Oil was extracted from the samples with acetone and then with 1:1 (by volume) petroleum ether and ethyl ether. The ether was flash-evaporated, and the extracted oil was analyzed for peroxide and free fatty acid content according to AOCS method (American Oil Chemists' Society, 1964). Organoleptic evaluations were performed on broiled portions by an 8-member panel using a 5-point scale for appearance, texture, and taste. The 5-point scale consisted of 5 points for excellent, 4 for very good, 3 for good, 2 for slightly good, 1 for borderline, and 0 for inedible. Appearance, texture, and taste were rated individually. Average scores of the 8-member taste panel are indicated in Tables 1, 2, and 3.

RESULTS

After 9 months' frozen storage, samples packed in vacuum received higher organoleptic scores than glazed fillets or glazed whole fish (Tables 1, 2, 3). Control samples packed in vacuum were rated 2.6, 2.3, and 2.5 as an average of the taste-panel results in appearance, flavor, and texture, respectively, after 9 months' storage. These scores compared to 2.0,

Table 1.—Organoleptic scores of Spanish mackerel (*Scomberomorus maculatus*) fillets packed in vacuum-sealed bags.

	2 weeks			2 months			3 months			6 months			9 months		
	A	F	T	A	F	T	A	F	T	A	F	T	A	F	T
Control	4.0	4.5	4.0	4.2	4.4	4.5	4.6	3.8	3.4	3.0	2.8	3.4	2.6	2.3	2.5
Tenox 4:															
Injected	3.8	3.6	3.6	3.8	3.8	3.8	4.4	4.0	4.2	2.8	3.0	3.6	1.3	2.0	1.3
Dipped	4.0	4.2	4.0	3.6	3.6	4.0	3.8	3.8	3.6	3.2	2.6	3.0	1.0	2.3	1.8
Tenox 6:															
Injected	3.8	4.0	4.2	3.2	3.6	3.8	3.8	3.8	4.4	3.2	2.6	3.0	1.1	2.3	1.8
Dipped	3.6	4.0	4.0	4.0	4.2	4.2	4.4	4.2	4.6	2.2	3.2	3.6	2.1	2.3	2.0
EDTA:															
Injected	4.0	4.0	4.0	4.0	3.4	4.0	3.8	3.4	3.8	3.2	3.2	3.2	3.0	3.3	3.0
Dipped	3.8	4.6	4.4	4.4	4.2	4.0	4.2	4.0	4.6	2.8	3.2	3.6	3.0	3.3	3.2
Tenox 4 + EDTA:															
Injected	4.0	4.2	4.2	4.0	3.4	3.6	3.8	3.8	4.4	2.4	2.2	3.6	2.7	3.0	2.5
Dipped	4.2	3.8	3.8	4.0	4.2	4.2	4.2	3.6	4.0	3.8	2.8	3.6	2.7	2.3	2.7
Tenox 6 + EDTA:															
Injected	4.2	3.8	4.0	4.2	4.2	4.2	4.2	3.4	3.8	3.8	3.0	3.4	2.2	1.7	2.5
Dipped	4.0	4.0	4.0	4.2	4.0	4.2	4.4	3.2	3.8	2.8	3.2	3.4	2.0	1.3	2.0

A = Appearance
F = Flavor
T = Texture

Table 2.—Organoleptic scores of glazed Spanish mackerel (*Scomberomorus maculatus*) fillets.

	2 weeks			2 months			3 months			6 months			9 months		
	A	F	T	A	F	T	A	F	T	A	F	T	A	F	T
Control	4.2	3.6	4.2	4.2	4.4	4.8	4.4	3.8	4.4	3.2	2.8	2.8	2.0	2.0	2.3
Tenox 4:															
Injected	4.2	3.4	4.0	3.6	3.8	4.4	4.0	3.8	4.0	1.7	1.7	2.5	1.4	1.3	1.6
Dipped	4.6	4.2	4.4	4.0	4.4	4.6	4.2	4.0	4.2	2.6	2.6	3.2	0.8	0.0	0.0
Tenox 6:															
Injected	4.2	4.0	4.4	3.8	4.0	3.6	3.8	4.2	4.0	3.0	3.0	2.8	0.8	0.0	1.2
Dipped	4.2	3.6	4.0	3.6	3.2	3.2	4.0	3.0	3.2	1.2	1.5	2.2	1.1	0.0	1.0
EDTA:															
Injected	4.4	4.4	4.4	4.0	4.2	3.6	4.0	4.2	3.6	3.4	3.4	4.0	3.0	2.7	3.0
Dipped	4.2	3.6	4.5	3.6	4.2	3.8	3.8	4.0	3.4	1.7	1.5	2.2	2.0	3.3	2.5
Tenox 4 + EDTA:															
Injected	4.2	3.8	4.0	4.2	3.0	3.8	4.0	3.8	3.6	2.0	3.0	3.0	2.5	3.0	1.5
Dipped	4.2	4.0	3.8	4.0	3.6	3.0	4.0	3.8	3.2	3.0	2.6	2.2	2.0	3.0	1.7
Tenox 6 + EDTA:															
Injected	4.2	4.2	4.4	4.0	3.8	3.6	4.0	3.8	4.2	3.4	3.2	3.0	1.5	0.3	1.2
Dipped	4.6	4.4	4.6	4.2	3.4	3.2	3.4	3.0	2.4	1.5	1.0	2.2	1.0	1.3	1.5

A = Appearance
F = Flavor
T = Texture

2.0, and 2.3, respectively, for glazed fillets, and 1.5, 1.0, and 1.8, respectively, for whole heads-off control samples. In some instances, control fillets packed in vacuum received better scores than samples treated with antioxidant mixtures not packed in vacuum. After 9 months' storage

Table 3.—Organoleptic scores of glazed whole heads-off Spanish mackerel (*Scomberomorus maculatus*).

	2 weeks			2 months			3 months			6 months			9 months		
	A	F	T	A	F	T	A	F	T	A	F	T	A	F	T
Control	4.2	4.4	4.2	3.6	4.0	4.6	4.0	3.8	3.8	3.6	2.6	3.0	1.5	1.0	1.8
Tenox 4:															
Injected	4.2	4.2	4.2	4.4	3.8	4.6	4.0	4.0	4.8	3.4	3.2	4.0	2.0	1.7	2.0
Dipped	4.0	3.8	4.4	3.8	3.8	4.2	3.8	2.2	3.4	2.8	2.4	2.4	1.3	1.3	2.1
Tenox 6:															
Injected	3.8	3.4	4.0	3.6	3.4	3.4	4.0	3.4	3.8	3.0	3.0	3.0	1.6	1.7	2.3
Dipped	4.4	4.6	4.6	4.4	4.0	4.6	4.2	3.0	3.4	3.6	3.2	3.6	0.8	1.3	1.6
EDTA:															
Injected	4.2	3.8	4.4	3.8	2.8	3.6	3.4	2.8	3.0	2.6	2.2	2.2	3.2	3.0	2.7
Dipped	4.4	4.6	4.4	3.8	3.8	4.2	3.6	2.4	3.6	3.2	3.6	3.4	3.2	3.0	2.7
Tenox 4 + EDTA:															
Injected	4.2	4.5	4.4	4.0	3.8	4.8	4.2	4.0	4.0	3.4	3.4	3.6	2.7	0.7	2.2
Dipped	4.0	3.2	4.2	3.6	3.0	2.6	4.0	3.0	3.0	2.6	2.6	3.2	2.5	2.3	2.2
Tenox 6 + EDTA:															
Injected	4.4	3.4	4.4	3.8	3.8	4.2	3.6	3.0	3.0	2.8	2.2	2.4	2.2	2.7	2.5
Dipped	4.4	4.6	4.6	4.2	4.4	3.8	4.0	3.6	3.6	3.6	3.2	3.6	1.7	1.3	2.2

A = Appearance
F = Flavor
T = Texture

most samples treated with Tenox 4 and Tenox 6 did not receive organoleptic scores equaling the control (Tables 1, 2, 3).

Rancidity was observed by the taste panel in most samples not packed in vacuum between the third and sixth month. Severe discoloration usually preceded the detection of rancidity by several weeks in samples treated with mixtures of antioxidants. This discoloration was exhibited by a general darkening of the flesh in the controls and a yellowish hue in samples treated with Tenox 4 and Tenox 6. Samples treated with EDTA, however, did not develop any rancid off-flavors during the storage period.

Figure 1 shows a typical flavor curve describing deterioration of flavor in vacuum-packed fillets during frozen storage. The Tenox 6 curve is typical of data obtained from samples treated with mixtures of the various antioxidants. Control fillets packed in vacuum show a more or less steady decline in flavor throughout the storage period (Table 1). The scores of samples treated with Tenox 6 decreased unevenly from 4.0 to 2.25 (Table 1). The flavor curve of fillets treated with EDTA and packed in vacuum remains higher than the controls from the third month to the ninth month (Table 1). At the third month, or about the time rancidity was first noticed in some samples, the control and Tenox 6 treated samples showed a slight increase in averaged scores.

The evaluation of texture by the taste panel throughout the storage period showed interesting results. Typical curves can be seen in Figure 2. The texture of the controls dropped in the ratings from 4.0 to 3.4 after 3 months' storage where it remained until the sixth month. Samples treated with Tenox 6 were rated superior to the controls in texture through

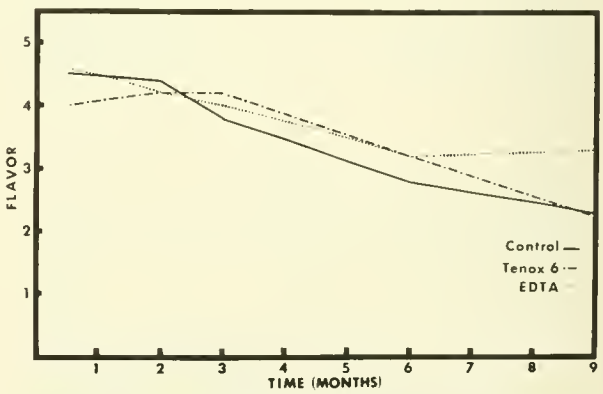

Figure 1.—Organoleptic score for flavor of Spanish mackerel fillets.

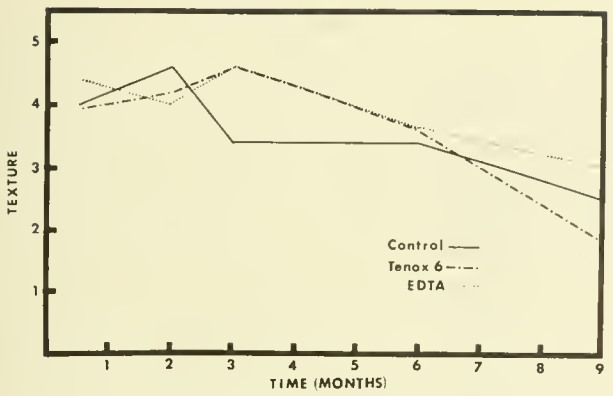

Figure 2.—Organoleptic score for texture of Spanish mackerel fillets.

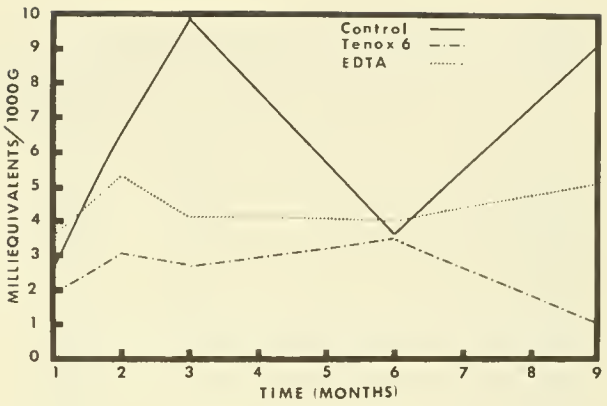

Figure 3.—Peroxide content of the oil fraction from Spanish mackerel.

the sixth month but fell below the controls on the ninth month. EDTA-treated samples remained superior to the controls in texture from the third to the ninth month. Samples treated with Tenox 4 plus EDTA and Tenox 6 plus EDTA, regardless of packaging technique and application method, scored the lowest in texture after 9 months' storage (Tables 1, 2, 3).

Peroxide content of the test pack generally followed the curves shown in Figure 3. Samples treated with Tenox 6 remained lower in peroxides than the controls or samples treated with EDTA (Tables 4, 5, 6). The initial increase of peroxides in the controls reached a high point in the third month (9.90 milliequivalents/1000 g) then decreased (3.60 milliequivalents/1000 g) in the sixth month. A large fluctuation in the peroxide content of the control samples continued throughout the test period (Tables 4, 5, 6). Samples treated with antioxidants or EDTA initially increased in peroxide content then remained static through the sixth month. Samples treated with Tenox 6 decreased in peroxide content after the sixth month period coinciding with increasing rancid off-flavors development. EDTA-treated samples increased in peroxide content after the sixth month period

Table 4.—Peroxide content of Spanish mackerel (*Scomberomorus maculatus*) packed in vacuum-sealed bags.

	2 weeks	2 months	3 months	6 months	9 months
			milliequivalents/1000 grams		
Control	2.50	6.60	10.92	3.60	9.14
Tenox 4:					
Injected	3.00	7.22	3.76	11.48	14.25
Dipped	3.34	4.04	17.50	10.22	3.02
Tenox 6:					
Injected	--	4.20	6.14	9.34	4.62
Dipped	1.54	3.04	2.70	9.00	1.14
EDTA:					
Injected	3.30	6.14	3.30	2.56	4.92
Dipped	3.62	5.34	4.04	12.00	5.05
Tenox 4 + EDTA:					
Injected	3.10	5.80	4.40	15.10	2.46
Dipped	1.96	4.72	4.50	4.00	2.60
Tenox 6 + EDTA:					
Injected	2.90	5.00	4.40	4.40	1.72
Dipped	1.70	4.90	3.52	6.50	4.20

Table 5.—Peroxide content of glazed Spanish mackerel (*Scomberomorus maculatus*) fillets.

	2 weeks	2 months	3 months	6 months	9 months
			milliequivalents/1000 grams		
Control	1.80	17.80	24.17	9.10	21.60
Tenox 4:					
Injected	2.42	11.72	12.00	3.30	48.80
Dipped	2.50	8.70	10.60	3.18	29.80
Tenox 6:					
Injected	3.00	8.84	12.20	18.12	20.60
Dipped	2.18	6.70	10.72	3.58	14.80
EDTA:					
Injected	3.20	5.00	12.00	4.32	4.92
Dipped	6.52	8.74	17.10	7.22	33.40
Tenox 4 + EDTA:					
Injected	3.90	16.87	7.80	5.40	16.65
Dipped	4.12	14.50	13.84	11.60	13.65
Tenox 6 + EDTA:					
Injected	3.70	10.66	14.92	9.20	9.60
Dipped	1.74	6.04	8.70	9.90	--

Table 6.—Peroxide content of glazed whole heads-off -Spanish mackerel (*Scomberomorus maculatus*).

	2 weeks	2 months	3 months	6 months	9 months
			milliequivalents/1000 grams		
Control	3.30	8.10	5.66	16.80	13.36
Tenox 4:					
Injected	3.60	8.02	9.02	18.90	41.30
Dipped	2.30	9.00	14.50	30.62	16.94
Tenox 6:					
Injected	6.92	6.84	9.98	11.52	15.42
Dipped	3.88	7.60	10.30	3.40	30.50
EDTA:					
Injected	3.84	11.40	3.22	20.90	33.50
Dipped	5.25	4.70	11.55	12.70	18.12
Tenox 4 + EDTA:					
Injected	3.70	8.74	6.80	24.38	19.30
Dipped	2.60	10.86	11.37	15.10	22.80
Tenox 6 + EDTA:					
Injected	3.22	7.56	2.60	20.50	18.85
Dipped	3.62	7.56	9.48	15.08	29.00

but no rancid off-flavors or odors were detected.

The free fatty acid content of the controls, Tenox 6, and EDTA-treated samples are shown in Figure 4. Samples treated with Tenox 4 and antioxidants plus EDTA showed similar curves (Tables 7, 8, 9). The free fatty acid content of the test pack varied considerably throughout the experiment. Samples treated with Tenox 6 increased steadily in free fatty acid content until the third month then rose steadily to the ninth month.

DISCUSSION

The variables that were compared throughout the experiment included (1) methods of applying the antioxidants and EDTA and (2) the packaging method (vacuum-packaging compared to glazing). Samples dipped or injected showed the same trends in peroxide and free fatty acid content throughout the experiments. Organoleptic evaluation of these samples was almost identical.

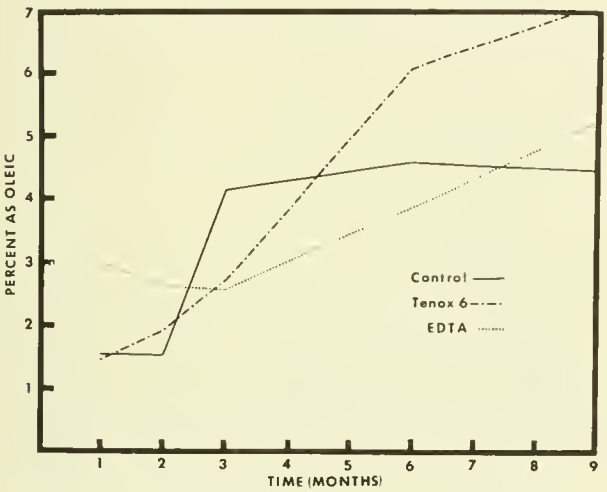

Figure 4.—Free fatty acid content of the oil fraction from Spanish mackerel fillets.

A difference in organoleptic scores between samples packed in vacuum or glazed was noted throughout the storage period. Samples packed in vacuum were rated organoleptically superior to glazed samples (Tables 1, 2, 3).

Samples treated with Tenox 4, Tenox 6, Tenox 4 plus EDTA, and Tenox 6 plus EDTA developed rancid flavors and odors after 6 months' frozen storage. Peroxide and FFA content of samples treated with these compounds did not correlate with the rancid flavors and odors that developed. This would indicate a deterioration similar to the observations of Castell et al. (1965), who reported that many off-odors are not usually identified with the development of oxidative rancidity. Castell et al. found that a considerable difference occurred in early developing off-odors when rancidity was activated by different chemical compounds, and they observed a different off-odor associated with the addition of different trace minerals. Castell et al. concluded that the mixture of trace minerals on fish acquired through environment and handling could account for the varied off-odors from deterioration found in commercial samples.

Castell and Spears (1968) observed a rapid increase in rancidity development with the addition of minute quantities of trace minerals.

Table 7.—Free fatty acid content of Spanish mackerel (*Scomberomorus maculatus*) fillets packed in vacuum-sealed bags.

	2 weeks	2 months	3 months	6 months	9 months
	% oleic	*% oleic*	*% oleic*	*% oleic*	*% oleic*
Control	1.55	1.54	4.15	4.60	4.45
Tenox 4:					
Injected	1.25	2.54	3.04	6.00	7.61
Dipped	1.12	1.85	6.21	3.51	3.70
Tenox 6:					
Injected	--	1.89	3.16	3.92	4.48
Dipped	1.46	1.85	2.73	6.65	12.51
EDTA:					
Injected	2.58	2.11	2.44	8.46	5.78
Dipped	2.98	2.61	2.54	8.84	5.21
Tenox 4 + EDTA:					
Injected	1.25	1.80	3.56	9.93	8.37
Dipped	1.35	1.94	4.26	3.87	7.00
Tenox 6 + EDTA:					
Injected	1.24	2.79	2.56	4.75	10.76
Dipped	1.31	2.45	2.62	7.63	7.05

Table 8.—Free fatty acid content of Spanish mackerel (*Scomberomorus maculatus*) glazed fillets.

	2 weeks	2 months	3 months	6 months	9 months
	% oleic	*% oleic*	*% oleic*	*% oleic*	*% oleic*
Control	0.62	2.21	6.15	2.45	6.66
Tenox 4:					
Injected	1.33	2.39	3.81	3.95	4.35
Dipped	1.74	2.03	4.37	4.05	4.54
Tenox 6:					
Injected	2.64	2.07	3.39	3.58	4.38
Dipped	2.00	1.58	3.22	6.08	6.54
EDTA:					
Injected	0.99	2.53	2.99	2.63	12.47
Dipped	1.87	3.33	2.80	7.38	5.05
Tenox 4 + EDTA:					
Injected	1.21	4.82	3.78	3.70	6.69
Dipped	1.70	1.85	2.91	6.01	6.27
Tenox 6 + EDTA:					
Injected	2.97	1.82	3.23	4.18	5.70
Dipped	1.65	4.08	3.89	4.61	5.84

Table 9.—Free fatty acid content of glazed whole heads-off Spanish mackerel (*Scomberomorus maculatus*).

	2 weeks	2 months	3 months	6 months	9 months
	% oleic	*% oleic*	*% oleic*	*% oleic*	*% oleic*
Control	1.33	2.14	2.75	5.58	4.24
Tenox 4:					
Injected	1.80	2.16	3.64	5.21	9.41
Dipped	1.67	2.13	2.57	5.01	5.17
Tenox 6:					
Injected	2.72	3.07	2.74	3.65	5.58
Dipped	1.09	2.47	4.37	3.56	6.91
EDTA:					
Injected	1.49	1.70	3.90	4.78	7.51
Dipped	1.98	3.38	5.21	2.36	4.63
Tenox 4 + EDTA:					
Injected	1.08	2.11	1.92	5.82	5.92
Dipped	0.94	2.10	5.40	4.55	5.08
Tenox 6 + EDTA:					
Injected	1.29	2.37	2.57	4.23	6.09
Dipped	1.44	2.69	4.22	4.81	8.11

The addition of a chelating compound such as EDTA would decrease the amount of trace minerals available. However, the chelation of trace minerals did not affect the initial production of peroxides and free fatty acid.

The development of rancidity in Spanish mackerel during frozen storage was retarded by the application of EDTA. The only criticism received by these samples from the taste panel throughout the storage period was in their appearance. A yellowish discoloration developed on and adjacent to the belly flap after the sixth month of storage. The concentration of EDTA applied to the sample may be a factor

in the development of this discoloration. Additional experiments using various salts of EDTA and different concentrations is described in Part II.

Part II. THE EFFECT OF VARIOUS EDTA COMPOUNDS UPON TEXTURE AND THE DEVELOPMENT OF RANCIDITY IN SPANISH MACKEREL FILLETS

MATERIALS AND PROCEDURE

Spanish mackerel were purchased locally with the exact age and area of capture being unknown. The overall condition of the fish was considered to be better than the average commercial samples. Samples were filleted and then dipped or injected with 250 ppm, 180 ppm, and 125 ppm of the following compounds: EDTA (ethylenediaminetetraacetic acid), $Ca(Na)_2EDTA$ (disodium calcium ethylenediaminetetraacetate), $(Na)_2EDTA$ (disodium ethylenediaminetetraacetate), $(Na)_4EDTA$ (tetrasodium ethylenediaminetetraacetate), and $(Ca)_2EDTA$ (dicalcium ethylenediaminetraacetate). The parameters for dipping and injecting were the same reported in Part I. After treatment all samples were packed in vacuum and stored at —10° F until analyzed. All EDTA compounds were obtained from Eastman Organic Chemical Co.

Samples were analyzed every 3 months for peroxide content, free fatty acid content, and organoleptically as previously stated.

RESULTS

The development of free fatty acids adhered to the same general pattern observed in Part I of this experiment for EDTA-treated fillets. Samples treated with $(Na)_4EDTA$ and $(Na)_2$-EDTA remained lower in free fatty acid content after 12 months of frozen storage than did samples treated with other compounds. Two samples surpassed the 6.88% free fatty acid as oleic found in the control at the 12th month. One sample was injected with 250 ppm $(Na)_2$-EDTA, one with 125 ppm $(Na)_4EDTA$ (Table 10).

The concentration of additive that appeared to best protect the fillets against free fatty acid formation was dependent upon the method of application (Fig. 5). Fillets dipped in $(Na)_4$-EDTA at the 180 ppm level remained lower in free fatty acid content throughout the storage period than did samples dipped in 250 ppm or 125 ppm level (Table 10). Samples injected with $(Na)_2EDTA$ at the 125 ppm level were better protected against free fatty acid formation than samples injected with higher concentrations of $(Na)_2EDTA$ (Table 10). Samples treated with $(Na)_4EDTA$ followed the same pattern of free fatty acid development as those treated with $(Na)_2EDTA$. Thus, after 12 months of frozen storage, fillets dipped or injected with 180 ppm $(Na)_4EDTA$ remained lower in free fatty acid content than the other samples treated with $(Na)_4EDTA$ (Table 10). Fillets dipped at the 125 ppm level in four of the five tested compounds remained lower in free fatty acid content than did the respective injected samples (Table 10). Those fillets injected with $(Ca)_2EDTA$ at the 180 ppm level were lower in free fatty acid content as oleic than $(Ca)_2EDTA$ dipped samples and were the exception to the rule (Table 10).

In short, the samples remaining lowest in free fatty acid content after 12 months' frozen storage were those treated with 180 ppm or 125 ppm of each of the respective compounds. Samples treated with 250 ppm of EDTA and its salts

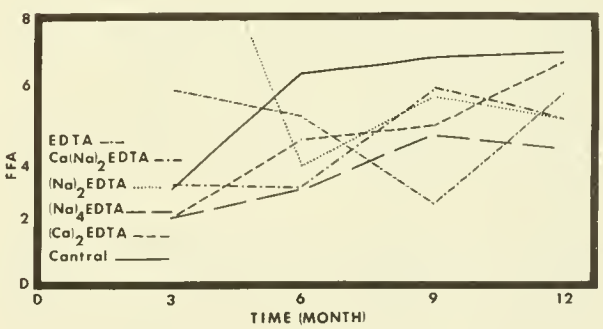

Figure 5.—Free fatty acid content of the oil from Spanish mackerel fillets treated with 180 ppm of EDTA and certain salts of EDTA.

Table 10.—Fatty acid content of Spanish mackerel (*Scomberomorus maculatus*) packed in vacuum-sealed bags.

	Dipped				Injected			
	3 months	6 months	9 months	12 months	3 months	6 months	9 months	12 months
	% oleic	*% oleic*	*% oleic*	*% oleic*	*% oleic*	*% oleic*	*% oleic*	*% oleic*
Control	2.81	6.35	6.81	6.88	--	--	--	--
EDTA:								
250 ppm	2.81	4.67	6.26	5.85	19.56	5.38	4.74	7.69
180 ppm	5.89	5.01	2.40	5.77	--	4.61	4.74	6.10
125 ppm	2.04	3.68	5.93	4.03	--	4.44	5.65	5.17
$Ca(Na)_2EDTA$:								
250 ppm	--	5.29	10.60	6.16	--	4.31	6.26	4.81
180 ppm	2.95	2.92	5.85	4.96	4.61	3.40	5.92	4.31
125 ppm	3.32	--	6.03	4.30	5.09	3.66	3.63	5.88
$(Na)_2EDTA$:								
250 ppm	10.02	4.24	5.61	9.25	2.12	2.44	4.31	4.36
180 ppm	35.90	3.54	5.62	4.96	2.04	4.39	5.84	5.72
125 ppm	2.01	3.25	5.44	3.51	3.03	3.52	4.89	4.09
$(Na)_4EDTA$:								
250 ppm	7.23	6.00	5.98	6.77	2.09	3.35	5.54	4.66
180 ppm	1.96	2.87	4.49	4.01	2.68	3.60	5.71	4.03
125 ppm	2.06	6.70	3.99	7.96	2.22	4.80	4.82	--
$(Ca)_2EDTA$:								
250 ppm	3.21	4.48	4.16	6.71	2.41	4.56	6.27	4.94
180 ppm	2.95	4.34	4.74	6.63	2.22	3.02	4.28	3.71
125 ppm	2.54	--	8.12	4.01	2.09	4.45	5.17	5.58

showed the highest free fatty acid content at the end of the storage period.

Fillets dipped with 180 ppm of $(Na)_2EDTA$, $(Na)_4EDTA$, and $(Ca)_2EDTA$ were lower in peroxide content than samples injected with these compounds after 12 months' frozen storage (Table 11). The peroxide formation in the samples increased up to the ninth month. A decrease in peroxide content from the ninth month to the 12th month was noted (Table 11).

The concentration of the various compounds used affected the peroxide content. Samples dipped with 180 ppm of $(Na)_2EDTA$ were found to have the lowest peroxide content after 12 months of frozen storage (Table 11). Peroxide formation was best retarded in these samples treated with 180 ppm of these compounds.

The organoleptic texture scores of samples dipped in 180 ppm of the various compounds can be seen in Figure 6 and Table 12. After 6 months of storage, the taste panel rated these samples in the range of 2.8 (EDTA) to 4.0 [$(Na)_2EDTA$]. Scores for these samples after 12 months of frozen storage were below 3.0. Three of the five samples treated with the different compounds, however, were rated equal to or better than the control in texture after 12 months of storage. These were those treated at the 180 ppm level of $(Ca)_2EDTA$, $(Na)_2EDTA$, and $(Na)_4EDTA$, and 125 ppm of $(Ca)_2EDTA$, $(Na)_4EDTA$, and $Ca(Na)_2EDTA$.

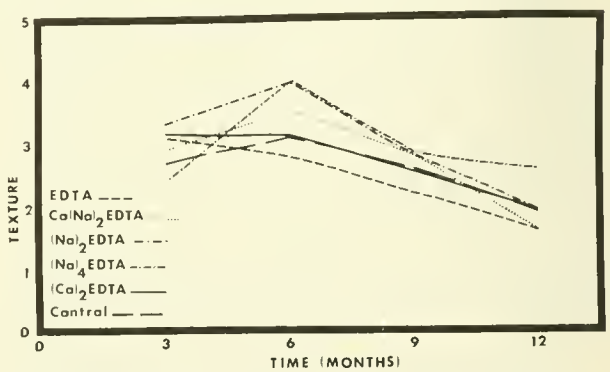

Figure 6.—Organoleptic score for texture of Spanish mackerel fillets treated with 180 ppm of EDTA and certain salts of EDTA.

Table 11.—Peroxide content of Spanish mackerel (*Scomberomorus maculatus*) packed in vacuum-sealed bags

	Dipped				Injected			
	3 months	6 months	9 months	12 months	3 months	6 months	9 months	12 months
	milliequivalents/1000 grams				*milliequivalents/1000 grams*			
Control	10.05	10.85	82.41	14.96	--	--	--	--
EDTA:								
250 ppm	0.00	8.37	14.82	14.27	0.00	7.54	13.75	17.77
180 ppm	0.00	9.54	9.95	18.84	--	6.23	9.55	9.25
125 ppm	37.58	6.43	43.90	12.16	--	--	80.24	15.28
$Ca(Na)_2$EDTA:								
250 ppm	--	11.26	59.09	14.87	--	6.03	107.03	12.56
180 ppm	23.91	5.43	115.58	27.64	7.43	--	69.91	13.71
125 ppm	8.84	--	10.07	18.71	0.00	4.02	18.37	27.09
$(Na)_2$EDTA:								
250 ppm	0.00	5.02	5.89	16.70	8.24	4.22	67.33	19.70
180 ppm	--	8.44	75.40	5.43	8.24	6.28	125.83	13.03
125 ppm	20.30	6.03	38.59	12.76	5.02	4.02	47.84	11.07
$(Na)_4$EDTA:								
250 ppm	3.81	7.04	57.48	15.00	7.43	5.02	52.06	14.67
180 ppm	2.41	5.02	43.64	12.46	7.43	3.62	40.10	13.87
125 ppm	12.66	8.04	51.05	24.12	12.66	6.36	38.79	--
$(Ca)_2$EDTA:								
250 ppm	16.28	7.37	88.26	16.75	12.46	8.04	56.66	16.18
180 ppm	--	7.54	56.64	15.33	2.01	8.04	38.55	21.55
125 ppm	9.44	--	12.76	15.56	5.02	21.86	8.80	21.51

Table 12.—Texture scores of Spanish mackerel (*Scomberomorus maculatus*) packed in vacuum-sealed bags.

	Dipped				Injected			
	3 months	6 months	9 months	12 months	3 months	6 months	9 months	12 months
Control	3.3	3.3	--	1.9	3.3	3.3	--	1.9
EDTA:								
250 ppm	3.1	3.1	2.6	1.9	2.5	2.6	2.6	1.9
180 ppm	3.1	2.8	2.2	1.6	3.0	2.6	2.2	1.6
125 ppm	3.1	3.1	2.6	1.6	3.0	2.5	2.6	1.9
$Ca(Na)_2$EDTA:								
250 ppm	3.3	3.1	2.8	1.6	3.5	3.5	3.0	1.9
180 ppm	2.9	3.5	2.8	1.6	3.7	3.3	3.2	1.6
125 ppm	2.7	2.3	2.8	1.9	3.4	3.8	2.8	1.9
$(Na)_2$EDTA:								
250 ppm	3.4	3.3	2.8	1.9	3.0	3.5	2.8	1.6
180 ppm	3.3	4.0	2.8	1.9	2.5	3.8	2.4	1.6
125 ppm	3.0	3.8	2.2	1.6	2.5	2.8	3.0	1.9
$(Na)_4$EDTA:								
250 ppm	2.1	3.1	2.8	1.9	3.7	3.3	3.2	1.9
180 ppm	2.4	4.0	2.8	2.6	3.5	3.5	3.4	2.9
125 ppm	3.1	3.5	3.0	2.9	3.3	3.6	3.4	1.9
$(Ca)_2$EDTA:								
250 ppm	2.7	4.1	2.4	1.6	2.8	2.8	3.0	1.9
180 ppm	2.7	3.1	2.6	1.9	3.1	3.6	2.8	2.9
125 ppm	2.8	3.6	--	2.9	2.8	3.8	3.0	2.9

DISCUSSION

Spanish mackerel fillets were treated with EDTA and four of its salts, vacuum packaged, and tested for the development of rancidity and tough texture. Analysis of free fatty acids and peroxides as well as organoleptic evaluation did not reveal the development of rancidity in any of the samples treated with $(Na)_2$EDTA or $(Na)_4$EDTA. The evaluation of data obtained from these analyses showed that several of these compounds, which are chemically closely related, protected Spanish mackerel fillets from discoloration and off-odors more effectively than others.

Although free fatty acids were found in irregular concentration throughout the storage period, those samples dipped in 180 ppm $(Na)_4$-EDTA were 2.87% lower in free fatty acid content than the controls (Table 10). Castell and Spears (1968) showed that the degradation of fish oil is directly related to available trace minerals. The addition of $(Na)_4$EDTA to Spanish mackerel fillets apparently reduced the amount of free trace minerals resulting in a much slower breakdown of triglycerides and lower peroxide content than normal.

Peroxide values varied considerably for each sample throughout the storage period. However, some generalizations can be made. A sharp increase in peroxide values occurred between the third month and the ninth month, after which decreases of different magnitudes were noted for all samples (Table 11). Two compounds, $(Na)_2$EDTA and $(Na)_4$EDTA, appeared to restrict the development of peroxides more effectively than the others.

Free fatty acid and peroxide values are not necessarily related to the absence or presence of rancidity. These analyses are indicators of the chemical degradation of the oil in the samples. Other chemical tests, such as TBA, also measure the chemical reactions occurring. In the final analysis, the only true evaluation of rancidity is its detection by a trained taste panel. Typical rancid odor and off-flavors did not develop in any sample during the storage period. Since the taste panel noticed the development of a woody texture in the fillets treated with EDTA in Part I, this experiment was designed to ascertain differences in texture which might result from the use of various salts of the parent EDTA. The taste panel preferred the texture of samples treated with $(Na)_2$EDTA and $(Na)_4$EDTA to samples treated with other compounds or the controls (Table 12). The general opinion of the panel was that these samples were not equal to fresh mackerel fillets but that they were superior to the other samples or to commercially frozen mackerel fillets.

Power et al. (1968) reported similar results from $(Na)_2$EDTA and $(Na)_4$EDTA dipped haddock fillets stored in ice. Haddock fillets dipped in 0.5% and 0.75% $(Na)_4$EDTA were rejected because of extreme tough texture. However, samples dipped in 1.0% $(Na)_4$EDTA remained acceptable throughout the experiment. Power found that although the texture in these fillets was not equal to fresh fillets it was not objectionable. The observed changes in the texture of Spanish mackerel fillets treated with EDTA salts, although not objectionable, remain a mystery. Chemical or other pathways by which these changes occur are at this point speculation.

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