NOAA TECHNICAL MEMORANDUM NMFS SEFC-75

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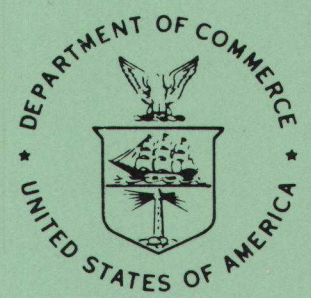

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U.S. DEPARTMENT OF COMMERCE
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National Marine Fisheries Service
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Malcolm Baldridge, Secretary
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John V. Byrne, Administrator
NATIONAL MARINE FISHERIES SERVICE
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Abstract

Blueback herring, Alosa aestivalis, were used in a pilot study of lipid oxidation during low-temperature storage to (1) assess the usefulness of a higher storage temperature (-2°C for 12 weeks) to accelerate and duplicate the effects of longer term low-temperature storage (-20°C for 12 months) on lipid oxidation, and (2) evaluate several different chemical methods to monitor lipid oxidation changes during low-temperature storage of fishery products. The effectiveness of TBHQ antioxidant was also evaluated at both storage temperatures. Results indicated that the lipid oxidation rate was about 4 times faster at -2°C; patterns of change were similar at the two temperatures with TBA and COP values increasing and eventually declining. Changes in fatty acid composition and iodine values were not useful measurements of lipid oxidation in this study. Measured TBA and COP values were consistently higher for untreated fillets, indicating greater lipid oxidation. Limited sensory evaluations indicated that TBHQ treatment was effective, but differences were not statistically significant.

Introduction

Most of the finfish species which are both abundant and little used for food in the southeastern region of the United States have the common problem of rapid lipid oxidation during storage. Many different methods have been used for the measurement of lipid oxidation (Gray, 1978) but none have proved satisfactory over the entire course of the oxidation process. There is a need to select and evaluate the more promising methods for measurement of lipid oxidation and to develop standard methods for determining the storage stability of target species. An accelerated test for the determination of frozen storage stability would be useful for processing studies with unfamiliar species.

Underutilized species of the herring (Clupeidae) family are of particular interest for commercial fishery development in the southeast region but in general they are not readily available locally. The blueback herring, Alosa aestivalis, which is available fresh and in good supply during spawning migrations, was selected for this study. Both the blueback herring and the alewife, A. pseudoharengus, are commonly known as river herring . The blueback herring is more common on the U.S. South Atlantic coast, but actually occurs along the coast from Florida to Nova Scotia (Hildebrand, 1963) and has often been confused with the alewife. Loesch and Lund (1977) described biological characteristics for spawning runs of bluebacks in the Connecticut River. In South Carolina the species is harvested for bait but the catch is regulated in order to provide forage for recreational species, primarily striped bass, Morone saxatilis.

The objectives of this study were to evaluate alternative methods for measurement of lipid oxidation during frozen storage at -20^{o}C and during an

accelerated storage test at -2°C. Effects of experimental treatments and storage characteristics for unfamiliar species could be determined in a shorter time period if oxidation patterns were similar but accelerated at the higher temperature. The protective effect of the antioxidant, tertiary butyl hydroquinone (TBHQ), was also investigated at the two temperatures.

Material and Methods

Raw Material

River herring, harvested at night from the Cooper River, were refrigerated overnight, then iced and transported the following morning to the laboratory. Sexually mature females, averaging slightly over one-half pound in weight (257 gm. and 26.1 cm. average fork length), were selected for use in this study. Roe contents averaged 15.4% of body weight and the average yield of (skin-on) fillets was 43.6%.

Proximate chemical analyses for both skinless and skin-on fillets are shown in Table 1. Fat content was determined by a chloroform: methanol extraction method (Smith, Ambrose, and Knobl, 1964) and protein, moisture and ash were determined by AOAC methods (AOAC, 1975). The presence of a hypodermal fat layer was indicated by the significantly higher fat content in the skin-on fillets in comparison with the skinless tissues.

Skin-on fillet pairs from individual fish were divided between untreated and antioxidant treated samples. Half of the fillets were treated by dipping for one minute in a 0.025% solution of TBHQ (Eastman Chemical Products, Inc.).[1/] This treatment has been shown to leave TBHQ residues within legal

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1/ The use of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

limits in skin-on fillets (Deng, et al., 1977). Treated and untreated fillets were sealed, four to a package, in 1.15 mil polyethylene Ziploc bags (Dow Chemical Co.).[1/] The bagged samples were placed into waxed cartons for storage at either -2°C or -20°C. The samples for the accelerated test were initially held at -20°C for 2 hours to accelerate the freezing process and then transferred to a freezer controlled at -2°C. The "superchilled" (-2°C) samples were evaluated at 0, 1, 3, 6, 9 and 12 weeks. Hard frozen (-20°C) samples were evaluated at 0, 1, 2, 6, 9 and 12 months.

Analytical Procedures

Lipids were extracted from 30 g samples of control and treated fillets with chloroform, methanol and water (Bligh and Dyer, 1959). The chloroform phase was recovered, concentrated to small volume in a rotary evaporator, transferred to a 10.0 ml volumetric flask and brought to volume with chloroform. One ml aliquots of this extract were used for gravimetric determination of lipid content and chemical analysis requiring the use of extracted lipids (Joseph and Seaborn, 1979). Stored samples were evaluated at the specified times by the following chemical analyses:

(1) TBA Value - The direct extraction method of Vyncke (1975) was used. A filtrate from the extraction of 20 g tissue with a 7.5% trichloracetic acid solution containing 0.1% of both propyl gallate and EDTA was reacted with thiobarbituric acid (TBA) solution and the absorbance read at 531 nm. The results were expressed as mg malonaldehyde/kg tissue.

(2) COP value - The conjugated oxidation products (COP) assay as described by Parr and Swoboda (1976) was used. Hydroperoxides, organic hydroxides and carbonyl oxidation products were determined on lipid extracts by ultraviolet absorbance measurements before and after reduction and dehydration reactions.

(3) Iodine Value - The Wijs method (AOCS, 1970), modified by the use of chloroform rather than carbon tetrachloride as the solvent, was used to chemically determine the iodine values of lipids extracted from the samples.

(4) Fatty Acid Composition - Extracted lipids were converted to methyl esters by saponification with 0.5 N KOH in methanol followed by treatment with boron trifluoride in methanol (14% by weight) as described by Metcalfe and Schmitz (1961). Fatty acid methyl esters were analyzed by gas-liquid chromatography (GLC) on a flexible fused silica wall coated open tubular (WCOT) column (50 m x 0.21 mm) coated with Carbowax 20-M. Individual fatty acids were identified from experience or the use of semi-logarithmic plots of retention times (Ackman, 1963) and appropriate standards.

In addition to the chemical measurements of lipid oxidation, sensory evaluations of the frozen samples were made at 0, 9 and 12 months. A 5-member taste panel rated the samples on the basis of color, flavor, rancidity, texture and overall acceptability, based on the principles of quantitative descriptive analysis (Stone, et al., 1974). The panel was given preliminary training in rancidity detection through the use of different concentrations of mullet oil, oxidized by bubbling air through it at 60^{o}C, in fresh vegetable oil. Samples were tasted, described and discussed by the panel.

Results and Discussion

Chemical Tests

The TBA values determined on both super-chilled (0-12 weeks) and frozen (0-12 months) river herring samples are shown in Figure 1. In the accelerated test at -2^{o}C, TBA values peaked after 6 weeks and then declined. At its maximum, the TBA value for the untreated (control) sample was 8.9 mg malonaldehyde/kg, almost three times as high as that of the TBHQ-treated sample,

but the value declined rapidly to 4 mg/kg at 12 weeks. The decline or leveling off of TBA values during frozen storage has been observed many times, and was discussed by Deng, et al. (1977). During frozen storage at -20°C, the TBA values reached a peak at about 6 months and again, the untreated sample value (2.7 mg/kg) was three times as great as that of the TBHQ treated sample.

The COP values (Figure 2) increased for longer time periods before declining. At -2°C, peaks were reached after 9 weeks and were followed by sharp declines. During frozen storage at -20°C, the COP value of the untreated sample peaked at 9 months but the value for the TBHQ treated sample did not peak before the end of the 12-month storage period. At both storage temperatures, the treated samples had consistently lower COP values than did the untreated controls.

The iodine values (IV), shown in Table 2, were too variable to be of use in evaluating lipid oxidation in this study. Although paired fillets were used for control and treated samples, significant, but unexplained, differences (0-10 units) in IV were observed between control and treated samples in the "accelerated" freezer study but much smaller differences (0 to 3 IV units) in the -20°C study. This variation is reflected in the table of IV values.

Fatty Acid Composition

The fatty acid compositions are tabulated in Table 3 for samples stored at -2°C and in Table 4 for the samples at -20°C. There were no significant trends in any of the major fatty acids and, in particular, there were no significant losses in polyunsaturates. There were some significant individual sample variations in the -2°C study, particularly in the third week when lower levels of 20:1W9 and 22:1W11 were measured, probably due to differences in the composition of the fish comprising the 3-week samples. The individual variation within and between samples in the -20°C study was much less.

Sensory Evaluations

The mean organoleptic ratings of the taste panelists at 0, 9 and 12 months are listed in Table 5. Statistical analysis of the data was based on observations of the five panelists who participated in all tests. A 3-way analysis of variance for the factors of treatment, storage time and panel member was done. The only significant differences found were among the individual panel members for their ratings of color, rancidity and acceptability. The average ratings suggested a milder flavor and greater acceptability for the TBHQ-treated samples, but statistical differences were not significant. The average rancidity rating for the untreated sample at 12 months was 44 percent higher than the rating for the TBHQ treated sample. Therefore, a pair-wise t test was done separately to test equality of means between the two groups, but no significant differences were found ($P > 0.05$).

Conclusions

(1) The accelerated frozen storage test at $-2^{o}C$ shows a pattern of TBA and COP values that is similar to the more conventional test at $-20^{o}C$.

(2) The untreated samples had higher TBA and COP values than the TBHQ treated samples and ratios were similar at the two temperatures, although the absolute values were higher and more variable for the $-2^{o}C$ samples.

(3) The results indicate that lipid oxidation proceeded four times as fast at $-2^{o}C$ as at $-20^{o}C$. For instance, the TBA values peaked at 6 months at $-20^{o}C$ and at 6 weeks at $-2^{o}C$. This is in line with the general rule that the rates of chemical reactions approximately double for each $10^{o}C$ rise in temperature.

(4) Fatty acid profiles and iodine values were not useful as measurements of lipid oxidation under the conditions used in this study.

(5) The TBHQ treatment appears to be effective in the inhibition of lipid oxidation as measured by TBA and COP values. The average values of taste panel ratings for flavor intensity, rancidity, and overall acceptability also indicate an advantage for the TBHQ treated samples, but variation between panel members was too great to demonstrate a statistically significant difference between samples.

(6) Based on chemical tests, the superchilled ($-2^{o}C$) storage of river herring appears to be a useful indicator of lipid oxidation patterns at $-20^{o}C$. It could be useful for the more rapid determination of the effects of chemical treatment on the oxidative stability of fishery products during frozen storage, but additional experimental work with other species would be required to determine the value of the method.

Acknowledgements

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Table 1. Proximate chemical analyses for fillets of river herring with and without skin (percent composition).

Sample	Protein	Fat	Ash	Moisture
Skinless fillet	18.08	4.62	1.19	76.75
Fillet, skin on	17.99	7.54	1.28	74.11

Table 2. Iodine values (Wijs method) for river herring during storage at -2°C and -20°C.

Temperature	Treatment	Storage Time (Weeks/Months) 0	1	3	6	9	12
-2°C (time in weeks)	Untreated	145.2	154.8	156.1	134.7	129.5	149.4
	TBHQ dip	-	161.0	157.5	134.4	137.2	159.3
-20°C (time in months)	Untreated	145.2	151.5	145.7	155.7	150.9	141.7
	TBHQ dip	-	151.4	147.2	158.4	150.3	147.7

Table 3. Major fatty acids of river herring stored at -2°C for 12 weeks.

Weeks storage:	0	1		3		6		9		12	
Sample:	I[a]	C[a]	T[a]	C	T	C	T	C	T	C	T
Fatty acids											
14:0	5.0	5.7	5.4	5.0	4.9	5.9	5.8	5.8	5.4	5.7	6.0
16:0	15.7	15.7	15.5	17.4	17.2	15.9	16.1	16.1	15.1	17.1	17.8
16:1W9	0.6	0.6		0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.9
16:1W7	3.5	4.1	4.1	4.4	3.7	3.7	4.3	4.3	3.5	3.7	4.8
18:0	3.3	2.6	2.7	3.1	2.5	2.5	2.7	2.8	2.4	3.2	3.1
18:1W9	17.1	13.9	13.7	14.4	14.2	12.1	14.1	14.5	11.8	15.7	13.9
18:1W7	2.4	2.9	2.8	3.1	3.0	2.5	2.6	2.7	2.4	2.6	3.2
18:2W6	1.4	1.5	1.5	1.1	1.1	1.5	1.4	1.4	1.5	1.5	1.3
18:3W3	1.0	0.9	0.9	0.7	0.7	1.2	1.0	0.9	1.2	1.1	0.9
18.4W3	1.6	1.5	1.5	1.2	1.3	2.1	1.7	1.6	2.0	1.7	1.3
20:1W11	1.0	1.2	1.2	0.8	0.8	1.3	1.1	1.1	1.1	0.7	1.0
20:1W9	8.2	8.8	8.7	5.7	5.5	10.2	8.2	8.3	10.5	7.8	7.1
20:1W7	0.6	0.5	0.5	0.6	0.6	0.6	0.4	0.4	0.6	0.5	0.7
20:4W6	0.6	0.6	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.7	1.2
20:5W3	7.6	7.7	7.9	9.1	9.2	7.1	7.9	7.7	7.4	7.5	7.3
22:1W11	7.4	7.9	7.9	5.1	4.9	9.5	7.9	8.1	9.8	6.6	6.1
22:1W9	1.1	0.9	0.9	0.7	0.7	1.0	0.9	0.9	1.1	1.0	0.8
22:1W7	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.2	0.1	0.2
22:5W3	1.6	1.8	1.8	1.9	1.9	1.5	1.6	1.7	1.6	1.7	1.6
22:6W3	11.3	11.7	11.9	14.1	14.3	10.3	11.3	11.1	11.3	11.1	11.8

[a](I = initial sample; C = untreated control; T = TBHQ dipped sample.)

Table 4. Major fatty acids of river herring stored at -20°C. for 12 months.

Months in Storage	0	1		3		6		9		12	
Sample: Fatty acids	I[a]	C[a]	T[a]	C	T	C	T	C	T	C	T
14.0	5.0	6.0	6.1	5.7	5.3	5.1	4.8	5.5	5.1	4.8	4.7
16:0	15.7	17.3	17.2	16.1	15.6	16.2	16.0	15.9	15.4	15.3	16.1
16:1W9	0.6	0.6	0.6	0.7	0.7	0.6	0.7	0.7	0.6	0.6	0.8
16:1W7	3.5	4.4	4.4	4.0	4.0	3.7	3.6	3.5	3.4	3.6	3.7
18:0	3.3	3.0	2.9	2.6	2.6	3.3	3.1	2.9	2.8	2.9	3.2
18:1W9	17.1	14.5	14.6	12.7	12.8	15.2	13.2	14.2	13.8	14.6	14.5
18:1W7	2.4	2.9	2.9	2.6	2.6	2.9	2.8	2.7	2.6	2.8	2.8
18:2W6	1.4	0.9	1.3	1.5	1.5	1.4	1.4	1.4	1.4	1.5	1.5
18:3W3	1.0	0.7	0.8	1.0	1.0	0.8	1.0	0.9	1.0	1.0	1.0
18:4W3	1.6	1.3	1.4	1.7	1.7	1.3	1.5	1.6	1.6	1.6	1.5
20:1W11	1.0	1.0	1.0	1.0	1.1	1.2	1.1	1.2	0.9	1.0	0.8
20:1W9	8.2	7.2	7.3	8.7	8.7	7.9	7.5	9.1	9.3	9.3	7.9
20:1W7	0.6	0.5	0.5	0.5	0.5	0.6	0.6	0.8	0.8	0.5	0.6
20:4W6	0.6	0.8	0.8	0.7	0.7	0.7	0.9	0.8	0.7	0.6	0.7
20:5W3	7.6	8.6	8.3	7.8	8.2	8.0	8.5	7.7	8.0	7.5	7.5
22:1W11	7.4	6.3	6.3	8.1	8.2	6.7	6.9	8.0	8.5	8.3	6.9
22:1W9	1.1	0.8	0.8	0.9	0.9	0.9	0.9	1.4	1.4	0.9	0.9
22:1W7	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.2
22:5W3	1.6	2.0	1.8	1.7	1.8	1.7	1.9	1.6	1.7	1.7	1.8
22:6W3	11.3	12.2	10.5	11.5	12.0	12.6	13.6	12.9	12.2	12.2	12.6

[1](I = initial sample; C = untreated control; T = TBHQ dipped sample.)

Table 5. Organoleptic ratings for river herring stored at -20°C (0-200 mm open scale).

Characteristic	0 Rating	200 Rating	Sample	Storage Time, Months 0	9	12
Flavor	Bland	Strong	TBHQ	89	109	120
			Control	94	116	131
Rancidity	Not Rancid	Very Rancid	TBHQ	*	57	63
			Control		61	91
Color	Light	Dark	TBHQ	98	93	98
			Control	105	90	103
Texture	Soft	Firm	TBHQ	59	104	90
			Control	61	100	79
Acceptability	Never Buy	Buy Often	TBHQ	*	82	85
			Control		77	72

* Not tested at zero time.

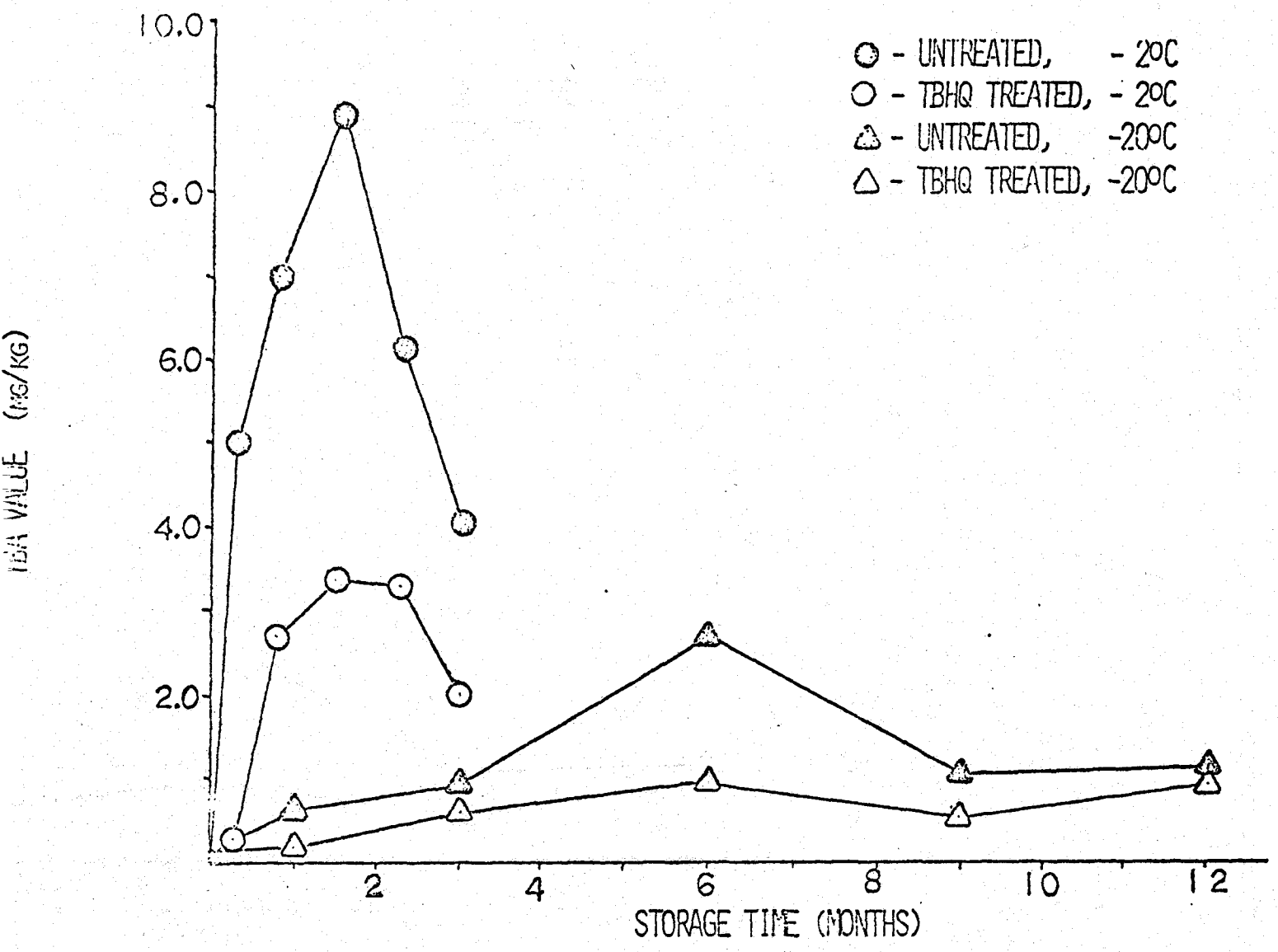

FIGURE 1. THIOBARBITURIC ACID (TBA) VALUES FOR RIVER HERRING FILLETS STORED AT -2°C OR -20°C, WITH OR WITHOUT TBHQ ANTIOXIDANT TREATMENT.

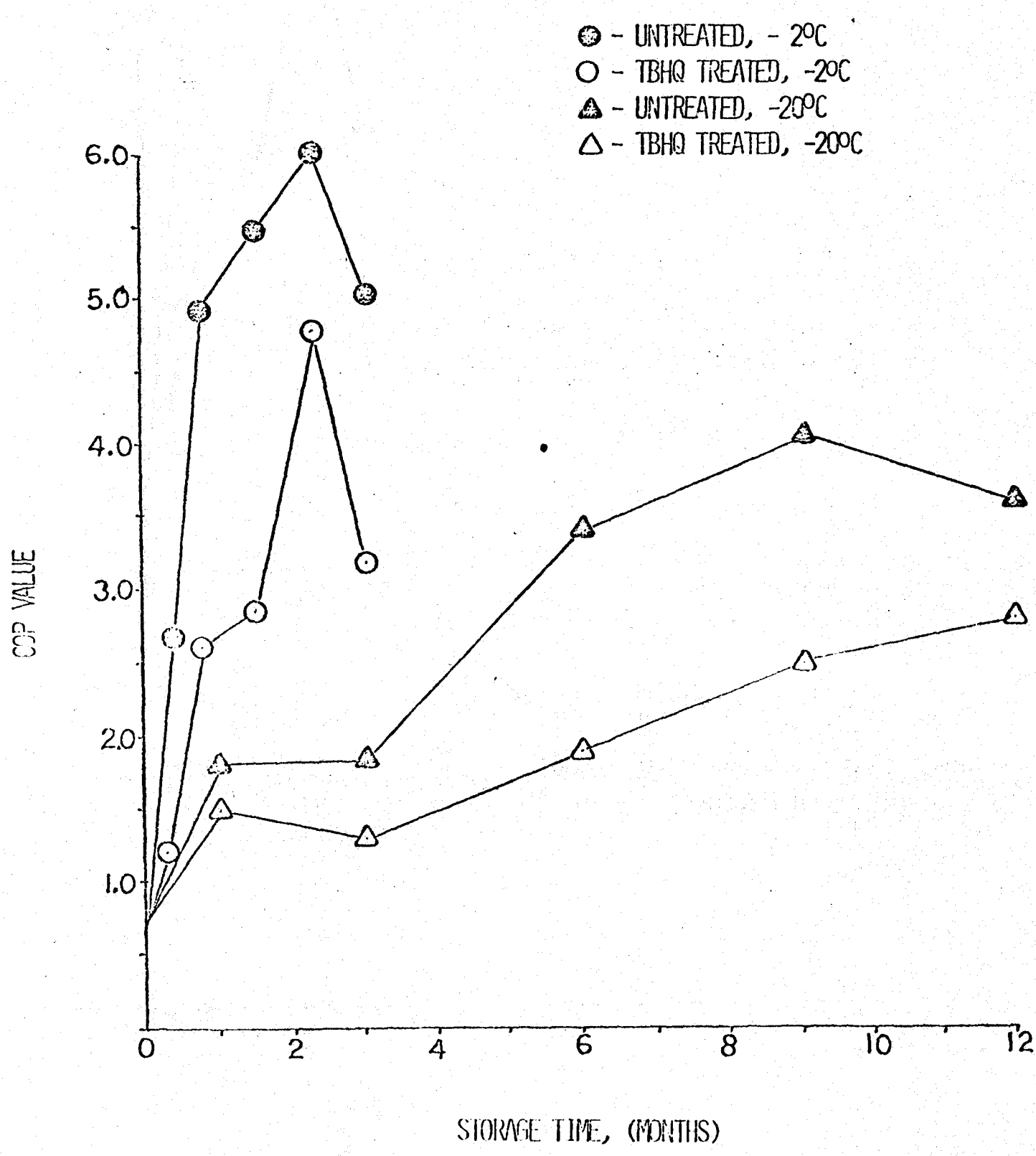

FIGURE 2. CONJUGATED OXIDATION PRODUCTS (COP) VALUES FOR RIVER HERRING STORED AT -2°C OR -20°C, WITH OR WITHOUT TBHQ ANTIOXIDANT TREATMENT.