NOAA TECHNICAL MEMORANDUM NMFS SEFC - 95

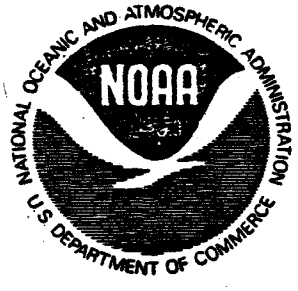
PRELIMINARY STUDIES IN MARINE LIPID OXIDATION

Jeanne D. Joseph and Gloria T. Seaborn

September 1982

U. S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries
Southeast Fisheries Center
Charleston Laboratory
P. O. Box 12607
Charleston, South Carolina 29412-0607

NOAA Technical Memorandum NMFS SEFC- 95



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Malcolm Baldrige, Secretary
National Oceanic and Atmospheric Administration
John Byrne, Administrator
National Marine Fisheries Service
William G. Gordon, Assistant Administrator for Fisheries

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ABSTRACT

Published information on current concepts of lipid oxidation mechanisms, oxidation products and their measurement by chemical and chromatographic means are reviewed. Strengths and weaknesses of classical methodology are discussed in some detail. Recently developed methodology is also described.

Several methods which appeared to be suitable for application to oxidized marine oils and lipids were selected and tested in conjunction with on-going studies of freezer-stored fish. The best of these methods were then applied in measuring lipid oxidation in refrigerator-stored light and dark muscle tissues of four species of fish and an oxidizing fish depot fat.

Although differences in rate and extent of lipid oxidation in the four species were evident, results of two studies of the mullet, Mugil cephalus, suggested that these differences were not species-specific. While oxidation was greater in dark muscle lipids than in those of light muscle in all four species, there appeared to be a direct relationship between initial fatty acid polyunsaturation and maximum TBA values attained in both light and dark muscle lipids. In contrast, high COP values were associated with greater fat content in light muscle, but this relationship was not evident in dark muscle tissues. Thus, it appears that fat content and lipid polyunsaturation, both of which probably vary seasonally in all species, determine the rate and extent of lipid oxidation in fish.

INTRODUCTION

It has been known for many years that fats and oils become rancid through autoxidation during storage and that this problem is accentuated in many marine fishes because of their content of highly unsaturated fatty acids. This problem, which may be particularly severe in fatty fish, can probably be overcome by the use of adequate packaging and storage methods, but in order to develop these methods, satisfactory means of evaluating the degree of lipid oxidation must be applied in storage or lipid stability studies.

Autoxidation, the spontaneous reaction of fatty acids (or other reactive organic compounds) with atmospheric oxygen, is believed to involve abstraction of an allylic hydrogen radical from a carbon atom adjacent to the double bond of a monoene, and between pairs of double bonds in the case of polyenes, followed by oxygen uptake by the carbon radical thus generated (Logani and Davies, 1980; Frankel, 1979, 1980). Classical mechanisms for oxidation of oleic (18:1ω9),[1/] linoleic (18:2ω6) and linolenic (18:3ω3) acids are shown in Figures 1-3 respectively (Dahle et al., 1962; Frankel, 1979, 1980). From the increasing complexity of the reactions, due to the increasing number of doubl bonds in the reactants, it is obvious that oxidation of the typical marine polyunsaturates, eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3) must be extremely complex, indeed. For example, upon oxidation, 20:5ω3 yields eight peroxy radicals and 22:6ω3, ten (Table 1) (Dahle et. al., 1962; Olcott, 1962).

1/ Short-hand notation for methylene-interrupted fatty acid structure indicates the number of carbon atoms in the molecule followed by the number of olefinic bonds. The ω number specifies the position of the ultimate olefinic bond nearest the terminal methyl group of the molecule.

SURVEY OF PUBLISHED METHODOLOGY

Numerous methods are available for assessing the extent of lipid oxidation in food products and biological systems, most of which have been developed for, and applied to, relatively simple vegetable oils, but many have one or more flaws which limit their applicability to marine fats and oils. Among the older non-subjective methods which have been most widely investigated and/or applied are:

1. Oxygen uptake.
2. Peroxide value (POV).
3. 2-Thiobarbituric acid reaction (TBA).
4. Diene conjugation

More recently, a number of other methods have been under investigation.

1. Conjugable oxidation products assay (COP).
2. Spectrophotometric and chromatographic measurement of various oxidation products.
3. Fatty acid compositional changes.

Oxygen uptake by an oil is a direct measurement of oxidation since one of the reactants may be quantified. This method has been utilized for many years, often, but not always, determined manometrically using the Warburg respirometer or a modification of this instrument (Stirton et al., 1945; Saunders et al., 1962; Marcuse and Fredriksson, 1968; Kwoh, 1971; Campbell et al., 1974; Romero and Morton, 1975; 1977a, b; Ke and Ackman, 1976; Morita et al., 1976; numerous other references). However, determination of oxygen uptake by an oxidizing oil gives no inf rmation as to the identity of the oxygenated products present at the time of analysis and is, therefore, a non-specific method for evaluating oxidation. Measurement of oxygen uptake by frozen whole fish has also been attempted but none could be detected over a two-month storage period (Keay _t al., 1972).

Measurements of POV and TBA have been in use for many years but th methods share a common flaw, in that each measures transitory compounds which appear during the course of oxidation (Gray, 1978; Logani and Davies, 1980). During the early stag s of oxidation, hydroperoxides appear and may be fairly accurately estimated by th POV m thod, but as oxidation continues, the hydroperoxides give rise to carbonyls (oxodienes) and hydroxy compounds not measurable by the official POV method of the American Oil Chemists' Society (AOCS).

The official POV method is a highly empirical titrametric procedure which is demanding in terms of sample size (5 g/replicate analysis). This rarely presents a problem in analysis of oxidized rendered oils but if oxidized lipids in fish or other animal tissues are to be determined, large quantities of tissues must be extracted to obtain sufficient lipid for analysis by the official method. It has also been noted that uniform sample weights must be used in order to obtain consistent results with th official POV method or modifications of it (Volz and Gortner, 1947; Link and Form , 1961). Recently, several spectrophotometric POV methods which appear to have promis have been published (Hicks and Gebicki, 1979; Asawaka and Matsushita, 1980a,b).

The TBA test is one of the more commonly used methods for determination of lipid oxidation, and numerous modifications of both the direct extraction method (Vyncke, 1975) and the steam distillation method (Tarladgis et al., 1960) have been reported. However, like the POV method, the TBA test, results of which are expressed as mg malonaldehyde per kg tissue or per kg oil, also measures only a portion of oxidation products including, but not limited to, malonaldehyde and other aldehydes.

Another serious flaw of the TBA method is that the amount of malonaldehyd produced in an oxidizing lipid appears to depend upon the degree of unsaturation of the component fatty acids (Dahl et al., 1962). These authors suggested that only peroxide radicals with an ethylenic bond located β,γ to the peroxide group undergo

cyclization and concommitant formation of malonaldehyde and that such peroxides arise only from autoxidizing fatty acids having three or more double bonds (compare Figures 2 and 3). As shown in Figure 3, the scheme of Dahle et al. (1962) indicates that of the four peroxides formed during autoxidation of 18:3ω3, only two will give rise to malonaldehyde. The number of isomeric peroxide and β, γ peroxide radicals for m re unsaturated fatty acids is shown in Table 1. Thus, TBA color developed fr m oxidized lipids of tissue samples will vary according to the fatty acid profile of each sample and at the same level of oxidation (i.e., same POV), the TBA value of a tissue rich in polyunsaturates will be greater than that of a tissue containing more saturated fatty acids (Figure 4).

More recently, a different series of reactions has been proposed as a more thermodynamically likely mechanism of malonaldehyde formation from oxidized trienes, but not from dienes, by way of intermediate, bicyclic endoperoxides (Figure 5) (Pryor et al., 1976). These compounds have structures which are similar to those of the intermediate endoperoxides produced during biosynthesis of prostaglandins and Pryor et al. (1976) noted that enzymes which catalyze prostaglandin biosynthesis from trienoic and tetraenoic fatty acids do not form endoperoxides from dienoic substrates.

Unlike the results of Dahle et al. (1962) and Pryor et al. (1976), de Koning and Silk (1963) reported that the TBA reagent reacts with malonaldehyde arising from autoxidizing fatty acids having more than a single double bond, and Castell et al. (1966) obtained a TBA reaction on an emulsion of oxidizing 18:2ω6. Tarladgis et al. (1960) and Caldironi and Bazan (1982) also observed the production of TBA- reactive material in oxidizing 18:2ω6. Ohkawa et al. (1978) investigated the discrepant results concerning TBA-reactivity of oxidized 18:2ω6 and found that this reaction was pH-dependent; color development was observed to be minimal at pH 1, the pH at

which the reaction is normally carried out, and increased to a maximum at pH 4. These investigators isolated the major oxidation product of 18:2ω6 by thin-layer chromatography and demonstrated that this product absorbed in the ultraviolet region of th spectrum (233 nm) indicating diene conjugation which develops concommitantly with peroxidation, and reacted with potassium iodide, a hydroperoxide indicator, but not with 2, 4- dinitrophenylhydrazine, a test for aldehydes. Recent work by these authors (Ohkawa, et al., 1978, 1979) and by Asawaka and Matsushita (1980a) hav applied these results to development of methods for quantitating hydroperoxides of oxidized lipids and fatty acids using the TBA reagent.

Depending upon specific experimental conditions and extent of oxidation, a multiplicity of secondary reaction products may be obtained; aldehydes including malonald hyde, ketones, alcohols, short-chained hydrocarbons, mono- and dicarboxylic fatty acids and epoxides have all been reported extensively in the literature, in addition to the endoperoxides suggested by Pryor et al. (1976) and the cyclic peroxid s d scribed by Porter et al. (1976, 1981). Since a number of these products react with the TBA reagent or give rise to malonaldehyde, again depending upon specific reaction conditions, it is evident that measurement of color development using this reagent is a crud , non-specific means of evaluating the extent of lipid oxidation, and should be limited to intraspecific comparisons.

As shown in Figures 2 and 3, when polyunsaturates undergo oxidation, double bond migration within the molecules occurs, producing conjugated double bond systems which absorb strongly in the ultraviolet region of the spectrum, dienes at 233 nm and trienes at 268 nm (Logani and Davies, 1980). Unfortunately, measurements of absorbance at wavelengths near 230 nm are somewhat uncertain because of the difficulty of establishing a background spectrum which is required for quantitation (Parr and Swoboda, 1976) although Recknagel and Goshal (1966) have suggested the use

of an unoxidized oil as a spectrophotometric blank in establishing a background spectrum. Also required for satsifactory quantitation is an accurate molar extinction coefficient (E_{max}) for the compound under study, but those available in the literatur for biological systems are only approximate values (Logani and Davies, 1980). Like TBA values, measurement of diene (or triene) conjugation is non-specific because, lacking accurate molar extinction coefficients, the magnitude of the absorbance change cannot be readily related to the extent of oxidation.

Two recent papers describe a spectrophotometric method for evaluating hydroperoxides and oxodienes in oxidizing lipid systems, the COP assay (Parr and Swoboda, 1976; Fishwick and Swoboda, 1977). The series of reactions involved in the assay is shown in Figure 6 (Fishwick and Swoboda, 1977). The first step in the analytic procedure, reduction of oxidized lipids by sodium borohydride, results in the disappearanc of the characteristic uv absorbance of carbonyl compounds (oxodienes) f oxidized polyunsaturates. This decrease in absorbance at 275 nm is known as th "oxodiene value." The sum of the increase in absorbance at 268 nm and 301 nm following the dehydration reaction is a measure of the formation of conjugated triene and tetraene chromophores, respectively, and is defined as the "COP value." The "COP ratio", the relative proportion of tetraene to triene products, is equal to the ratio of the absorbance change at the two wavelengths and gives useful information as to the degree of unsaturation of the oxidized fatty acids. A disadvantage of the method is that results are expressed in absorbance units for a 1% lipid solution rather than in molar units and, lacking accurate extinction coefficients, conversion to more meaningful numerical data is not possible. As with methods described earlier, the method is useful for intraspecific comparison.

A number of investigators have attempted to identify and quantitate a variety of oxidation products, primarily volatile and non-volatil carbonyls, isolated by chromatographic methods (Lillard and Day, 1964; Ellis et al., 1968; McGill et al., 1977;

Caporaso and Sink, 1978; Kunsman et al., 1978). Some analyses of volatile carbonyls have made use of gas-chromatographic effluent splitters which permitted organoleptic evaluation of the chromatographically separated components (McGill et al., 1977). The major problem with these methods is that, in many cases, experimental conditions such as heat and low pH tend to decompose the hydroperoxide percursors to the carbonyl products being assayed, giving results which are too high (Logani and Davies, 1980). Many of these methods are too time-consuming or complex to be feasible for routine assay (Gray, 1978). As noted by Gray (1978), although the non-volatil carbonyls are the probable precursors of volatile carbonyls, they make no direct contribution to off-flavors of rancid oils and it might be more appropriate to analyze volatil oxidation products. The magnitude and complexity of such an analysis was well illustrated by the work of McGill et al. (1977) who isolated and identified 96 volatil compounds, more than half of which were carbonyls, from cold-stored cod fillets. As in the analysis of non-volatile carbonyls, degradation of precursors to analytes may still present experimental difficulty (Gaddis et al., 1966; McGill and Hardy, 1977).

In model systems, short-chained hydrocarbons, products of hydroperoxide decomposition, appear very early in metal catalyzed autoxidation, before aldehydes are d tectabl (Dumelin and Tappel, 1977). Pentane is the primary product of 6 fatty acid xidation and ethane and/or ethylene of ω 3 fatty acid oxidation. These hydrocarbons may be analyzed by gas-liquid chromatographic (GLC) head-space analysis (Dumelin and Tappel, 1977), and a recent paper describes a relatively simple trap for collecting and concentrating volatile short-chained hydrocarbons for subsequent GLC analysis (Singleton and Pattee, 1980). Injection of oxidized oil directly onto the GLC column and identification of the eluted volatile hydrocarbons has also been described (Scholz and Ptak, 1966; Jarvi et al., 1971). Either of these methods might be quite suitable for detecting the onset of oxidation in low-temperature st rage studies in which oxidation would be expected to be delayed and minimal in extent.

Evaluation of the loss of polyunsaturated fatty acids as a result of lipid oxidation has perhaps received less attention than deserved as an oxidation assay method (however, see May and McCay, 1968a,b; Shono and Toyomizu, 1973a,b; Hardy et al., 1979; Ke et al., 1975) although GLC analyses of fatty acids present relatively few problems. With the use of an appropriate internal standard such as 23:0 and a column liquid phase which will separate the even-carbon chain lengths (EGSP-Z, Silar 5-CP or Carbowax 20M for example)[2/], polyunsaturates such as 18:4ω3, 20:5ω3, 22:4ω6, 22:5ω3 and 22:6ω3 can be quantitated with a high degree of accuracy which is enhanced if analyses are carried out on glass or fused silica wall-coated open tubular columns. The related approach of measuring iodine values (IV) or calculating them from fatty acid GLC data (Ackman, 1966) also seems to have potential, particularly if these methods should be coupled with other methods such as TBA and COP assays (however, see reservations described in the next section). A recent review states, with regard to this approach,

> A major difference between this and other methods is that it involves a direct analysis of the tissue lipids themselves as opposed to the detection of products resulting from lipid peroxidation. In this respect, this technique provides one of the most direct methods for detection and measuring the extent of lipid peroxidation in biological systems. (Logani and Davies, 1980).

Nevertheless, it is essential that the original fatty acid composition of the unoxidized lipids be known if this method is to be applied.

In 1978, a computer search produced over 2000 pertinent published documents on lipid oxidation and numerous new papers are published monthly in a number of international journals. Therefore, the foregoing comments have not been intended as an exhaustive review of lipid oxidation methodology or published literature, but, rather, as a relatively brief review of the more common methods in use at present and

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2/ Specification of trad -names or corporations does not constitute endorsement by NOAA or NMFS.

newer methods which are still undergoing development. That which follows in the next section is an account of our application and evaluation of some of the methods described.

PRELIMINARY LABORATORY EVALUATION OF METHODOLOGY

R.G. Ackman, 1980 recipient of the Kaufmann Memorial Award, stated in his memorial lecture,

> ...it is fitting that I close the Kaufmann memorial lecture with some remarks on potential improvements in publication of lipid material. On a lighter note I suggest that no titles be permitted to start with 'A simple apparatus...' or 'A simple method...' My own contribution to this genre was in fact a gross misuse of the word. (Ackman, 1980).

We concur with Dr. Ackman's implication that few publications so titled do indeed describe simple methods or equipment. Nevertheless, it is our long-term goal to provide such methods for assessing the extent of lipid oxidation in marine products for we believe that such methods are very much needed by seafood technologists and the seafood industry in general, for quality control. Therefore, the methods we have selected for initial evaluation, with few exceptions, have required only equipment generally available in most modern laboratories and reagents commonly on hand or available at reasonable cost. We have tended to avoid methods utilizing toxic chemicals such as benzene, diazomethane and a number of others, and for the comfort of our co-workers, have, so-far, avoided methods requiring use of pyridine. Because of the rising costs of organic solvents and subsequent disposal problems after use, we have concentrated our initial efforts on the application of "micro-" methods.

We consider this technical memorandum to be a preliminary progress report since, as noted in the first section, there are a number of recently published methods and several older methods which might be satisfactorily applied to analysis of oxidized marine lipids. Methods devised for simple model systems or relatively simple vegetable oils frequently require modification before application to highly complex marine oils and much time is often required to evaluate the accuracy and precision of the modified method. Furthermore, because interest in lipid oxidation and the effects of oxidized fats on human health is international in scope, new methodology is published virtually monthly, and the potential is great that improved or more

applicable methods will be available in the future. It is therefore essential that we be prepared to evaluate new methods which appear to offer advantages over thos currently in use.

Initially, no effort was made to perfect the official AOCS POV method for routine analysis of marine oil hydroperoxides since it is so demanding in terms of sample size. Recently, however, a series of oxidized and discolored fish oils became available to us in quantity, and they permitted us to compare POVs obtained using the official method with those obtained on samples of 0.5 g or less with appr priate modifications in methodology (see methodology section). We found the modified method acceptable for determination of POV when sample size must be limited.

A recently published spectrophotometric POV method (Asawaka and Matsushita, 1980b) requires a sample weight of 200 mg or less and interfering lipids and carotenoids are extracted into an organic solvent. We evaluated this method, using cumene hydroperoxide and H_2O_2 as reactants, and preliminary results agreed fairly well with those of the official method. However, oxidized oils frequently caused formation of cloudy solutions during color development. It is interesting that th authors did not suggest the use of N_2 or other inert atmosphere which Link and Formo (1961) considered indispensible to any iodometric procedure. In addition, Asawaka and Matsushita (1980b) did not mention the threshold level of I_2 below which no blue color develops upon addition of starch indicator (Swoboda and Lea, 1958).

Application of the TBA method (Vyncke, 1975) to a variety of freezer-stored fish tissues has presented no serious difficulty other than occasional development of turbidity in the reactant solutions. Both the direct extraction method (Vyncke, 1975) and the distillation method (Tarladgis et al., 1960) give satisfactory results although the former method is less complex and more rapid than the latter. However, some investigators consider the distillation method of MacLean and Castell (1964) to give more meaningful and reproducible results since experimental conditions result in

complet degradation of hydroperoxides to TBA-reactive material (R. G. Ackman, personal communication). Because TBA values, after first reaching a maximum, decline as oxidation progresses, perhaps because of reactions between malonaldehyd (and other aldehydes) and tissue proteins and/or amino acids, interpretation of th experimental data does present problems. Nevertheless, because the method is so widely used in food technology, it seems advisable to continue to include it in future storage and stability studies while keeping its limitations in mind.

Sinc future studies on marine lipid stability may include analyses of oils extracted from fish tissues prior to induced oxidation, the recently published TBA method of Ke and Woyewoda (1979) was investigated. Ke and Woyewoda (1979) found that reproducibility of the method was satisfactory (relative standard deviation, 2-7%) and they observed that a variety of potentially interfering lipid soluble substances, when pr sent at up to 25% of the total sample, gave a relative deviation of less than 2%. We experienced no difficulty in applying the method to oxidized mullet oil (see n xt section) and we consider it a suitable method for measuring TBA values of oxidized fish oils.

The COP assay requires measurement of ultraviolet absorbance at three different wave-lengths, 268, 275 and 301 nm, for calculation of oxodiene value, COP value and COP ratio (Parr & Swoboda, 1976). Rather than reading absorbance at the three specified wavelengths, we normally obtain ultraviolet spectra between 220 and 400 nm (Cary 219 spectrophotometer) of the unreacted, reduced and dehydrated solutions, and measure absorbances directly from the charts. Inspection of spectra obtained on oxidizing lipids of refrigerator-stored fish muscle revealed that absorbance at 317 and 350 nm also increases with storage time (Figure 7), perhaps indicating the development of conjugated pentaene and hexaene chromophores (Hammond and Lundberg, 1953; O'Connor, 1955).

The assay, as published, apparently was developed primarily for vegetable oils which generally contain large amounts (45-65%) of 18:2ω6, whereas most marine oils usually contain less than 2% total dienes. Therefore, the COP value, which is the sum of the increase in triene absorption (from dienoic fatty acids) and tetraene absorption (from fatty acids continuing three or more double bonds) may not be entirely suitabl as a measurement of oxidation in marine lipids. It might be more appropriate to consider only the increase in tetraene absorption or to include absorption at th longer wavelengths. More experience is needed with a variety of marine oils before this question can be answered satisfactorily.

A considerable amount of time was spent in evaluating declining iodin values as a quantitative indicator of ongoing lipid oxidation. In our experience, neither the official AOCS method, nor a modification of it recommended for use with oxidized oils (Cocks and van Rede, 1966), adequately measures oxidation; both methods giv valu s which are substantially, and probably significantly, higher than that which can be calculated from GLC data of fatty acid composition (Figure 8). Oxidized fatty acids do not interfere with gas chromatography of non-oxidized acids since, because of their oxygenated functions, they are too polar to elute from the mildly-polar c lumn in use or because they decompose into shorter chained compounds in the heated injection port, in which case they are eluted with the solvent. Therefore, determination of the loss in polyunsaturation of an oil by GLC of the constituent fatty acids and c mputer calculation of IV appears to be the more direct means of evaluating the extent of lipid oxidation. These values could then be compared with uv spectrophotometric measurements of tetraene conjugation which would provide a possible basis for expressing tetraene conjugation in terms of molar loss of unsaturation. The COP value could then be used for inter-specific comparisons.

A method described at the 1980 ISF/AOCS World Congress was not commented on in the first section of this report because no publication describing the methodology is availabl as yet. However, an abstract has been published (Kaitaranta and Ke, 1980). The oral presentation indicated that marine oils are oxidized in air and oxidation measured by determination of oxygen uptake (weight), TBA values and increas in polar oxidation products by Iatroscan thin layer chromatography/flame ionization detection (TLC/FID) chromatrography. This combination of methods would appear to be promising for our use in fish oil stability studies, because oxygen uptak is a direct measurement affording meaningful although non-specific numerical results and suitable TBA methods and the necessary chromatographic instrumentation ar available.

LIPID OXIDATION IN REFRIGERATOR-STORED FISH TISSUES

I. Introduction

One of the major deterrents to fuller commercial or recreational utilization of Southeast finfish is that a number of species present in abundance, seasonally, rapidly becom rancid and develop off-flavors soon after capture unless handled properly or processed promptly. Development of procedures for control of rancidity such as improved packaging, application of antioxidants or determination of proper storage conditions, is impeded by the lack of suitable analytic methods for measuring oxidativ rancidity or lipid stability in fish tissues.

The selection of target species, mullet (Mugil cephalus), croaker (Micropogonias undulatus, blue fish (Pomatomus saltatrix) and king mackerel (Scomberomorus cavalla) was based upon; (1) seasonal availability in fresh condition, (2) present or potential comm rcial or recreational value and (3) a reputation for rapid rancidity development. Our experimental design was devised to overcome certain problems inherent in earli r investigations of lipid oxidation in fish tissues. Generally, these studies have been carried out on fish fillets treated with various antioxidants and stored in a freezer at -20°C for 9 to 12 months, with quarterly analysis. In an effort to reduce the time required for evaluation of lipid stability, our samples have all been held in a refrigerator at 2°C. Because of differences in lipid and hematin content of light and dark muscle tissue, each tissue has been evaluated separately. In order to overcome problems in interpretation of experimental data arising from differences in composition of individual fish, homogeneous samples of light and dark muscle tissues from a number of fish of each species were prepared by mincing the frozen tissues in a domestic food processor prior to packaging and storage.

Our work during the past two years has been designed to further evaluate the availabl methodology for measuring oxidation in fish lipids by comparing

differences in rate and degree of oxidati n in the target species, to the extent permitted by the methodology. With succeeding studies, we have also attempted to broaden our investigation to the extent allowed by our facilities and equipment. For example, as described in a later section, we are now able to apply both TLC and Iatroscan TLC/FID chromatography to examine the changes in fatty acid c mposition of the major esterified lipid classes during refrigerated storage, and consequently, we are better able to sort out changes due to hydrolysis from those resulting from lipid oxidation. Although we have encountered some problems in quantitation of lipid classes, we find that TLC/FID analyses provide useful information for monitoring lipid stability.

II. Materials and Methods

A. Sample preparation

Mullet for the first study (I) were caught at Murrell's Inlet, S.C., 13 October 1980. King mackerel, mullet (second study, II), and bluefish were caught in Charleston, S.C. harbor 22 July 1981, 9 November 1981 and 17 Nov mber 1981, r spectively. The croakers were landed 8 April 1981 at Beaufort, N.C. All fish were iced immediately after being taken from the water and were headed, gutted and stored at -40°C within 24 hours after capture. On the first day of each study, the fish were removed from the freezer and allowed to thaw somewhat before sample preparation was begun.

All fish, dissecting equipment and work surfaces were washed with 70% isopropanol prior to dissection. Light and dark muscle tissues were separated, with particular care taken to exclude dark from light tissue. The separated tissues were placed in a freezer at -40°C until they were partially frozen (ca 30 minutes) and then were comminuted in a chilled domestic food processor. This partial freezing prevented overheating of the tissues in the processor and resulted in a finely minced, homogenous sample. The samples were weighed into high-quality polyethylene zip-lok

bags and flattened to uniform thickness, prior to storage in a single layer in th refrigerator at 2°C. On the day of sample preparation (day 0), baseline analyses w r carried out on reserved tissue samples. At intervals (see tables for sampling intervals), samples were removed from the refrigerator for chemical and chromatographic analyses of oxidation products.

B. Routine Chemical Procedures

1.) Tissue lipids. Lipids were extracted from the tissues by the Bligh and Dyer method (1959). Sample sizes ranged from 30 - 50 g for light muscle and 15 - 30 g for dark muscle, depending on the amount of tissue available and the lipid content. The chloroform ($CHCl_3$) phase was recovered, dried and evaporated to a small volum in a rotary evaporator at 37°C. The concentrated extract was transferred quantitatively to a 10.0 ml volumetric flask and brought to volume with $CHCl_3$. Aliquots of each solution were taken for gravimetric determination of lipid content and for chemical analyses. The remainder was used for lipid class analyses by TLC/FID chromatography and for fatty acid analyses by GLC.

2.) Depot lipid. When filleting the mullet for the first study, an extraperitoneal layer of fatty tissue, approximately 1 cm. thick, was observed surrounding the abdominal cavity in most of the fish. This fatty tissue and belly-flap tissu were extracted with acetone. The lipids were transferred to hexane by addition of water, dried, concentrated somewhat and stored at -40°C. For induction of oxidation, the oil solution was removed from the freezer, warmed to ambient temperature and completely stripped of solvent in a rotary evaporator at 37°C. About 750 ml of a clear, pale yellow oil with a pleasant odor was obtained. Two 5 g samples were accurately weighed into shallow glass dishes (91 mm. diameter). These samples were used for gravimetric determination of oxygen uptake. For chemical and

chromatographic analyses, 85 mg oil samples were weighed into 20 ml beakers, providing a similar surface area to weight ratio as that of the 5 g samples. All samples were placed in a forced-air oven at 60°C. At the times indicated in Fig. 14, the two 5 g samples were cooled in a dessicator, weighed and returned to th oven. Less frequently, two of the 85 mg samples were used for replicate free fatty acid determination (Ke and Woyewoda, 1978) and a third 85 mg sample provided ample material for COP assay (Parr and Swoboda, 1976) TBA value (Ke and Woyewoda, 1979) and fatty acid analyses by GLC.

C. Chemical Analyses of Oxidation Products

Conjugable oxidation products (COP) were determined on 3 ml or less of the $CHCl_3$ extract by the method of Parr and Swoboda (1976). Ultraviolet spectra of th COP assay products were obtained with a Cary 219 spectrophotometer at a scan rate of 1 nm/sec and a spectral band width of 0.5 nm.

Peroxide values were determined using a modification of the official AOCS method. Nitrogen (N_2) was bubbled through 10 ml of solvent ($CHCl_3$: acetic acid, 1:3, v:v) contained in a 50 ml Erlenmeyer flask. After about 1 min., a 2.0 ml aliquot of the $CHCl_3$ extract, containing 85-300 mg total lipid, was added followed by 0.5 ml of a freshly prepared saturated solution of KI. After a reaction time of one min., 10 ml of H_20 was added, N_2 flow discontinued and the liberated iodine was titrated with 0.01 N sodium thiosulfate from a micro-buret using starch indicator. Blank determinations produced no color in one min.

Additional samples of light and dark muscle tissue were extracted with trichloracetic acid for the determination of TBA values by the method of Vyncke (1975).

D. Chromatographic Methods

1.) Preparative thin-layer chromatography. Samples for determination of fatty acid composition of individual lipid classes were obtained by preparative TLC.

Approximately 25 mg of extracted lipid was streaked on a Silica-gel G plate and d veloped in hexane: diethyl ether: acetic acid (80:20:1, v:v:v). Following development, the plates were air dried and visualized under uv light after spraying with 2, 7-dichlorofluoroscein. The triacylglycerols and free fatty acids were extracted from the silica gel with $CHCl_3$:hexane (1:1, v:v). The polar lipids were extracted with methanol containing a small amount of H_2O.

2) Gas-liquid chromatography. Methyl esters of the component fatty acids, from individual lipid classes and total lipid extracts, were prepared by th m thod of Metcalf et al. (1966) and analyzed in a Hewlett-Packard 5830A gas chromatograph equipped with a 50 m x 0.21 mm flexible fused silica wall-coated open tubular column coated with Carbowax 20-M (Quadrex Corp., Norwalk CN). Helium carrier pressure was 60 psig and column flow, 1 ml/min. Separation was carried out isothermally at 210° C; injector and detector temperatures were maintained at 300° C. The component esters were identified by means of a computer program containing equival nt chain length (ECL) values of authentic standards and esters of cod liver oil (Ackman and Burgher, 1963). Odd-carbon chained unsaturated esters of mullet lipids wer isolated by silver nitrate/TLC ($AgNO_3$/TLC) and rechromatographed to d termine d gree of unsaturation. The GLC data were used to calculate the IV of the oxidizing esters by the method of Ackman (1966).

3.) TLC/FID. Lipid classes were quantified by the Iatroscan TLC/FID system (Iatroscan Laboratories, Tokyo, Japan). The hydrogen flow rate to the detector was 160 ml/min and the air flow, 2000 ml/min. Chromarods bearing the s parated lipid classes were scanned at a rate of 0.31 cm/sec. In the earlier studies, a Fisher Omniscribe recorder-integrator was used at a sensitivity of 10 mV. Later, this was replaced by a Hewlett-Packard 3390A reporting integrator. Type SII

chromarods, st red routinely in 6N sulfuric acid (H_2SO_4), were rinsed thoroughly with distilled H_20, oven dried for 10 min at 110° C and blank scanned immediately before the samples were applied. The rods were spotted with 10-20 µg total lipid in heptane: $CHCl_3$ (1:1, v:v) and developed for 30 min in hexane: ether: acetic acid (85:15:0.02, v:v:v) which separates triacylglycerols, free fatty acids, sterols and total polar lipids. This latter group, which remains at the site of application when this solvent mixture is used for separation, consists principally of the native phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and some minor phospholipids, but includes increasing amounts of polar oxidation products in oxidizing lipids. After d velopment, the rods were oven dried for 4 min. at 110° C before scanning. For separation of PC and PE from oxidation products, the rods were then spotted with a 30 µg lipid sample, developed in hexane: diethyl ether: acetic acid and scanned down to, but not including, the area occupied by the polar lipids. The polar lipids were then separated by development in $CHCl_3$: methanol: acetic acid: water (65:25:9:3, v:v:v:v) for one hour and quantified by a final scan. Standards were included in each run for tentative identification of sample components and determination of detector response factors. Values for lipid class composition presented in the tables are averages of four determinations and are given to two decimal places for comparative purposes only; this degree of accuracy is not implied.

III. Results.

A. Lipid Content and Class Composition

1. Croaker. Of all the species evaluated to date, the croaker has been lowest in fat content (Table 2). In addition, the amount of lipid which could be extracted from light muscle declined substantially as refrigerated storage time increased. Despite prolonged centrifugation of the extraction solutions, the

methanol-water upper phase remained cloudy and the odor of chloroform in this phas was very strong. Hardy et al. (1979) have reported losses of about 40% of total lipid extracted from light muscle of cold-stored cod as compared with that extractable from fresh fish. They postulated that the loss was due to retention of free fatty acids in the upper phase which is discarded in the Bligh and Dyer procedure. This seems to be th most logical explanation for our results also, since lipid class analyses of light muscle lipids showed losses of triacylglycerols and polar lipids but no corresponding increas in free fatty acids (Table 2). Similar, but less prominent, trends in total and esterified lipids of dark muscle were also observed. Little, if any, significant decrease in total and esterified lipids of dark muscle was observed.

2. King mackerel. As observed in the croaker study, there was a decrease in the amount of lipid extracted from king mackerel light muscle tissu during the course of the study, but a somewhat smaller decrease in extracted lipid of dark tissue (Table 3). Results of the lipid class analysis suggest that, as in the croak r, hydrolysis of light muscle esterified lipids with subsequent loss of free fatty acids into the discarded aqueous methanol phase is the most likely explanation. In dark muscl lipids, triacylglycerols declined substantially, but polar lipids, after an initial decline, increased during the latter stage of the study. Since the polar lipids measured in this study consisted of both native phospholipids, principally PC and PE, and polar oxidation products (POP), it seems probable that losses of phospholipids due to hydrolysis and oxidation were obscured by the accumulation of POP during the latter stage of the study.

3. Mullet. Two studies were carried out on rate and extent of lipid oxidation in mullet tissues and major differences were observed in the tissue lipids prior to and during oxidation. In study I the lipid content of the tissues was much greater than in those of study II. In the first study, light tissues contained about five

times as much fat and dark tissues, three times as much as those of the second study (Tables 4 and 5). This is surprising since the fish used in the two studies were captured during the autumn months, October and November, of succeeding years, 1980 and 1981. In the first study, no decline in the amount of lipid extracted, due to loss of free fatty acids into the aqueous methanol phase, was detected for either light or dark tissues, and no increase in free fatty acids, measured by the method of Ke and Woyewoda (1978), could be demonstrated. When the first study was conducted, we had not yet perfected the new technology of TLC/FID chromatography and, consequently, no attempt was made to quantify lipid class composition of the tissue lipids.

Prior to study II, we were able to develop suitable solvent systems and technology to separate not only the naturally-occurring major lipid classes, triacylglycerols, free fatty acids, sterols and polar lipids, but also the phospholipids, PE and PC, from POP. As lipid oxidation progressed, during study II, triacylglycerols of light muscle declined only slightly whereas the total polar lipids declined by about 50% and free fatty acids increased correspondingly (Table 5). In dark muscle lipids, triacylglycerols decreased by about two-fifths, but there was a steady, significant increase in polar lipids, similar to that observed in king mackerel dark tissue lipids. Separation and quantitation of the polar lipids demonstrated that PE and PC were lost during refrigerated storage and that the increase in polar lipids was due to development of POP. In addition, hydrolysis of esterified lipids in both light and dark muscle lipids was indicated by a significant increase in the percentage of free fatty acids.

In addition, in study II, the fatty acid compositions of triacylglycerols, total polar lipids and free fatty acids were determined in mullet light and dark tissue lipids extracted from baseline (T_i) samples and those stored for 24 days (T_f) and are listed in Table 6 as mg fatty acid/100 mg lipid. The column labeled "Totals" in this table is the

sum of each of the fatty acids found in the three lipid classes. While losses in fatty acids of th esterified classes can be attributed to hydrolysis and/or oxidation, any increase in free fatty acids is attributable only to hydrolysis of esterified lipids, although the increase may be minimized by subsequent oxidation of the free fatty acids after hydrolysis. Decreases in "totals" reflect losses due solely to oxidation. The results of this analysis gave information on the substrates for these degradative reactions. In light muscle lipids, little loss of triacylglycerols occurred, but substantial losses of polar lipids, due to hydrolysis and oxidation, were evident. The data sugg st that the most labile polar lipids were those which contained 16:0 and 20:4ω6, 20:5ω3, 22:5ω3 or 22:6ω3 (PUFA) as constitutent fatty acids. In contrast, in dark muscl lipids, both triacylglycerols and polar lipids were lost, due to hydrolysis and oxidation. The triacylglycerols most subject to loss were those containing 16:0, 16:1ω7, 18:1ω9 and PUFA, while the labile polar lipids contained only PUFA.

4. Bluefish. There appeared to be little if any hydrolysis of light muscl lipids of bluefish during the nine-day storage study as no difficulty was experienced in lipid extraction and no appreciable amount of free fatty acids developed (Table 7). Similarly, there was little if any loss of triacylglycerols although losses of PE and PC wer substantial, and the increase in total polar lipids indicated accumulation of POP. In contrast, lipid extraction of dark tissue on the ninth day of the study yielded less lipid than earlier extractions and free fatty acid levels were less than that observed earlier, suggesting that free fatty acids may have been discarded with the aqueous upper phase. Some losses of triacylglycerols were observed during the latter days of the study as a result of oxidation and/or hydrolysis and about 85% of the phospholipids were also lost.

As in the second of the mullet studies, fatty acid compositions of esterified lipids and free fatty acids were determined in bluefish light and dark muscle extracts (T_i and T_f) and are listed in Table 8. In light muscle lipids, mod st amounts of

triacylglycerols and polar lipids were lost as a result of hydrolysis and/or oxidation, but only molecules containing PUFA were affected by these reactions. In dark muscle lipids, on the other hand, losses of triacylglycerols and polar lipids were much greater than in light muscle lipids and molecules containing 16:0 and PUFA were found to be the most labile.

B. Chemical Analyses of Oxidation Products.

1. Croaker. Both TBA and COP values of dark muscle lipids reached a maximum by the end of the second week of storage, but declined thereafter, perhaps as a result of binding of the oxidation products with tissue proteins (Table 9, Fig. 9). In light muscle lipids, maximum values were attained slightly later. There appeared to be a linear relationship between storage time and loss of polyunsaturation in light muscle lipids, but a similar relationship in the dark muscle lipids was obscured by questionable values for PUFA which were not improved by re-analysis. Changes in the fatty acid composition with storage time are shown in Tables 10 and 11.

2. King mackerel. In both light and dark muscle lipids, TBA and COP values and losses in PUFA increased linearly with storage time, with no evidence of a plateau or decline in values (Table 12, Fig. 10). Tables 13 and 14 list the major fatty acids of oxidizing king mackerel lipids.

3. Mullet. In the two mullet studies, considerable differences were observed in the extent of oxidation, as measured by the chemical assays (Tables 4 and 15, Figs. 11 and 12). While TBA values of light muscle lipids did not differ appreciably in the two studies, the TBA value in dark muscle lipids was substantially lower after one week in study I. However, COP values were consistently much higher, at equivalent storage periods, in lipids of both tissues in the first study. Changes in the COP spectra of mullet II dark tissue lipids as a function of refrigerator-storage time are illustrated in Fig 7 and are representative of those obtained on oxidizing light and dark tissue

lipids of the other three species. Peroxide values (POVs) of dark tissue lipids in study II did not reach a plateau or decline during the study as did the COP value and th POVs of light muscle lipids.

Depot oil, extracted from extraperitoneal and belly-flap tissues of mullet used in th first study were also subjected to oxidation at 60°C and evaluated by the sam methods applied to the oxidizing tissue lipids (Table 16, Fig. 13). In addition, however, oxygen uptake was measured by gravimetry during the course of the 16 day study (Fig. 14). No weight gain could be detected during the first six days of the experiment nor was any loss in PUFA observed during this induction period (Table 15). Between six and nine days, however, oxygen uptake occurred at 0.064 μm/g/day (correlation coefficient = 0.966) but increased to 0.892 μm/g/day (correlation coefficient = 0.999) after nin days. Similar changes were observed in TBA and COP values and losses in PUFA. Major even-carbon fatty acids of the mullet tissue lipids observed in study II are listed in Tables 17 and 18.

4. Bluefish. For some unknown reason, results of the chemical analyses were less satisfactory than in those of the other three species. This was particularly troublesom with regard to TBA and COP values of dark muscle lipids on the seventh day, both of which were substantially lower than anticipated (Table 19, Fig. 15). The trend in both light and dark muscle lipids appeared to be toward increasing oxidation products with no imminent plateau except, perhaps for POVs, but unfortunately, insufficient tissue was available to design an experiment of greater duration. Changes in fatty acid composition with storage time are shown in Tables 20 and 21.

IV. Discussion

On unsatisfactory element of the design of these studies has been the use of minced tissues because this processing undoubtedly introduces air which probably

accelerates lipid oxidation in the tissues to some extent and may also increase bacterial contamination which has been shown to inhibit oxidation (Castell and MacLean, 1964). However, there seems to be no other way to minimize compositional differences which can be substantial between individual fish. The use of larg numbers of fish in each study to reduce the effects of this variation in composition was precluded by limited availability of fresh fish in some studies (croaker, king mackerel and bluefish) but equally so by the costs, both of purchase and disposal, of the required large volumes of organic solvents.

Another reason for our decision to use small tissue sample sizes was predicated by the difficulty of dissecting light and dark muscle tissues cleanly but yet rapidly to minimize oxidative degradation prior to baseline analyses. As a result, how v r, w lacked sufficient lipid extract to replicate the chemical analyses more than twic , although sufficient extract was available to replicate the chromatographic analyses a number of times, if necessary. We investigated both accuracy and precision of the ch mical methods during our preliminary evaluation of methodology with g nerally satisfactory results, but we recognize that mere duplication of analytic data does not constitute a firm basis for detailed comparisons. Consequently, we have limited our discussion of the experimental data to some general conclusions, rather than to perceived differences in oxidation rate or extent between the species.

Although there seem to be differences in rate and extent of lipid oxidation in tissues of croaker, mullet, bluefish and king mackerel, these differences do not appear to be species-specific. This is clearly shown by the different results obtained in the two mullet studies but even this comparison might be questioned since tissu lipid content was so different in the two studies.

Two factors which certainly affect the extent of lipid oxidation in fish tissues are lipid content and the initial percentage of polyunsaturated fatty acids in the lipids. When the four species which have been studied are ranked according to lipid content,

initial percentage of PUFA and maximum TBA and COP values attained (Table 22), certain relationships are clarified. First, there is an inverse relationship between total lipid content and PUFA, because the structural phospholipids, rich in PUFA, are relatively constant in amount from species to species and high fat content indicates storage of the less unsaturated triacylglycerols in the tissues. In both light and dark fish muscle tissue, there appeared to be a direct relationship between initial PUFA percentages and maximum TBA values reached. The observations of Dahle et al. (1962) described in the first section of this report, offer one explanation for this relationship. Dahle et al. (1962) found that the amount of color developed by the TBA reagent in solutions of purified, oxidizing fatty acids was directly related to th number of olefinic bonds present in the fatty acids, and they postulated that this was due to greater yield of malonaldehyde from oxidized PUFA (Table 1, Figs. 3 and 4). Thus, it is possible that high maximum TBA values of oxidizing fish lipids may be mor indicative of high initial percentages of PUFA in the tissues than of the overall extent of oxidation in the tissues. As noted in an earlier section, TBA values have limited utility in inter-species comparisons.

The COP assay has been used to evaluate extent of oxidation in a variety of food pr ducts, including some fishery products, and a direct relationship between COP and peroxid values was demonstrated but no comparison between COP and TBA values was made (Parr and Swoboda, 1976). Based upon the rankings in Table 22, there appears to be no direct relationship between the maximum TBA and COP values measured in these studies. Instead, there was an obvious, direct relationshp between maximum COP values and total lipid content in light muscle lipids. Since triacylglycerols contain three fatty acid residues succeptible to oxidation, but phospholipids only two, and since phospholipid content is fairly constant from species to species, it seems reasonable that COP values, expressed as absorbance of a one percent solution, might

be greater in oxidized lipids of fatty fish. However, this relationship between COP values and lipid content was not evident in dark muscle tissues.

As described earlier, Parr and Swoboda (1976) considered that the magnitude of the COP ratio was an indication of the degree of unsaturation of the oxidizing substrate. Fishwick and Swoboda (1977) reported that the COP ratio of a pure conjugated triene, from oxidized 18:2ω6, was zero while that of a pure conjugated tetraene from 18:3ω3 or more unsaturated PUFA was 2.8. Thus, the COP ratio would give valuable information in the analysis of an oxidized seed oil such as soy oil in which 18:2ω6 and 18:3ω3 are the only PUFA. However, in marine oils, 16, 18 and 20 carbon dienes rarely total more than 2% of total fatty acids, but substantial absorption du to the presence of conjugated trienes was observed in all of our oxidation studies. Representative spectra are illustrated in Fig. 7. Based upon accepted acylic mechanisms for lipid peroxidation and the chemical reactions of the COP assay as d scribed by Parr and Swoboda (1976), it would appear that both conjugated trien s and conjugated tetraenes could be formed during the assay of oxidized marine PUFA, d pending upon the specific site of peroxidation (Fig. 16). Thus, peroxidation of either of the terminal ethylenic bonds would result in formation of conjugated trienes while that of the more centrally-located olefinic bonds would yield conjugated tetraenes. In this case, the ratio of absorbance at 301 nm to that at 268 nm would depend upon preferential site peroxidation. However, a COP spectrum of reduced and dehydrated oxidized 22:6ω3 (initially > 99% purity) showed almost no conjugated triene absorption (COP ratio = 2.7) and was virtually identical with a published spectrum of similarly treated oxidized herring lipids (Fig. 7). Therefore, another explanation is needed for the sizable conjugated triene absorption observed in all of our spectra.

Since saturated fatty acids are far less prone to oxidation than PUFA, it has been suggested that 16:0 (May and McCay, 1968a, b) or the sum of 14:0, 16:0 and 18:0 (Ke et al., 1975) might serve as native internal standards in the assessment of PUFA

oxidation. However, we encountered losses of 16:0 during the oxidation of both bluefish and mullet II lipids, due, perhaps, to intramolecular bonding of fatty acid constituents in oxidizing triacylglycerols and phospholipids or possibly to development of polymers involving unesterified 16:0. Regardless of the explanation, our results suggest that it is not advisable to use native saturated fatty acids as internal standards.

It has been well established that tissue components can act as accelerants of lipid oxidation. Hematin and metal ions are two well-known examples. We have no means for evaluating the effects of such components in our fish tissue studies, but a comparison of the oxidation rate in the mullet depot fats with that of the tissue lipids indicates the importance of non-lipid tissue components in promoting oxidation. However, the possibility cannot be ruled out that tocopherols in the depot fat, obtained by way of the food web, conferred stability and prolonged the induction period of the mullet oil in the absence of tissue accelerants.

CONCLUSION

Some years ago, during a symposium on lipids and their oxidation, Dr. W.O. Lundberg, now retired as Director of the Hormel Institute, cited a very perceptiv observation by Dr. George Burr, co-discoverer with his wife, Dr. Mildred Burr, of the essential fatty acids. Dr. Burr told Dr. Lundberg,

> ... when you get through working with rancidity, your children will hav an opportunity to work on it. And when they are through, their children can work on it... (Schultz et al., 1962).

To appreciate the implication of this comment, as it relates to marine oils, it is necessary to understand that these scientists were concerned with oxidation of fatty acids containing a maximum of four olefinic bonds, and this remains true for most of today's specialists in this field. Inherent difficulties encountered in analyses of oxidized fats and oils are vastly compounded by the presence of the characteristic PUFA, 20:5ω3 and 22:6ω3, in marine lipids.

Seafoods have been important in the diet of coastal residents of many cultur s for hundreds of years, but insufficient information on proper processing and packaging methods to protect their quality may have reduced the availability or appeal of seafoods for those who have not been inhabitants of coastal areas. However, results of recent investigations suggest that enhanced utilization of seafoods in the diet may have pr viously unrecognized nutritional benefit. Potentially, marine lipids, particularly those rich in 20:5ω3, may have significant therapeutic value in dietary treatment of cardiovascular disease (Dyerberg et al., 1975; Bang et al., 1976; von Lossonczy et al., 1978; Dyerberg et al., 1978; Dyerberg and Bang, 1979; Saynor and Verel, 1980; Sand rs et al., 1981; Gunby, 1982). Therefore, despite the difficulties and uncertainties in analysis of oxidized marine lipids, continued development of adequat analytic methods is essential to permit protection of the nutritive value of seafoods and to enhance their availability and appeal t all segments of the population.

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TABLE 1. NUMBER AND TYPE OF ISOMERIC PEROXIDO RADICALS FORMED IN THE AUTOXIDATION OF METHYLENE-INTERRUPTED POLYENES.[1]

Polyenes	Isomeric peroxido radicals	β,γ-Unsat'd peroxido radicals	Percentage of β,γ-unsat'd radicals
Tetraene	6	4	67
Pentaene	8	6	75
Hexaene	10	8	80

[1] (Dahle *et al.*, 1962).

Table 2 . LIPID CONTENT AND COMPOSITION OF LIGHT AND DARK FISH TISSUES.

Croaker (*Micropogonias undulatus*)

Storage (days):	0	3	7	10	17
Tissue:			- Light Muscle -		
Lipid content (%):[1]	0.94	0.89	0.81	0.81	0.60
Class composition[1]					
Triacylglycerols:	0.36	0.31	0.28	0.21	0.13
Free fatty acids:	0.01	0.05	0.04	0.04	0.04
Sterols:	0.02	0.02	0.02	0.02	0.04
Polar lipids:	0.55	0.51	0.47	0.54	0.39
Tissue:			- Dark Muscle -		
Lipid content (%):[1]	1.96	1.70	1.64	1.94	1.72
Class composition[1]					
Triacylglycerols:	1.06	0.92	0.92	1.17	0.76
Free fatty acids:	0.03	0.13	0.09	0.08	0.13
Sterols:	0.02	0.05	0.03	0.04	0.07
Polar lipids:	0.84	0.68	0.59	0.65	0.76

[1] Grams/100 g tissue.

Table 3 . LIPID CONTENT AND COMPOSITION OF LIGHT AND DARK FISH TISSUES.

King mackerel (Scomberomorus cavalla)

Storage (days):	0	4	7	11	14	18
Tissue:			- Light Muscle -			
Lipid content (%):[1]	2.25	1.24	1.32	1.38	1.48	1.46
Class composition[1]						
Triacylglycerols:	1.48	0.69	0.77	0.79	0.89	0.79
Free fatty acids:	0.01	0.01	0.01	0.01	0.01	0.01
Sterols:	0.01	0.01	0.01	0.01	0.01	0.01
Polar lipids:	0.77	0.51	0.52	0.54	0.54	0.62
Tissue:			- Dark Muscle -			
Lipid content (%):[1]	5.05	4.24	3.92	4.22	4.39	4.41
Class composition[1]						
Triacylglycerols:	3.59	2.96	2.58	2.56	2.77	2.32
Free fatty acids:	0.01	0.13	0.16	0.21	0.11	0.18
Sterols:	0.04	0.03	0.04	0.05	0.03	0.06
Polar lipids:	1.42	1.12	1.14	1.42	1.52	1.86

[1] Grams/100 g tissue.

Table 4 . OXIDATION INDICES IN LIGHT AND DARK FISH TISSUES.

Mullet (I) (*Mugil cephalus*)

Storage (days):	0	4	6	8	12	15
Tissue:			- Light Muscle -			
Lipid content (%):[1]	5.13	4.59	5.20	5.82	5.74	5.76
TBA Value:[1]	0.01	0.09	0.13	0.24	0.29	0.33
PUFA:[2]	14.8	14.4	13.8	13.8	13.4	12.4
COP Assay[3]						
Oxodiene Value:	-	0.2	0.6	0.7	0.9	1.2
COP Value:	0.7	4.9	11.5	17.8	20.7	24.2
COP Ratio:	0.3	1.5	1.7	1.6	1.7	1.5
Tissue:			- Dark Muscle -			
Lipid content (%):	15.00	14.15	14.63	14.67	15.46	14.55
TBA Value:[1]	0.01	0.54	0.63	0.68	0.69	0.73
PUFA:[2]	15.4	13.0	11.7	11.7	10.2	8.2
COP Assay[3]						
Oxodiene Value:	0.1	1.0	1.5	1.7	1.6	3.4
COP Value:	1.1	17.2	24.7	28.6	34.4	44.5
COP Ratio:	1.1	2.5	1.6	1.5	1.4	1.2

[1] Millimoles/100 g oil.

[2] Percent of total fatty acids: sum of 20:4, 20:5, 22:5 and 22:6.

[3] Absorbance units for a one percent solution.

Table 5 . LIPID CONTENT AND COMPOSITION OF LIGHT AND DARK FISH TISSUES.

Mullet (II) (*Mugil cephalus*)

Storage (days):	0	2	7	14	21	24
Tissue:			- Light Muscle -			
Lipid content (%):[1]	1.03	1.08	1.01	1.04	1.06	0.87
Class composition[1]						
Triacylglycerols:	0.56	0.57	0.44	0.52	0.55	0.45
Free fatty acids:	0.01	0.02	0.04	0.14	0.17	0.20
Sterols:	0.05	0.04	0.04	0.04	0.05	0.03
Polar lipids:	0.42	0.45	0.48	0.33	0.29	0.19
PC:	0.29	0.31	0.29	0.14	0.07	0.05
PE:	0.03	0.04	0.04	0.02	0.03	0.02
Tissue:			- Dark Muscle -			
Lipid content (%):[1]	6.52	6.93	6.03	5.29	6.06	5.95
Class composition[1]				[2]		
Triacylglycerols:	5.28	5.34	2.83	-	2.97	2.92
Free fatty acids:	0.01	0.26	0.27	-	0.34	0.32
Sterols:	0.05	0.06	0.05	-	0.06	0.08
Polar lipids:	1.17	1.32	2.89	-	2.73	2.62
PC:	0.46	0.19	0.21	-	0.07	0.05
PE:	0.14	0.04	0.01	-	0.01	0.01

[1] Grams/100 g tissue.

[2] Unexplainable abberant results.

TABLE 6. MAJOR FATTY ACIDS IN THE TRIACYLGLYCEROL, FREE FATTY ACID AND POLAR LIPID FRACTIONS OF MULLET (II) TISSUES.

Lipid:	Triacyl-glycerols		Free Fatty Acids		Polar Lipids		Totals	
Sample:	Ti	Tf	Ti	Tf	Ti	Tf	Ti	Tf
Tissue:	- Light Muscle -							
Fatty Acids:	Mg/100 Mg Lipid							
12:0	0.3	0.4	0.5	0.2	-	-	0.9	0.6
14:0	3.4	3.2	0.3	0.5	0.5	0.1	3.8	3.8
15:0	2.7	2.6	0.1	0.5	0.5	0.1	3.3	3.2
16:0	11.2	11.0	0.6	5.3	7.9	1.0	19.7	17.3
16:1w7	9.2	8.7	0.2	1.0	0.5	0.5	9.9	10.2
18:0	1.1	1.1	0.2	1.4	1.7	0.3	3.0	2.8
18:1w9	3.6	3.4	0.3	0.8	1.3	0.6	5.2	4.8
18:1w7	1.9	1.8	0.1	0.5	0.7	0.3	2.7	2.6
18:2w6	1.1	1.0	0.1	0.3	0.6	0.1	1.7	1.5
20:4w6	1.3	1.3	0.1	1.4	3.5	1.9	4.9	4.6
20:5w3	2.5	2.3	0.1	1.9	4.2	0.9	6.8	5.1
22:5w3	1.9	1.9	-	1.1	2.7	0.9	4.6	3.9
22:6w3	1.1	1.1	-	1.4	5.0	1.5	6.1	4.0
Tissue:	- Dark Muscle -							
Fatty Acids:	Mg/100 Mg Lipid							
12:0	0.2	1.7	0.2	0.1	-	0.3	0.4	0.6
14:0	5.1	3.6	0.1	0.3	0.1	0.6	5.3	4.5
15:0	3.4	2.4	0.1	0.3	0.1	0.4	3.2	3.0
16:0	16.6	12.1	0.5	2.3	2.2	2.4	19.3	16.8
16:1w7	14.4	9.8	0.2	1.2	0.1	1.2	14.8	12.2
18:0	1.5	1.0	0.2	1.0	1.8	0.6	3.5	2.6
18:1w9	5.1	3.7	0.2	0.8	0.7	0.8	6.1	5.3
18:1w7	2.8	1.9	0.1	0.6	0.8	0.5	3.8	3.0
18:2w6	1.7	1.0	0.1	0.2	0.2	0.2	2.0	1.3
20:4w6	1.9	0.8	0.1	0.3	1.4	0.2	3.4	1.3
20:5w3	4.6	1.6	0.2	0.4	1.2	0.2	5.9	2.1
22:5w3	3.0	1.0	-	0.3	1.7	0.2	4.7	1.4
22:6w3	2.0	0.6	-	0.2	2.4	0.2	4.4	1.0

Table 7 . LIPID CONTENT AND COMPOSITION OF LIGHT AND DARK FISH TISSUES.

Bluefish (*Pomatomus saltatrix*)

Storage (days):	0	2	4	7	9
Tissue:			- Light Muscle -		
Lipid content (%):[1]	2.58	2.61	2.66	2.72	2.57
Class composition[1]					
Triacylglycerols:	1.75	1.81	1.98	1.79	1.63
Free fatty acids:	0.01	0.01	0.02	0.02	0.02
Sterols:	0.01	0.01	0.02	0.01	0.01
Polar lipids:	0.80	0.78	0.64	0.87	0.89
PC:	0.57	0.52	0.43	0.27	0.26
PE:	0.07	0.07	0.05	0.01	0.01
Tissue:			- Dark Muscle -		
Lipid content (%):[1]	6.49	6.47	6.64	6.45	4.50
Class composition[1]					
Triacylglycerols:	5.46	5.38	5.09	5.07	3.17
Free fatty acids:	0.02	0.11	0.11	0.15	0.09
Sterols:	0.04	0.05	0.05	0.06	0.05
Polar lipids:	0.95	0.87	1.28	1.08	1.14
PC:	0.47	0.26	0.19	0.12	0.10
PE:	0.16	0.01	0.01	0.01	0.01

[1] Grams/100 g tissue.

TABLE 8. MAJOR FATTY ACIDS IN THE TRIACYLGLYCEROL, FREE FATTY ACID AND POLAR LIPID FRACTIONS OF BLUEFISH TISSUES.

Lipid:	Triacyl-glycerols		Free Fatty Acids		Polar Lipids		Totals	
Sample:	Ti	Tf	Ti	Tf	Ti	Tf	Ti	Tf
Tissue:				- Light Muscle -				
Fatty Acids:				Me/100 Me Lipid				
14:0	1.7	1.7	-	0.1	0.2	0.2	1.8	2.0
15:0	0.3	0.3	-	-	0.1	0.3	0.3	0.6
16:0	15.6	15.0	-	1.1	3.7	3.2	19.3	19.3
16:1w7	4.1	3.9	-	0.2	0.2	0.2	4.3	4.4
18:0	4.9	5.1	-	0.3	1.1	0.9	6.0	6.3
18:1w9	19.6	18.4	-	0.9	1.5	1.7	21.2	20.9
18:1w7	2.3	2.1	-	0.1	0.3	0.3	2.5	2.5
18:2w6	0.5	0.5	-	0.1	0.1	0.1	0.6	0.6
20:4w6	0.8	0.7	-	0.1	0.7	0.4	1.5	1.2
20:5w3	2.6	2.2	-	0.2	1.0	0.6	3.6	3.0
22:5w3	1.3	1.1	-	0.1	0.3	0.2	1.6	1.4
22:6w3	4.8	4.0	-	0.3	4.9	3.0	9.7	7.3
Tissue:				- Dark Muscle -				
Fatty Acids:				Me/100 Me Lipid				
14:0	2.3	2.1	-	0.2	0.2	0.4	3.0	2.7
15:0	0.4	0.4	-	0.1	0.1	0.1	0.5	0.5
16:0	19.4	17.5	-	1.7	3.9	2.2	26.2	21.3
16:1w7	5.4	4.7	-	0.3	0.2	0.3	5.6	5.3
18:0	5.9	5.1	-	0.7	1.9	0.9	7.8	6.7
18:1w9	22.8	21.2	-	1.7	2.1	1.7	24.9	24.5
18:1w7	2.7	2.4	-	0.3	0.5	0.3	3.2	2.9
18:2w6	0.7	0.5	-	0.1	0.1	0.1	0.8	0.7
20:4w6	1.1	0.7	-	0.1	0.7	0.1	1.8	2.0
20:5w3	3.3	2.1	-	0.1	1.1	0.2	4.5	2.4
22:5w3	1.7	1.0	-	0.1	0.7	0.1	2.4	1.2
22:6w3	6.4	3.8	-	0.2	6.6	0.9	12.9	4.9

Table 9 . OXIDATION INDICES IN LIGHT AND DARK FISH TISSUES.

Croaker (*Micropogonias undulatus*)

Storage (days):	0	3	7	10	17
Tissue:			- Light Muscle -		
TBA Value:[1]	0.02	0.24	0.72	0.71	0.79
Iodine Value:	148.9	127.4	110.1	124.9	115.8
PUFA:[2]	29.8	28.1	28.1	26.8	24.4
COP Assay[3]					
Oxodiene Value:	0.2	0.5	1.5	1.2	1.1
COP Value:	1.2	4.4	13.5	15.0	11.8
COP Ratio:	1.6	1.8	2.2	2.5	2.4
Tissue:			- Dark Muscle -		
TBA Value:[1]	0.02	1.48	1.45	1.00	1.06
Iodine Value:	163.7	158.0	157.4	152.4	144.5
PUFA:[2]	25.9	19.5	15.9	19.1	16.2
COP Assay[3]					
Oxodiene Value:	1.2	3.1	4.9	2.6	2.6
COP Value:	1.7	22.3	33.3	25.6	22.7
COP Ratio:	1.3	2.0	1.8	2.2	2.0

[1] Millimoles/100 g oil.

[2] Percent of total fatty acids: sum of 20:4, 20:5, 22:5 and 22:6.

[3] Absorbance units for a one percent solution.

Table 10 . MAJOR FATTY ACIDS OF LIGHT STORED FISH TISSUES.

Croaker (*Micropogonias undulatus*)

Storage (days):	0	3	7	10	17
Tissue:	- Light Muscle -				
Fatty Acids	Weight Percent Composition				
Saturates					
14:0	1.3	1.4	1.3	1.3	1.2
16:0	22.3	22.6	23.1	23.4	22.8
18:0	5.8	5.8	6.4	6.9	7.2
Monoenes					
16:1w7	8.1	8.0	8.2	8.3	8.2
18:1w9	10.7	10.9	11.0	11.9	11.8
18:1w7	2.6	2.6	2.8	2.9	2.9
20:1w11	1.7	1.8	1.7	1.8	1.9
20:1w9	0.9	1.0	0.9	1.0	1.1
20:1w7	1.4	1.6	1.4	1.6	1.8
22:1w11	0.0	0.0	0.0	0.0	0.0
22:1w9	0.0	0.2	0.2	0.0	0.0
22:1w7	0.0	0.0	0.1	0.0	0.0
Dienes					
18:2w6	1.0	1.1	1.1	1.1	1.1
20:2w6	0.7	0.7	0.7	0.7	0.7
Trienes					
18:3w3	0.3	0.2	0.3	0.2	0.3
20:3w6	0.3	0.3	0.3	0.3	0.3
20:3w3	0.2	0.2	0.1	0.3	0.2
Tetraenes					
18:4w3	0.5	0.3	0.4	0.2	0.2
20:4w6	4.9	4.8	4.9	4.7	4.2
20:4w3	0.4	0.4	0.3	0.3	0.3
22:4w6	1.6	1.7	1.5	1.5	1.4
Pentaenes					
20:5w3	6.6	6.2	6.4	5.8	5.1
22:5w6	1.6	1.6	1.5	1.4	1.5
22:5w3	3.7	3.6	3.5	3.4	3.2
Hexaenes					
22:6w3	14.6	13.5	13.3	12.9	11.8

[1] Values of zero represent less than 0.1 percent of total fatty acids.

Table 11 . MAJOR FATTY ACIDS OF DARK STORED FISH TISSUES.

Croaker (*Micropogonias undulatus*)

Storage (days):	0	3	7	10	17
Tissue:	- Dark Muscle -				
Fatty Acids	Weight Percent Composition				
Saturates					
14:0	1.6	1.5	1.7	1.6	1.5
16:0	22.2	23.1	25.9	23.1	23.3
18:0	6.4	6.9	7.4	7.4	7.6
Monoenes					
16:1w7	9.9	9.9	11.2	10.3	11.5
18:1w9	12.4	13.3	14.7	13.7	14.2
18:1w7	3.2	3.4	3.7	3.6	3.8
20:1w11	1.9	2.2	2.4	2.1	2.4
20:1w9	1.1	1.4	1.3	1.2	1.4
20:1w7	1.7	1.7	2.1	1.9	2.2
22:1w11	0.0 [1]	0.0	0.0	0.0	0.0
22:1w9	0.1	0.3	0.3	0.3	0.1
22:1w7	0.1	0.2	0.2	0.2	0.1
Dienes					
18:2w6	1.0	1.0	1.1	1.1	1.1
20:2w6	0.8	0.8	0.8	0.8	0.8
Trienes					
18:3w3	0.3	0.3	0.3	0.3	0.3
20:3w6	0.2	0.2	0.2	0.3	0.2
20:3w3	0.1	0.2	0.1	0.2	0.2
Tetraenes					
18:4w3	0.4	0.4	0.4	0.3	0.2
20:4w6	3.8	3.2	2.6	3.0	2.8
20:4w3	0.2	0.3	0.2	0.3	0.3
22:4w6	1.3	1.1	0.9	1.1	1.1
Pentaenes					
20:5w3	5.6	4.5	3.7	4.4	3.8
22:5w6	1.1	0.9	0.5	0.8	0.7
22:5w3	3.7	3.1	2.5	3.1	2.8
Hexaenes					
22:6w3	12.9	8.8	5.9	8.5	6.9

[1] Values of zero represent less than 0.1 percent of total fatty acids.

Table 12 . OXIDATION INDICES IN LIGHT AND DARK FISH TISSUES.

King mackerel (Scomberomorus cavalla)

Storage (days):	0	4	7	11	14	18
Tissue:			- Light Muscle -			
TBA Value:[1]	0.05	0.20	0.36	0.42	0.67	1.06
Iodine Value:	169.7	168.9	168.1	163.4	161.8	160.3
PUFA:[2]	33.3	32.7	32.7	31.2	30.1	30.2
COP Assay[3]						
Oxodiene Value:	0.4	–	0.2	0.1	0.8	1.2
COP Value:	1.5	2.3	4.6	15.1	12.8	14.4
COP Ratio:	1.5	1.3	2.0	1.8	1.9	2.4
Tissue:			- Dark Muscle -			
TBA Value:[1]	0.07	0.67	1.39	1.73	1.73	2.26
Iodine Value:	146.4	152.4	146.7	135.6	134.1	126.2
PUFA:[2]	29.7	27.9	26.2	23.2	22.5	20.1
COP Assay[3]						
Oxodiene Value:	–	0.9	1.7	0.4	3.0	3.4
COP Value:	1.4	11.5	13.6	16.9	20.7	22.9
COP Ratio:	1.3	1.7	1.7	1.3	1.5	1.2

[1] Millimoles/100 g oil.

[2] Percent of total fatty acids: sum of 20:4, 20:5, 22:5 and 22:6.

[3] Absorbance units for a one percent solution.

Table 13 . MAJOR FATTY ACIDS OF LIGHT STORED FISH TISSUES.

King mackerel (Scomberomorus cavalla)

Storage (days):	0	4	7	11	14	18
Tissue:	- Light Muscle -					
Fatty Acids	Weight Percent Composition					
Saturates						
14:0	2.1	2.0	2.0	2.1	2.1	2.1
16:0	23.4	24.1	24.0	24.7	24.8	25.1
18:0	8.7	9.0	9.1	9.4	9.4	9.5
Monoenes						
16:1w7	3.0	2.8	2.7	2.8	2.9	2.9
18:1w9	11.8	12.2	12.1	12.4	12.5	12.5
18:1w7	3.1	3.1	3.1	3.2	3.2	3.2
20:1w11	0.1	0.1	0.1	0.1	0.1	0.1
20:1w9	1.0	1.0	1.1	1.1	1.1	1.1
20:1w7	0.2	0.2	0.2	0.2	0.2	0.2
22:1w11	0.2	0.2	0.2	0.2	0.2	0.2
22:1w9	0.0 [1]	0.0	0.0	0.0	0.0	0.0
22:1w7	0.0	0.0	0.0	0.0	0.0	0.0
Dienes						
18:2w6	1.0	1.0	1.0	1.0	1.0	1.0
20:2w6	0.2	0.2	0.2	0.2	0.2	0.2
Trienes						
18:3w3	0.4	0.4	0.4	0.4	0.4	0.4
20:3w6	0.1	0.1	0.1	0.1	0.1	0.1
20:3w3	0.1	0.1	0.1	0.1	0.1	0.1
Tetraenes						
18:4w3	0.4	0.4	0.4	0.4	0.4	0.4
20:4w6	2.7	2.8	2.8	2.8	2.7	2.7
20:4w3	0.2	0.2	0.2	0.2	0.2	0.2
22:4w6	0.5	0.5	0.5	0.5	0.5	0.5
Pentaenes						
20:5w3	4.6	4.6	4.5	4.4	4.3	4.2
22:5w6	1.3	1.3	1.4	1.3	1.3	1.3
22:5w3	1.7	1.8	1.7	1.6	1.6	1.6
Hexaenes						
22:6w3	24.2	23.7	23.6	22.4	22.1	21.7

[1] Values of zero represent less than 0.1 percent of total fatty acids.

Table 14 . MAJOR FATTY ACIDS OF DARK STORED FISH TISSUES.

King mackerel (Scomberomorus cavalla)

Storage (days):	0	4	7	11	14	18
Tissue:	- Dark Muscle -					
Fatty Acids	Weight Percent Composition					
Saturates						
14:0	2.2	2.3	2.5	2.7	2.7	2.8
16:0	21.7	22.8	23.9	25.3	25.2	26.3
18:0	10.0	10.4	10.8	11.2	11.4	11.7
Monoenes						
16:1w7	3.2	3.4	3.5	3.7	3.7	3.9
18:1w9	13.7	14.3	14.7	15.1	15.3	15.9
18:1w7	3.7	3.8	3.9	4.0	4.1	4.2
20:1w11	0.2	0.2	0.2	0.2	0.2	0.2
20:1w9	1.2	1.3	1.3	1.3	1.3	1.3
20:1w7	0.3	0.3	0.3	0.3	0.3	0.3
22:1w11	0.3	0.3	0.3	0.3	0.3	0.3
22:1w9	0.1[1]	0.1	0.1	0.1	0.1	0.1
22:1w7	0.0	0.0	0.0	0.0	0.0	0.0
Dienes						
18:2w6	1.1	1.1	1.1	1.1	1.1	1.0
20:2w6	0.2	0.2	0.2	0.2	0.2	0.2
Trienes						
18:3w3	0.4	0.4	0.4	0.4	0.4	0.3
20:3w6	0.1	0.1	0.1	0.1	0.1	0.1
20:3w3	0.1	0.1	0.1	0.1	0.1	0.1
Tetraenes						
18:4w3	0.4	0.4	0.4	0.3	0.3	0.3
20:4w6	2.5	2.4	2.3	2.0	2.0	1.8
20:4w3	0.2	0.2	0.2	0.1	0.2	0.1
22:4w6	0.6	0.5	0.4	0.3	0.4	0.4
Pentaenes						
20:5w3	4.0	3.8	3.7	3.2	3.2	2.9
22:5w6	1.3	1.2	1.0	0.9	0.9	0.8
22:5w3	2.0	1.8	1.7	1.5	1.5	1.3
Hexaenes						
22:6w3	17.2	19.8	18.6	16.4	15.8	14.1

[1] Values of zero represent less than 0.1 percent of total fatty acids.

Table 15 . OXIDATION INDICES IN LIGHT AND DARK FISH TISSUES.

Mullet (II) (*Mugil cephalus*)

Storage (days):	0	2	7	14	21	24
Tissue:			- Light Muscle -			
TBA Value:[1]	0.03	0.07	0.11	0.26	0.50	0.37
POV:[2]	–	1.8	3.0	9.3	9.7	5.9
Iodine Value:	154.9	151.7	154.6	148.3	145.7	145.1
PUFA:[3]	29.1	27.7	28.8	26.1	24.9	25.0
COP Assay[4]						
Oxodiene Value:	–	–	0.6	–	–	–
COP Value:	1.2	1.2	2.3	6.1	12.1	7.7
COP Ratio:	0.3	1.0	1.5	1.9	1.6	2.0
Tissue:			- Dark Muscle -			
TBA Value:[1]	0.00	0.62	1.45	2.10	1.65	1.38
POV:[2]	–	5.9	18.0	22.5	32.3	39.9
Iodine Value:	133.1	126.5	117.4	116.7	104.1	109.1
PUFA:[3]	22.0	19.3	15.7	15.1	11.2	12.8
COP Assay[4]						
Oxodiene Value:	–	–	1.9	2.1	2.6	2.2
COP Value:	0.9	8.4	22.4	22.9	21.5	20.6
COP Ratio:	1.9	1.6	1.5	1.3	1.3	1.5

[1] Millimoles/100 g oil.

[2] Milliequivalents/100 g oil.

[3] Percent of total fatty acids: sum of 20:4, 20:5, 22:5 and 22:6.

[4] Absorbance units for a one percent solution.

TABLE 16. OXIDATION INDICES IN MULLET DEPOT OIL.

Storage (Days):	0	.25	1	2	3	6	9	13	16
TBA Value:[1]	0.01	0.01	0.01	0.01	0.01	0.04	0.09	0.50	0.76
PUFA:[2]	13.6	13.5	13.4	13.4	13.5	13.8	13.1	10.9	7.9
Free Fatty Acids:	1.3	1.2	1.2	1.2	1.4	1.8	1.9	3.0	6.4
COP Assay[3]									
Oxodiene Value:	-	-	-	0.1	0.1	1.0	1.2	2.7	6.2
COP Value:	1.2	0.9	1.5	1.3	1.8	2.9	5.3	11.6	13.2
COP Ratio	1.3	1.7	1.1	1.4	1.6	1.5	1.6	1.2	1.0

1 Millimoles/100 g oil.

2 Percent of Total fatty acids: sum of 20:4, 20:5, 22:5 and 22:6.

3 Absorbance units for a 1% lipid solution.

Table 17 . MAJOR FATTY ACIDS OF LIGHT STORED FISH TISSUES.

Mullet (II) (*Mugil cephalus*)

Storage (days):	0	2	7	14	21	24
Tissue:	- Light Muscle -					
Fatty Acids	Weight Percent Composition					
Saturates						
14:0	3.6	3.9	3.4	4.0	4.2	4.1
16:0	20.6	20.9	20.8	21.4	21.9	21.6
18:0	3.0	3.2	3.5	3.6	3.7	3.8
Monoenes						
16:1w7	10.4	10.8	10.5	11.0	11.2	11.7
18:1w9	5.6	5.5	5.4	5.7	5.5	5.5
18:1w7	3.1	3.0	3.0	3.0	3.1	3.3
20:1w11	0.1	0.1	0.1	0.1	0.1	0.1
20:1w9	0.2	0.2	0.2	0.2	0.2	0.2
20:1w7	0.3	0.3	0.3	0.3	0.3	0.3
22:1w11	0.2	0.2	0.2	0.2	0.1	0.1
22:1w9	0.2	0.2	0.2	0.2	0.2	0.2
22:1w7	0.0[1]	0.0	0.0	0.0	0.0	0.0
Dienes						
18:2w6	1.9	1.8	1.8	1.8	1.8	1.7
20:2w6	0.2	0.2	0.2	0.1	0.1	0.1
Trienes						
18:3w3	0.8	0.8	0.8	0.8	0.7	0.7
20:3w6	0.4	0.4	0.4	0.4	0.4	0.4
20:3w3	0.1	0.1	0.1	0.1	0.1	0.1
Tetraenes						
18:4w3	1.0	1.0	1.0	1.0	0.9	0.9
20:4w6	5.8	5.6	5.9	5.5	5.6	5.5
20:4w3	0.6	0.5	0.5	0.5	0.5	0.5
22:4w6	1.1	1.0	1.0	0.9	0.9	0.9
Pentaenes						
20:5w3	8.4	8.1	8.3	7.8	7.5	7.4
22:5w6	1.1	1.1	1.1	1.1	1.0	1.0
22:5w3	5.8	5.8	5.9	5.5	5.3	5.3
Hexaenes						
22:6w3	7.5	7.1	7.4	6.8	6.6	6.6

[1] Values of zero represent less than 0.1 percent of total fatty acids.

Table 18 . MAJOR FATTY ACIDS OF DARK STORED FISH TISSUES.

Mullet (II) (*Mugil cephalus*)

Storage (days):	0	2	7	14	21	24
Tissue:	- Dark Muscle -					
Fatty Acids	Weight Percent Composition					
Saturates						
14:0	5.1	5.3	5.7	5.8	6.2	6.0
16:0	19.2	19.9	21.0	21.2	22.8	22.2
18:0	3.1	3.1	4.0	3.2	3.5	3.4
Monoenes						
16:1w7	15.2	15.8	16.6	16.7	17.8	17.4
18:1w9	6.0	6.2	6.3	6.5	7.0	6.8
18:1w7	3.7	3.8	3.8	3.9	4.2	4.0
20:1w11	0.2	0.2	0.2	0.2	0.2	0.2
20:1w9	0.3	0.3	0.3	0.3	0.3	0.3
20:1w7	0.4	0.4	0.4	0.4	0.4	0.4
22:1w11	0.1	0.1	0.1	0.1	0.0	0.0
22:1w9	0.2	0.1	0.1	0.1	0.1	0.1
22:1w7	0.0[1]	0.0	0.0	0.0	0.0	0.0
Dienes						
18:2w6	2.1	2.1	2.1	2.1	2.0	2.0
20:2w6	0.2	0.2	0.2	0.2	0.2	0.2
Trienes						
18:3w3	1.2	1.2	1.1	1.2	1.1	1.0
20:3w6	0.3	0.3	0.3	0.3	0.3	0.3
20:3w3	0.1	0.1	0.1	0.1	0.1	0.1
Tetraenes						
18:4w3	1.6	1.6	1.5	1.4	1.2	1.3
20:4w6	3.5	3.2	2.9	2.8	2.3	2.5
20:4w3	0.6	0.5	0.5	0.5	0.4	0.4
22:4w6	0.9	0.8	0.7	0.7	0.5	0.6
Pentaenes						
20:5w3	6.6	6.3	5.5	5.3	4.4	4.8
22:5w6	0.8	0.7	0.6	0.5	0.4	0.5
22:5w3	5.3	4.8	4.1	4.4	3.2	3.5
Hexaenes						
22:6w3	5.1	4.3	3.5	3.5	2.6	3.0

[1] Values of zero represent less than 0.1 percent of total fatty acids.

Table 19 . OXIDATION INDICES IN LIGHT AND DARK FISH TISSUES.

Bluefish (Pomatomus saltatrix)

Storage (days):	0	2	4	7	9
Tissue:			- Light Muscle -		
TBA Value:[1]	0.02	0.04	0.11	0.47	0.57
POV:[2]	0.9	0.7	1.5	10.7	13.8
Iodine Value:	125.9	128.1	126.5	120.5	122.4
PUFA:[3]	19.8	20.3	20.1	18.2	18.7
COP Assay[4]					
Oxodiene Value:	-	-	-	0.6	1.4
COP Value:	1.6	1.2	3.8	13.7	18.6
COP Ratio:	1.2	1.4	1.5	2.9	2.0
Tissue:			- Dark Muscle -		
TBA Value:[1]	0.01	0.53	0.72	0.91	1.61
POV:[2]	0.6	6.1	19.9	25.7	28.6
Iodine Value:	120.5	116.4	122.1	116.7	106.3
PUFA:[3]	17.8	16.7	18.5	16.7	13.9
COP Assay[4]					
Oxodiene Value:	-	0.5	2.1	1.1	1.8
COP Value:	1.0	5.7	12.7	14.6	30.9
COP Ratio:	1.1	1.9	2.6	2.4	1.6

[1] Millimoles/100 g oil.

[2] Milliequivalents/100 g oil.

[3] Percent of total fatty acids: sum of 20:4, 20:5, 22:5 and 22:6.

[4] Absorbance units for a one percent solution.

Table 20 . MAJOR FATTY ACIDS OF LIGHT STORED FISH TISSUES.

Bluefish (Pomatomus saltatrix)

Storage (days):	0	2	4	7	9
Tissue:	- Light Muscle -				
Fatty Acids	Weight Percent Composition				
Saturates					
14:0	2.3	2.3	2.4	2.3	2.4
16:0	23.1	22.6	23.4	22.8	23.7
18:0	6.7	6.6	6.9	6.7	6.9
Monoenes					
16:1w7	6.2	6.1	6.3	6.1	6.4
18:1w9	25.8	25.5	26.1	25.9	26.5
18:1w7	3.1	3.0	3.1	3.1	3.2
20:1w11	0.3	0.3	0.2	0.2	0.3
20:1w9	1.9	1.8	1.8	1.8	1.9
20:1w7	0.4	0.4	0.4	0.4	0.4
22:1w11	0.4	0.4	0.4	0.4	0.5
22:1w9	0.1	0.1	0.1	0.1	0.1
22:1w7	0.0	0.0	0.0	0.0	0.0
Dienes					
18:2w6	0.7	0.7	0.7	0.7	0.7
20:2w6	0.2	0.2	0.2	0.2	0.2
Trienes					
18:3w3	0.4	0.4	0.4	0.4	0.4
20:3w6	0.1	0.1	0.1	0.1	0.1
20:3w3	0.1	0.1	0.1	0.1	0.1
Tetraenes					
18:4w3	0.6	0.6	0.6	0.5	0.6
20:4w6	1.7	1.8	1.8	1.6	1.7
20:4w3	0.4	0.5	0.4	0.4	0.4
22:4w6	0.6	0.7	0.5	0.5	0.5
Pentaenes					
20:5w3	4.6	4.8	4.8	4.4	4.4
22:5w6	0.6	0.6	0.5	0.5	0.5
22:5w3	2.0	2.1	2.0	1.9	2.0
Hexaenes					
22:6w3	11.4	11.6	11.5	10.4	10.7

Values of zero represent less than 0.1 percent of total fatty acids.

Table 21 . MAJOR FATTY ACIDS OF DARK STORED FISH TISSUES.

Bluefish (Pomatomus saltatrix)

Storage (days):	0	2	4	7	9
Tissue:	- Dark Muscle -				
Fatty Acids	Weight Percent Composition				
Saturates					
14:0	3.3	3.0	2.5	2.3	2.9
16:0	22.7	24.0	21.9	22.3	24.3
18:0	10.8	6.7	7.0	7.3	7.5
Monoenes					
16:1w7	5.7	7.1	6.5	6.6	7.2
18:1w9	23.3	25.1	25.7	26.8	27.1
18:1w7	3.0	3.2	3.2	3.3	3.5
20:1w11	0.3	0.3	0.3	0.3	0.3
20:1w9	1.8	1.7	2.1	2.1	2.0
20:1w7	0.4	0.4	0.5	0.5	0.5
22:1w11	0.5	0.4	0.6	0.5	0.6
22:1w9	0.1	0.1	0.1	0.1	0.1
22:1w7	0.0[1]	0.0	0.0	0.0	0.0
Dienes					
18:2w6	0.7	0.8	0.8	0.8	0.8
20:2w6	0.2	0.2	0.2	0.2	0.2
Trienes					
18:3w3	0.4	0.4	0.4	0.4	0.4
20:3w6	0.1	0.1	0.1	0.1	0.1
20:3w3	0.1	0.1	0.1	0.1	0.1
Tetraenes					
18:4w3	0.6	0.7	0.6	0.6	0.6
20:4w6	1.5	1.5	1.5	1.5	1.3
20:4w3	0.4	0.4	0.4	0.4	0.3
22:4w6	0.7	0.6	0.7	0.7	0.4
Pentaenes					
20:5w3	4.1	4.2	4.3	4.1	3.4
22:5w6	0.7	0.5	0.6	0.6	0.4
22:5w3	2.1	1.9	2.3	2.1	1.7
Hexaenes					
22:6w3	10.1	9.1	10.3	9.0	7.5

[1] Values of zero represent less than 0.1 percent of total fatty acids.

TABLE 22. SPECIES RANKED ACCORDING TO LIPID CONTENT, INITIAL PUFA PERCENTAGE AND MAXIMUM TBA AND COP VALUES ATTAINED.[1]

Lipid Content	PUFA	TBA Value	COP Value
Tissue:	- Dark Muscle -		
Mullet (I)	Mackerel	Mackerel	Mullet (I)
Bluefish	Croaker	Mullet (II)	Croaker
Mullet (II)	Mullet (II)	Bluefish	Bluefish
Mackerel	Bluefish	Croaker	Mackerel
Croaker	Mullet (I)	Mullet (I)	Mullet (II)
Tissue:	- Light Muscle -		
Mullet (I)	Mackerel	Mackerel	Mullet (I)
Bluefish	Croaker	Croaker	Bluefish
Mackerel	Mullet (II)	Bluefish	Mackerel
Mullet (II)	Bluefish	Mullet (II)	Croaker
Croaker	Mullet (I)	Mullet (I)	Mullet (II)

[1] Ranked in decreasing order.

Fig. 1. Autoxidation of oleic acid (18:1 ω9).

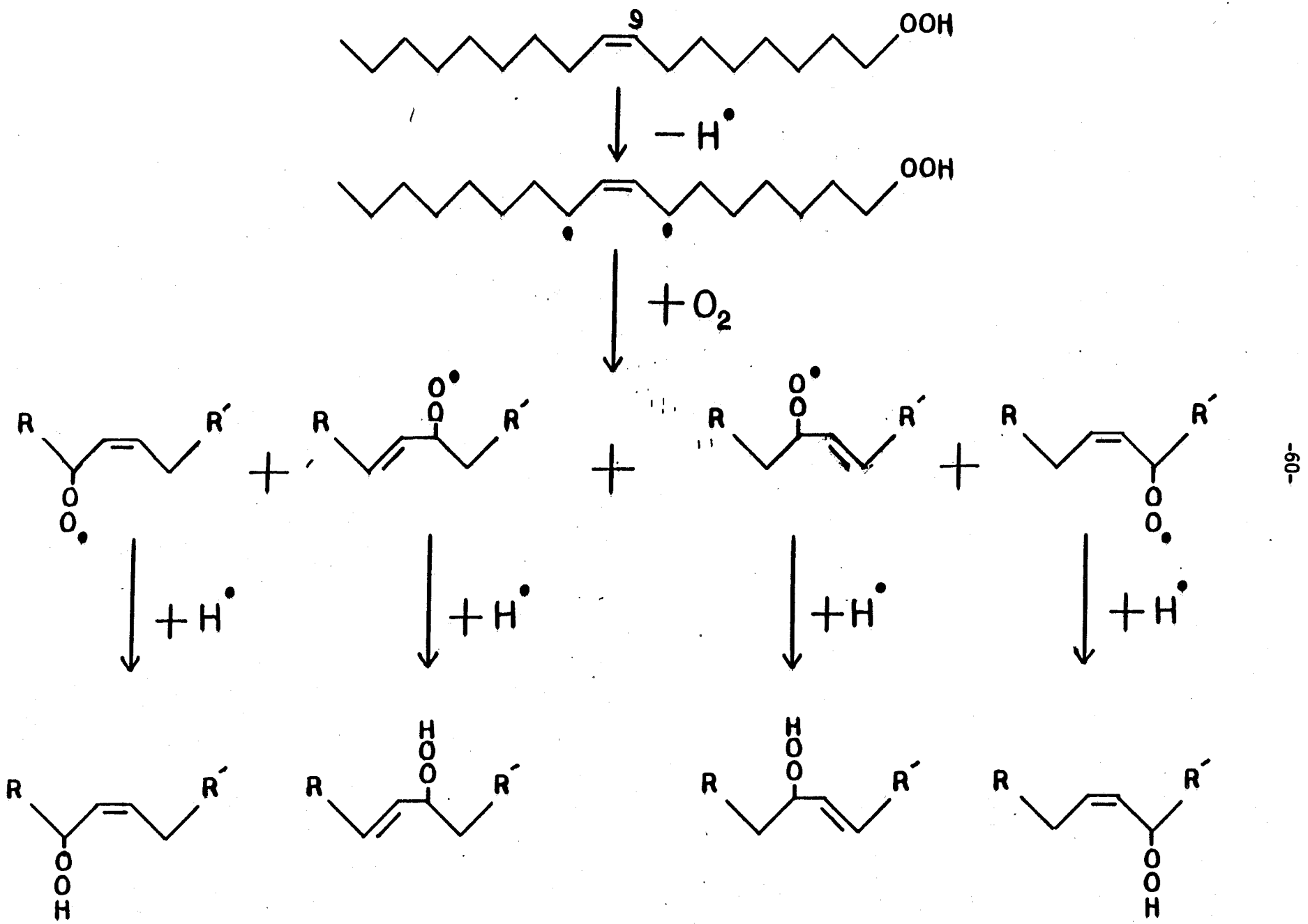

9
OOH
$-H^{\bullet}$
OOH
$+O_2$
R
R´
O
O
$+H^{\bullet}$
HO

Fig. 2. Autoxidation of linoleic acid (18:2ω6).

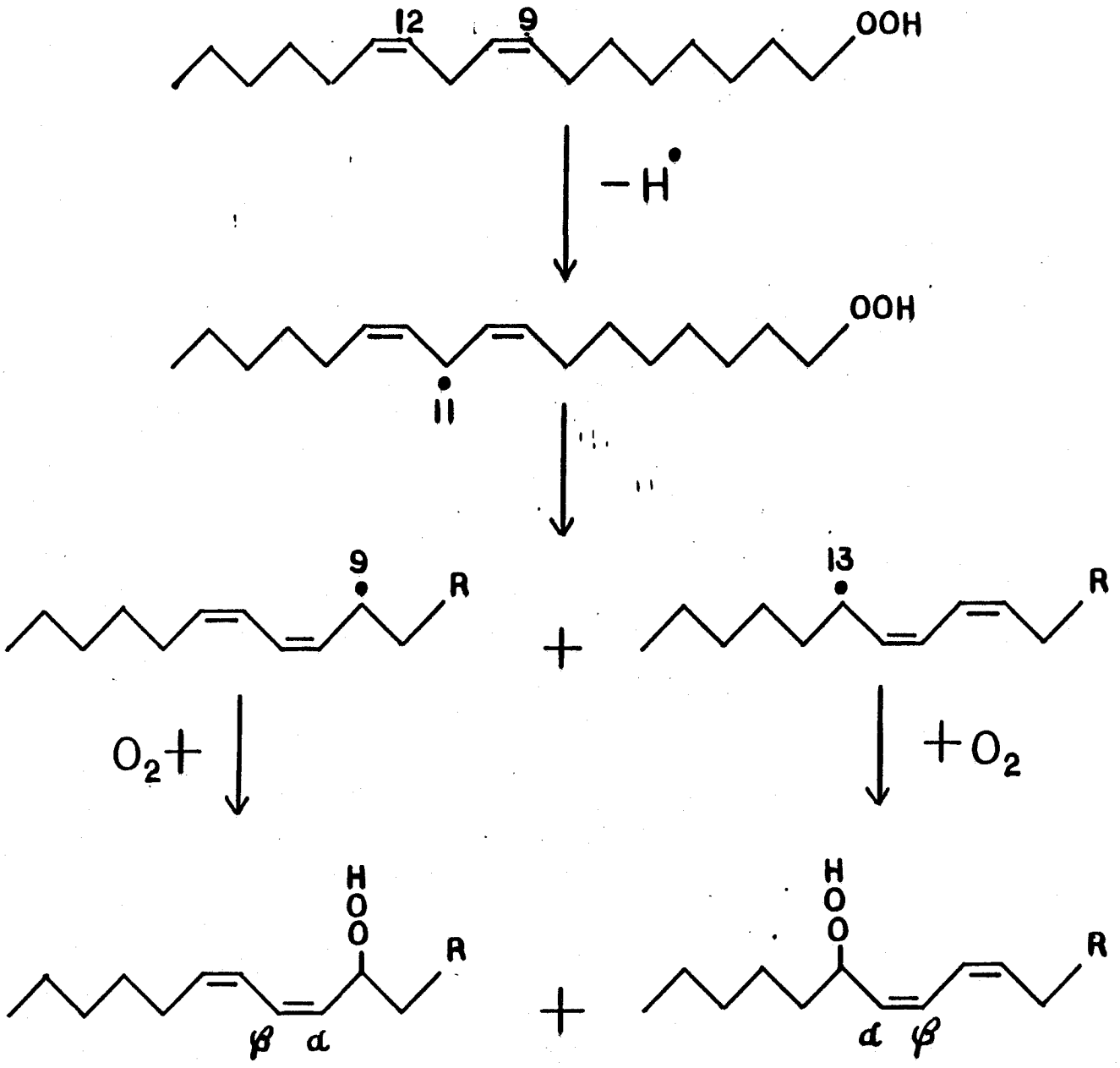

12
9
OOH
$-H^{\bullet}$
OOH
11
9
R
13
R
O_2+
$+O_2$
H
O
O
R
β α
H
O
O
R
α β

Fig. 3. Autoxidation of linolenic acid (18:3 ω3) and biosynthesis of malonaldehyde.

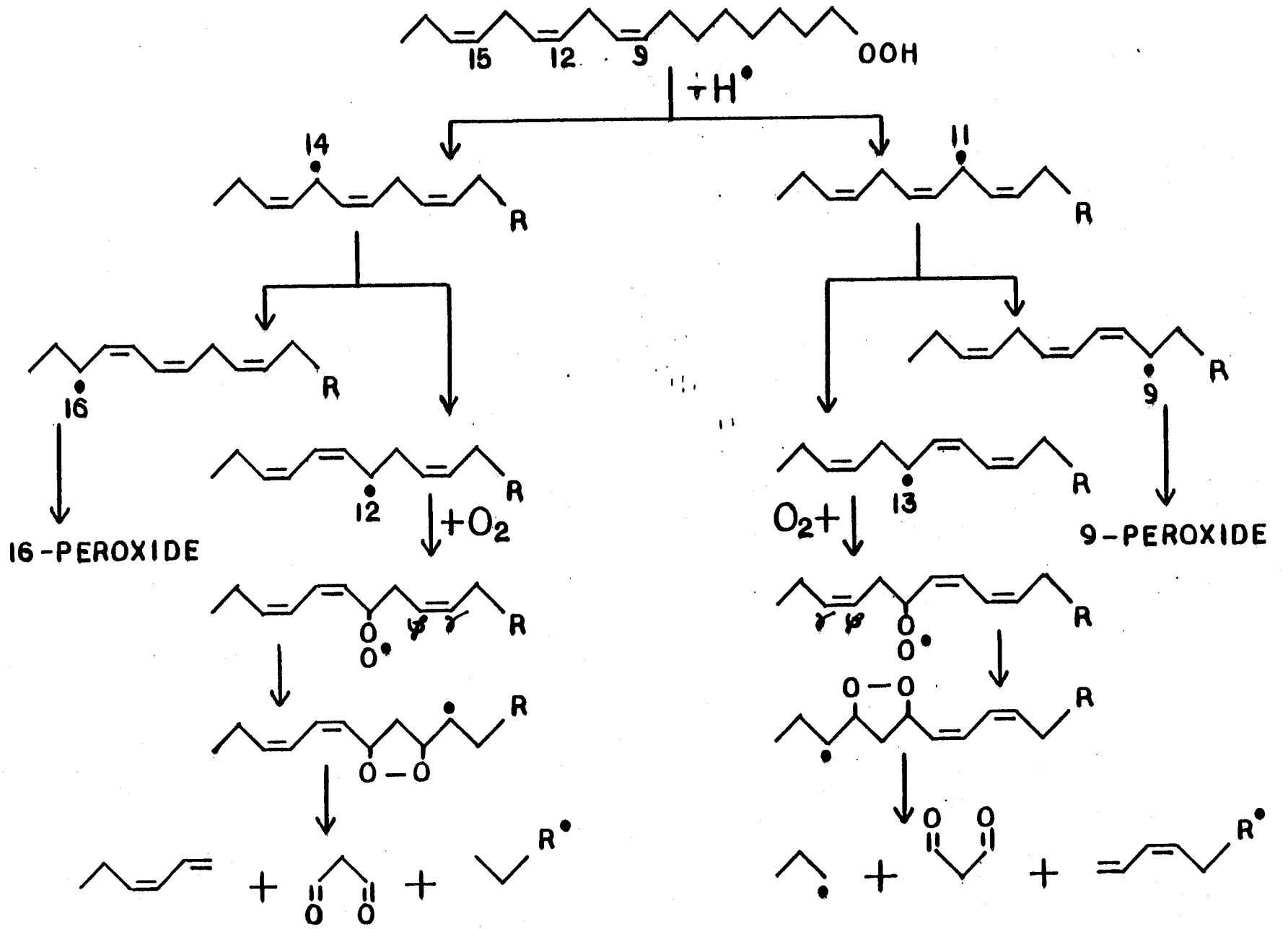

15
12
9
OOH
+H•
14
11
R
16
12
13
9
16-PEROXIDE
9-PEROXIDE
+O2
O2+
O
O•
O—O
R•

Fig. 4. Relationship between TBA Value and degree of fatty acid polyunsaturation (taken from Dahle et al., 1962).

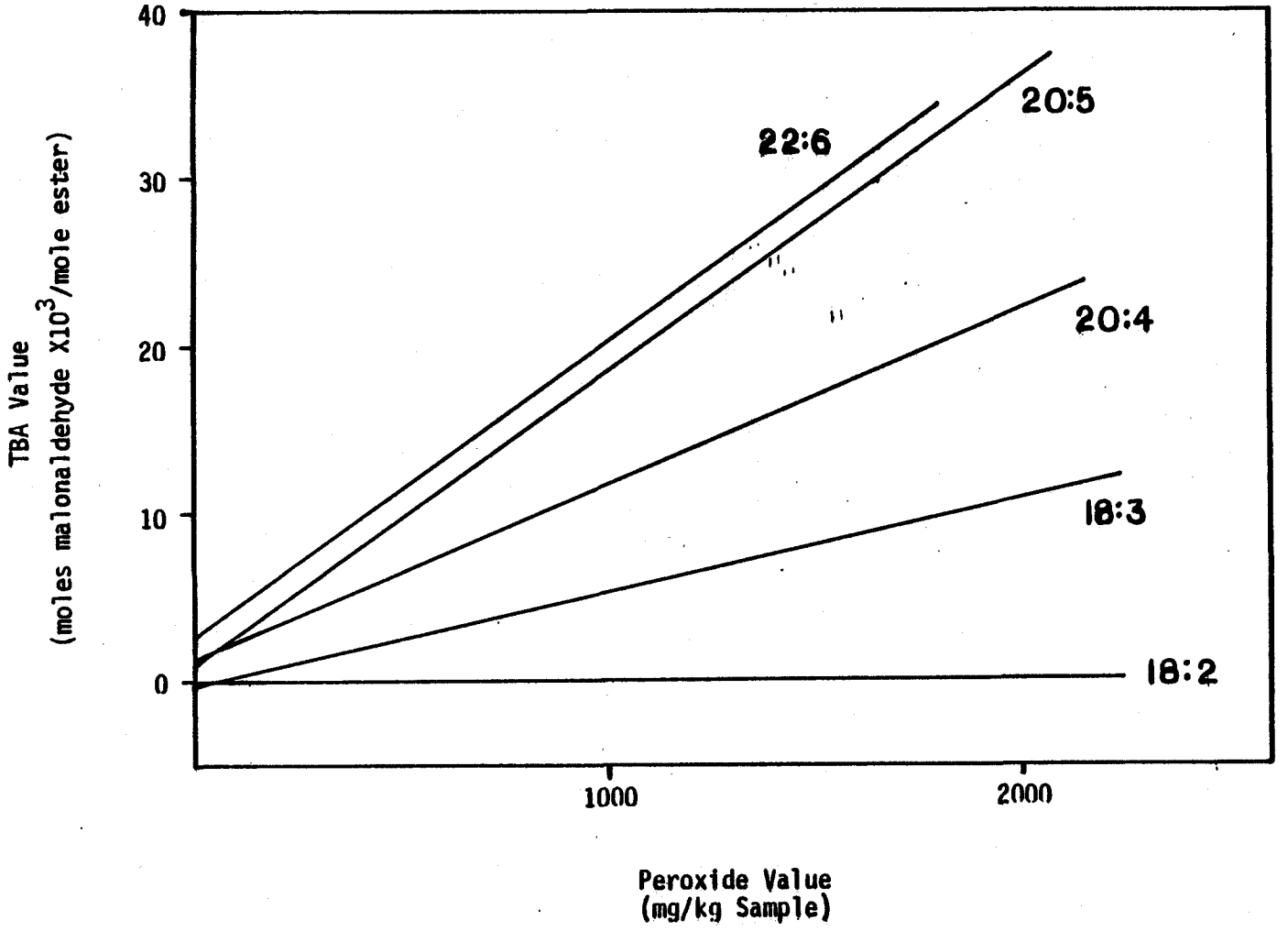

40
30
20
10
0
1000
2000
22:6
20:5
20:4
18:3
18:2
TBA Value
(moles malonaldehyde X10^3/mole ester)
Peroxide Value
(mg/kg Sample)

Fig. 5. Proposed biosynthesis of malonaldehyde by way of cyclic endoperoxides (Pryor et al., 1976).

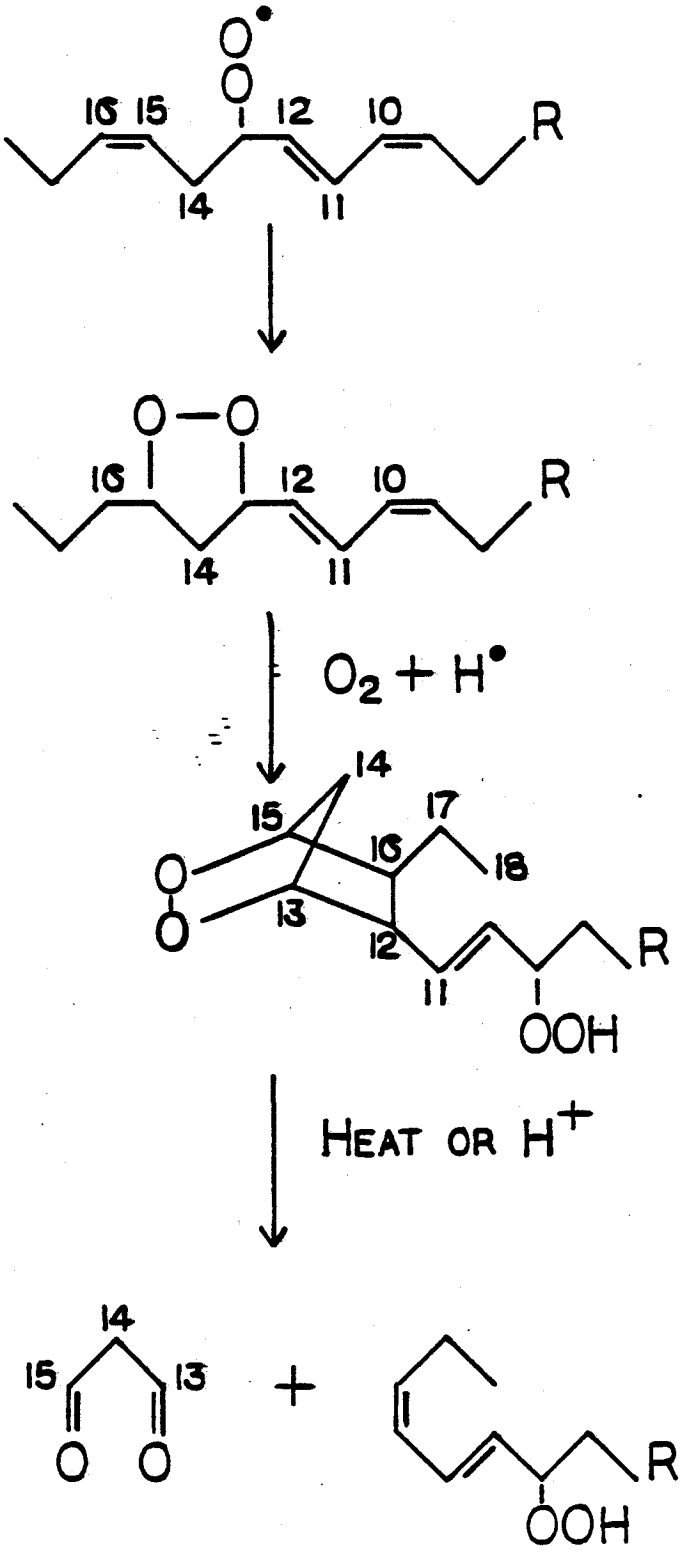
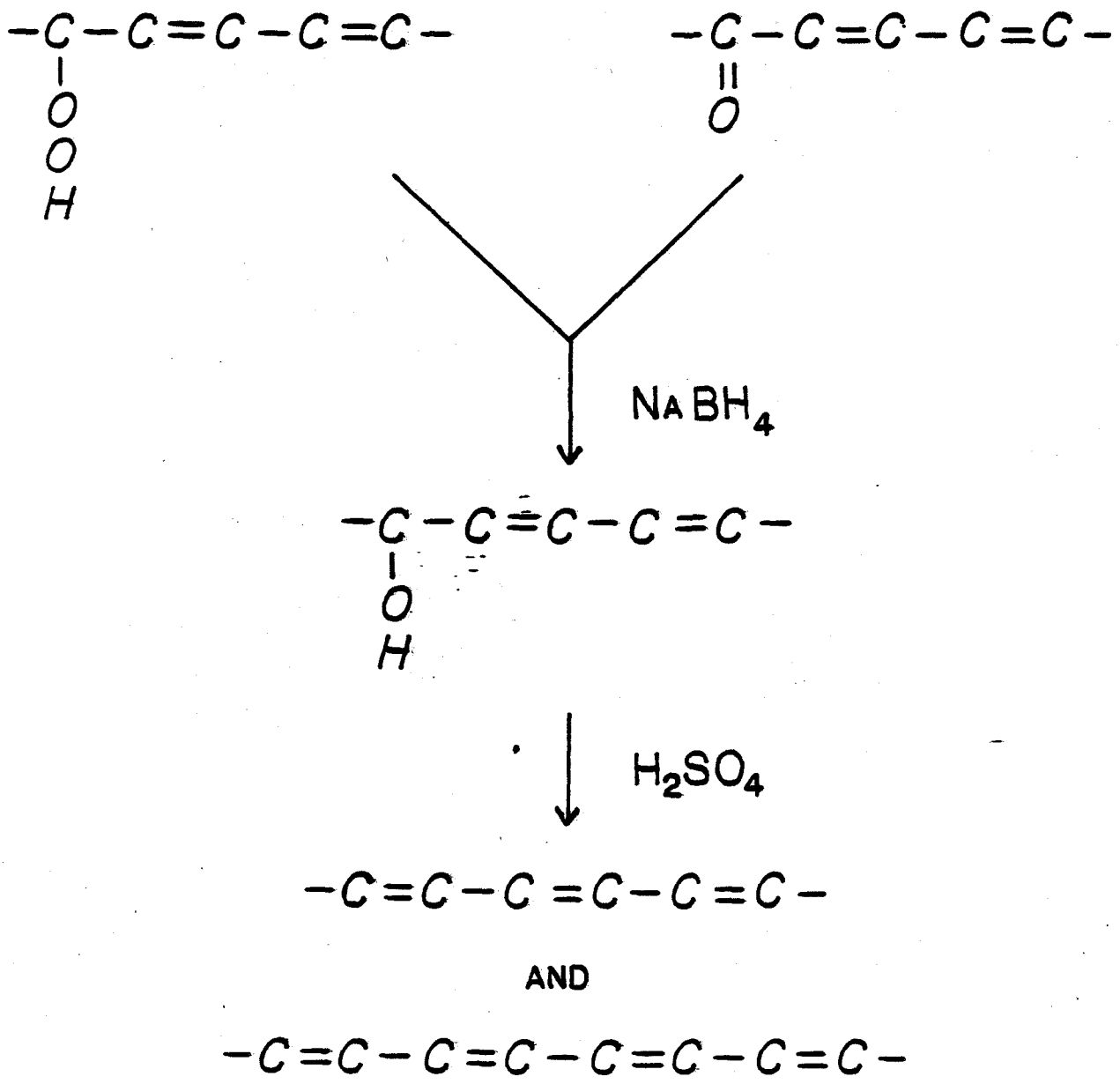

16 15
12
10
R
14
11
O—O
$O_2 + H^{\bullet}$
17
18
OOH
HEAT OR H^+

Fig. 6. Chemical reactions of the COP assay (Fishwick and Swoboda, 1977).



AND

Fig. 7. COP assay spectra obtained from oxidizing dark muscle lipids of mullet after 0 (I), 2 (II), 7 (III), 14 (IV), 21 (V) and 24 (VI) days refrigerated storage. Dilutions and spectrophotometer range settings selected to keep all absorbance peaks on scale.

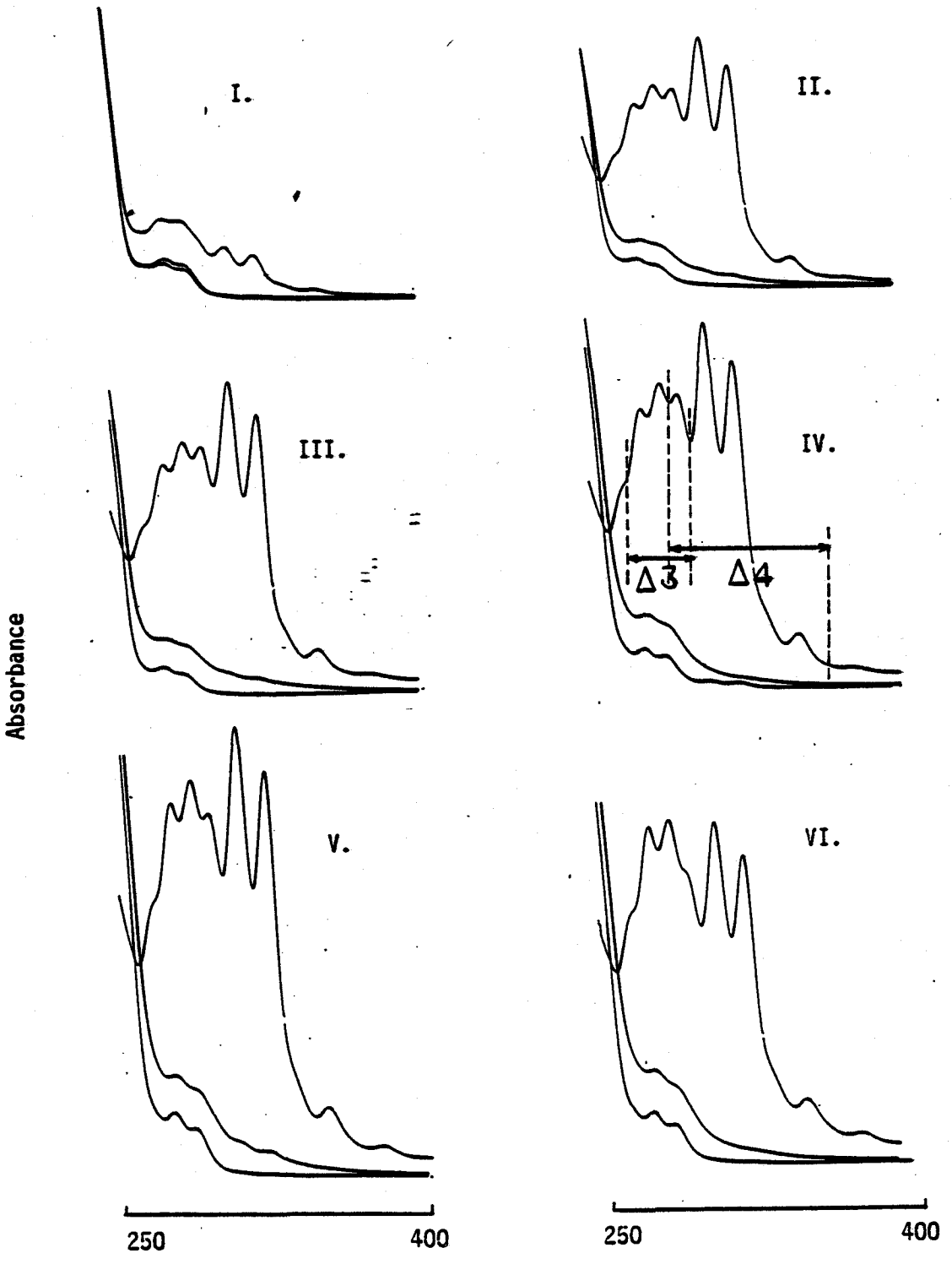

I.
II.
III.
IV.
Δ3
Δ4
V.
VI.
Absorbance
250
400
250
400
Wavelength (nm)

Fig. 8. Iodine Values of oxidizing menhaden oil as measured by the official AOCS method (●——●), modified methodology for oxidized fats (Δ--Δ) and calculated from GLC compositional data (o——o).

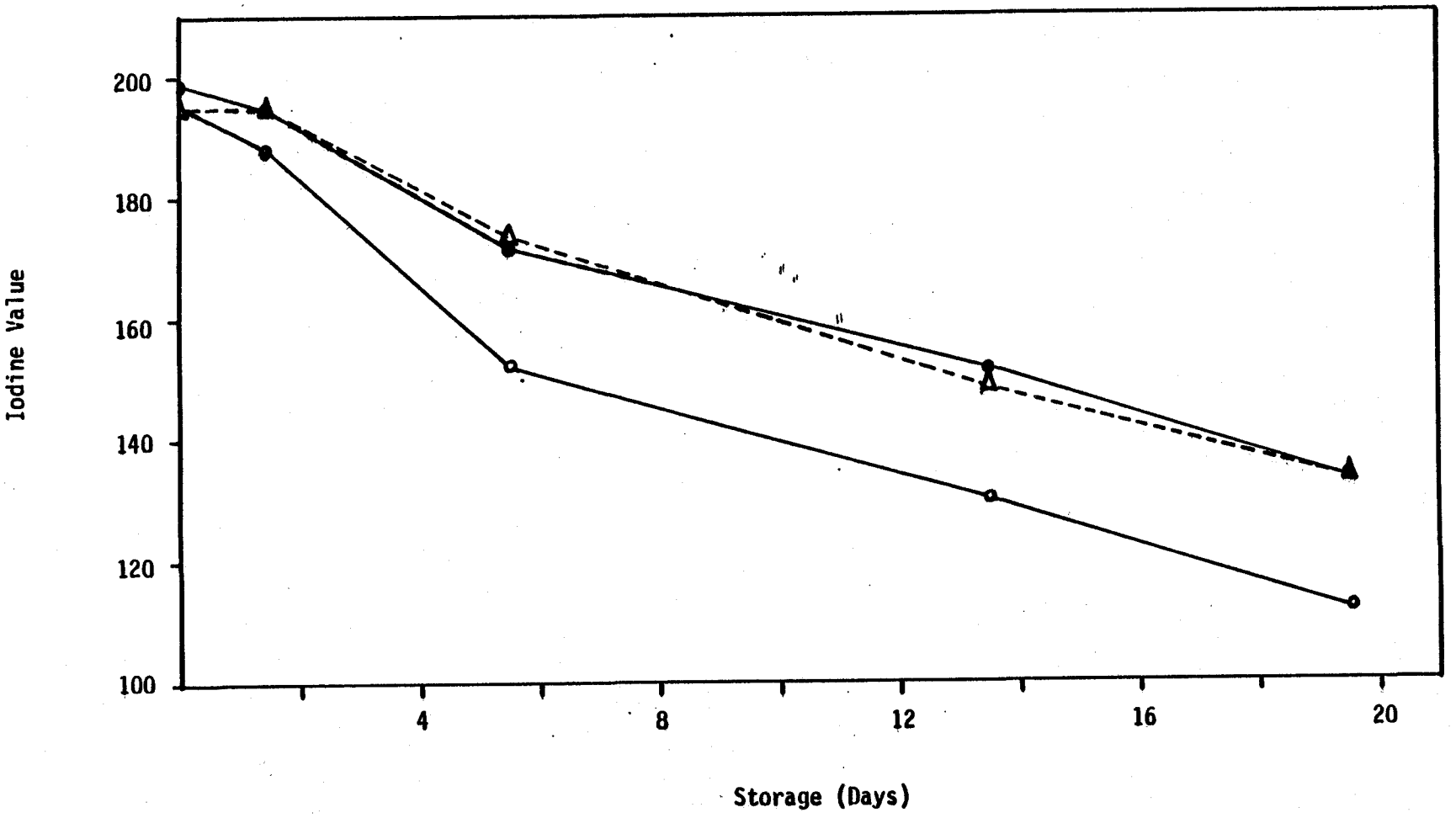

200
180
160
140
120
100
Iodine Value
4
8
12
16
20
Storage (Days)

Fig. 9. Oxidation indices of oxidizing light and dark muscle lipids of croaker.

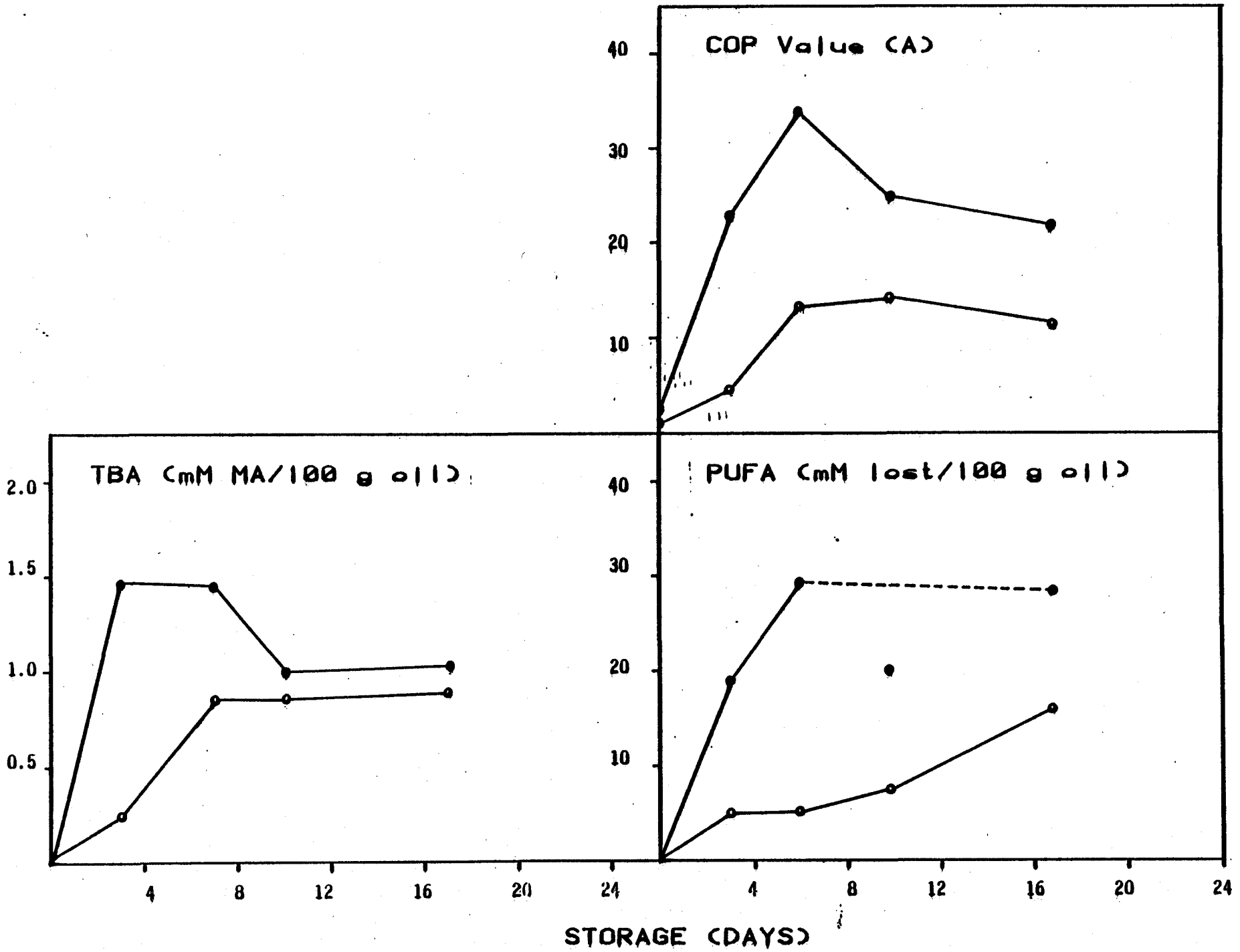

COP Value (A)
40
30
20
10
TBA (mM MA/100 g oil)
2.0
1.5
1.0
0.5
PUFA (mM lost/100 g oil)
40
30
20
10
4
8
12
16
20
24
STORAGE (DAYS)

Fig. 10. Oxidation indices of oxidizing light and dark muscle lipids of king mackerel.

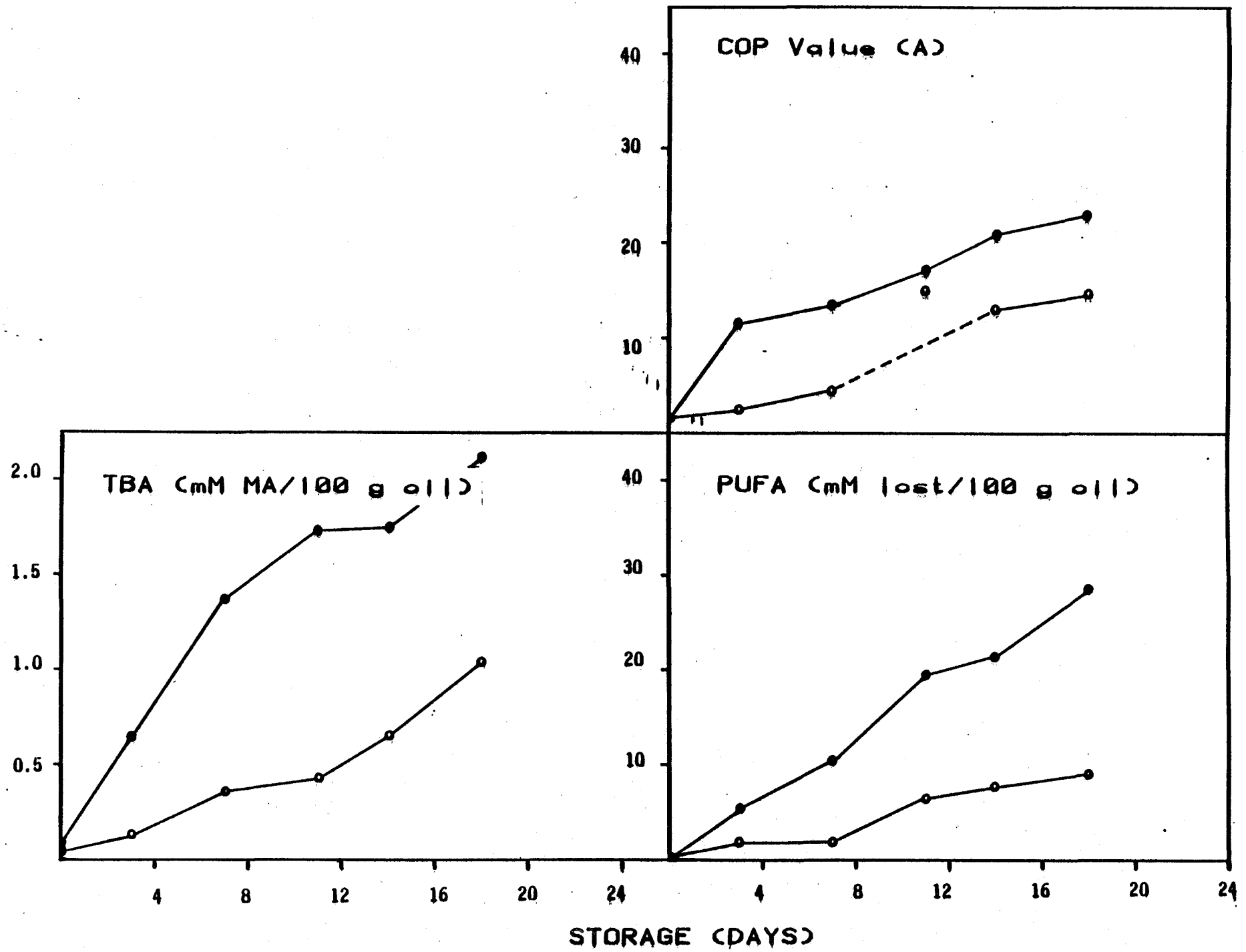

COP Value (A)
40
30
20
10
TBA (mM MA/100 g oil)
2.0
1.5
1.0
0.5
PUFA (mM lost/100 g oil)
40
30
20
10
4
8
12
16
20
24
4
8
12
16
20
24
STORAGE (DAYS)

Fig. 11. Oxidation indices of oxidizing light and dark muscle lipids of mullet (study I).

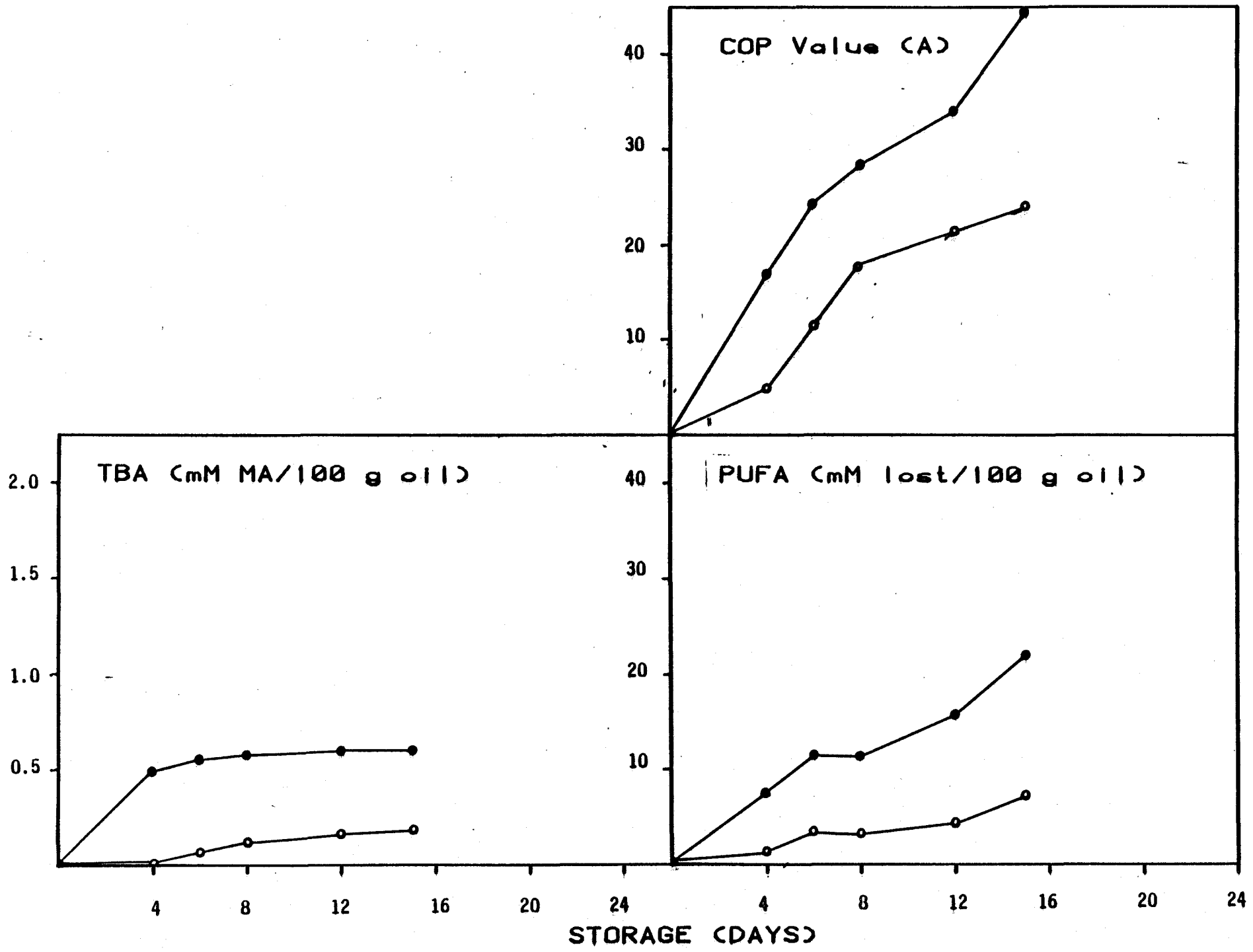

COP Value (A)
40
30
20
10
TBA (mM MA/100 g oil)
2.0
1.5
1.0
0.5
PUFA (mM lost/100 g oil)
40
30
20
10
4
8
12
16
20
24
4
8
12
16
20
24
STORAGE (DAYS)

Fig. 12. Oxidation indices of oxidizing light and dark muscle lipids of mullet (study II).

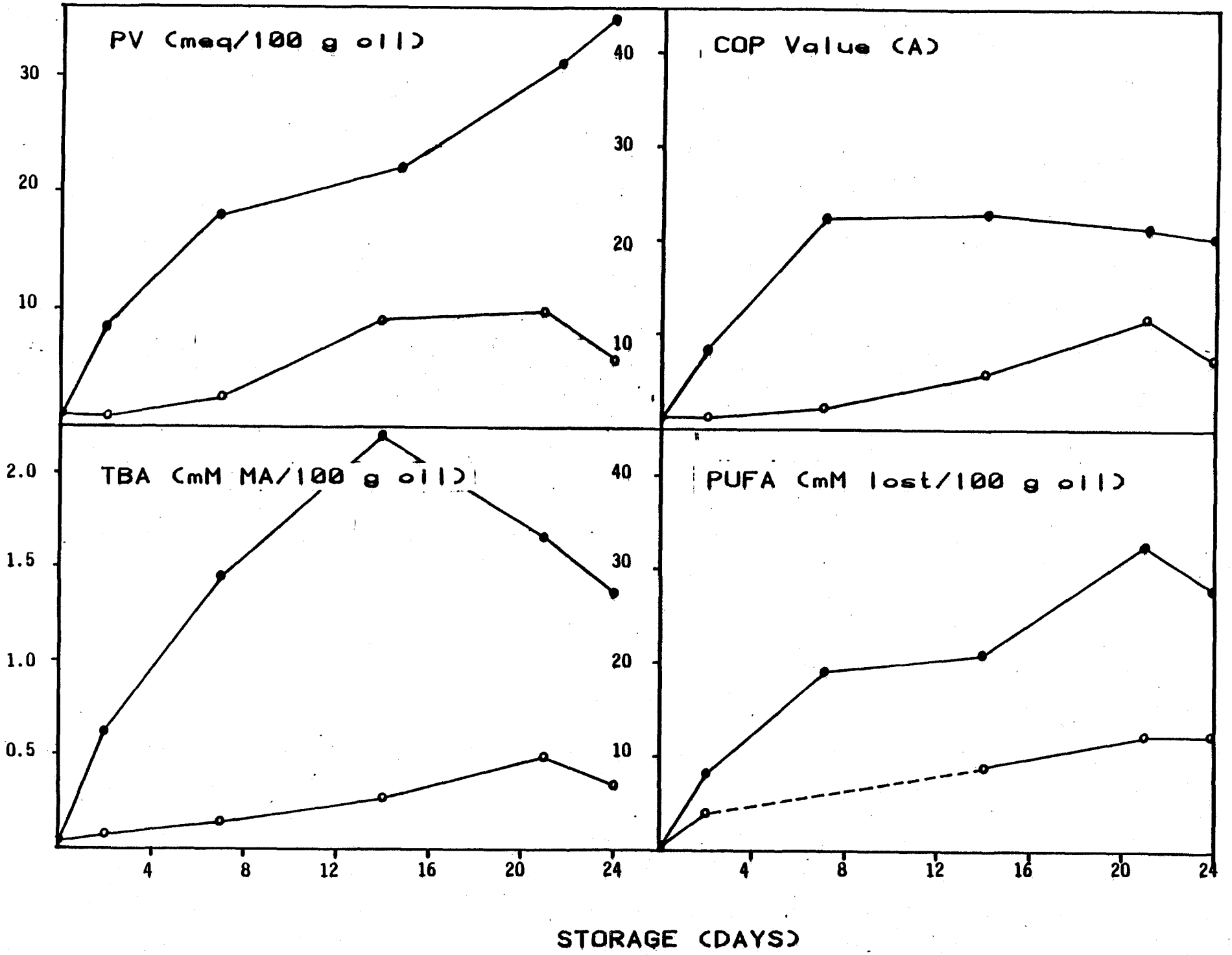

PV (meq/100 g oil)
COP Value (A)
TBA (mM MA/100 g oil)
PUFA (mM lost/100 g oil)
30
20
10
40
30
20
10
2.0
1.5
1.0
0.5
40
30
20
10
4
8
12
16
20
24
4
8
12
16
20
24
STORAGE (DAYS)

Fig. 13. Oxidation indices of oxidizing mullet depot oil (study I).

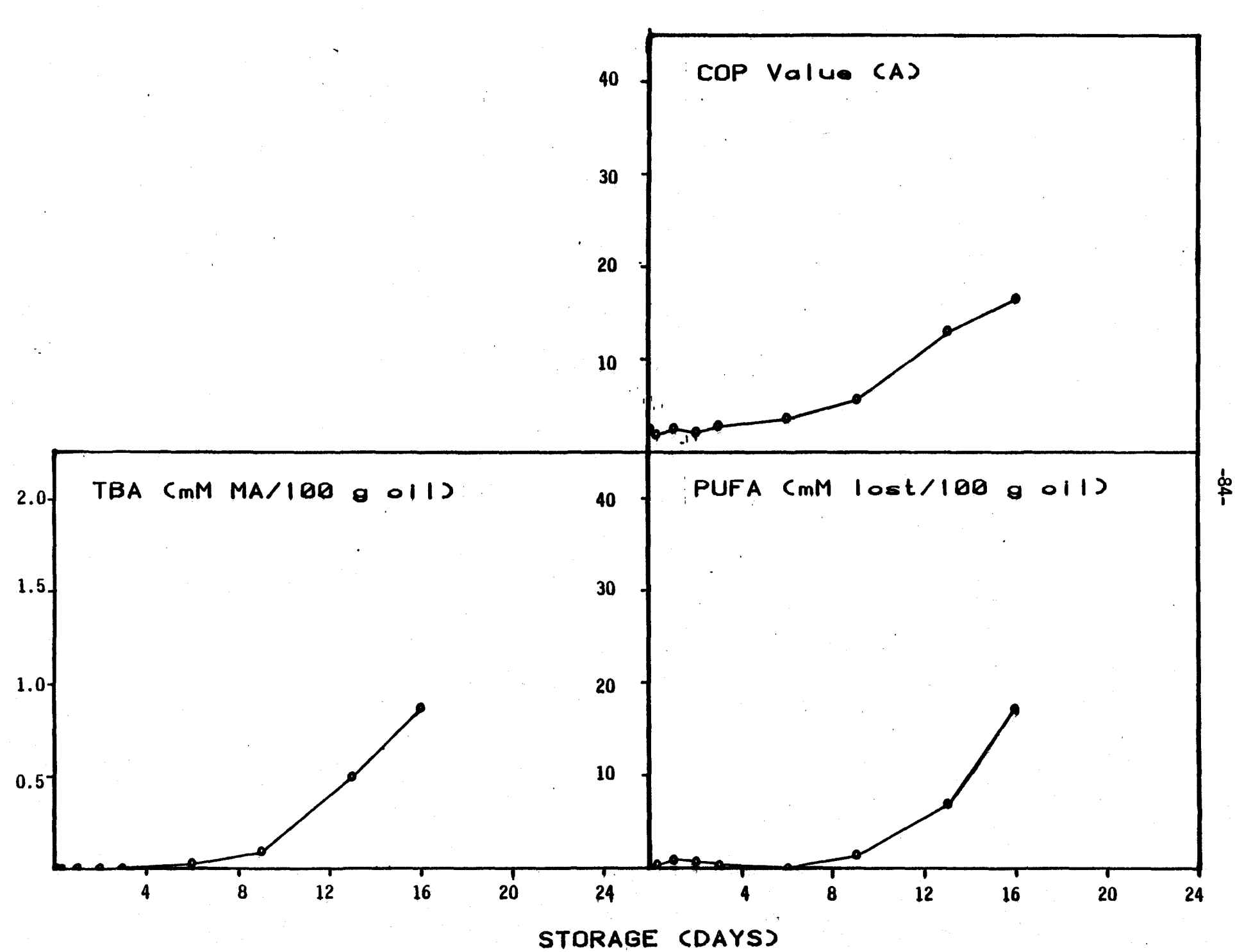

COP Value (A)
40
30
20
10
TBA (mM MA/100 g oil)
2.0
1.5
1.0
0.5
PUFA (mM lost/100 g oil)
40
30
20
10
4
8
12
16
20
24
4
8
12
16
20
24
STORAGE (DAYS)

Fig. 14. Oxidation of mullet depot oil as measured by oxygen uptake.

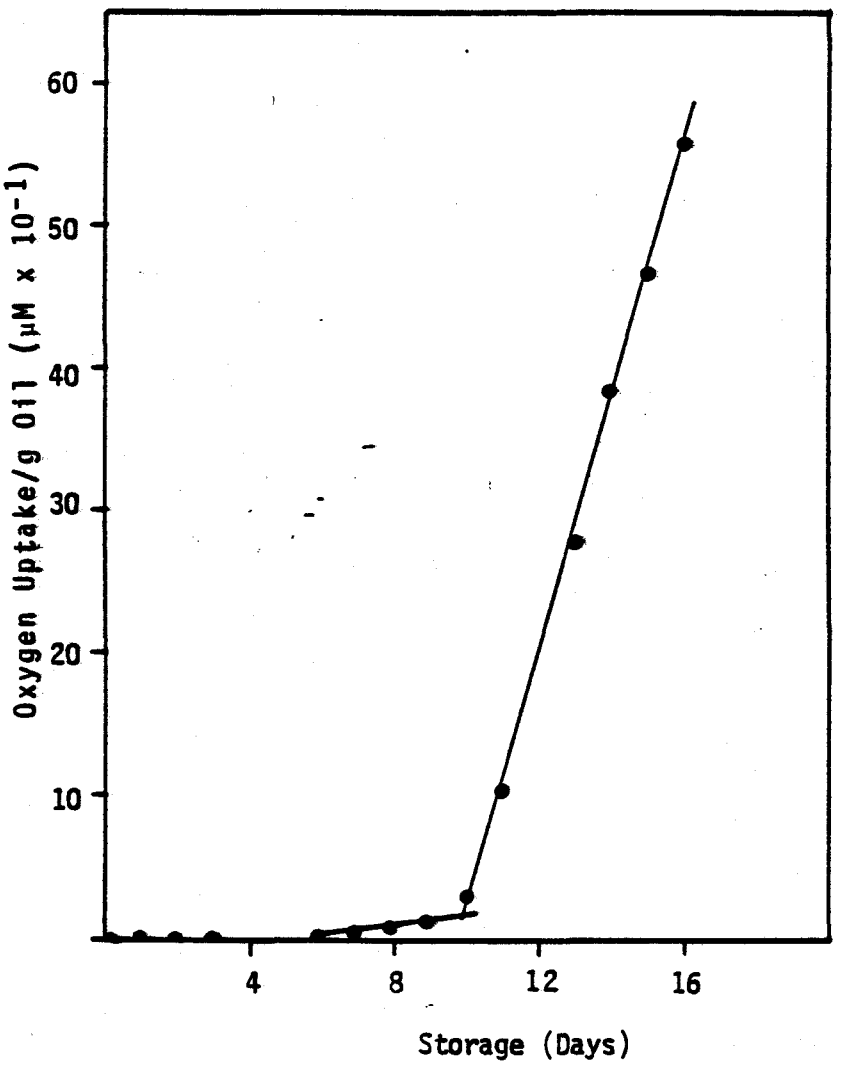

60
50
40
30
20
10
Oxygen Uptake/g Oil (μM x 10⁻¹)
4
8
12
16
Storage (Days)

Fig. 15. Oxidation indices of oxidizing light and dark muscle lipids of bluefish.

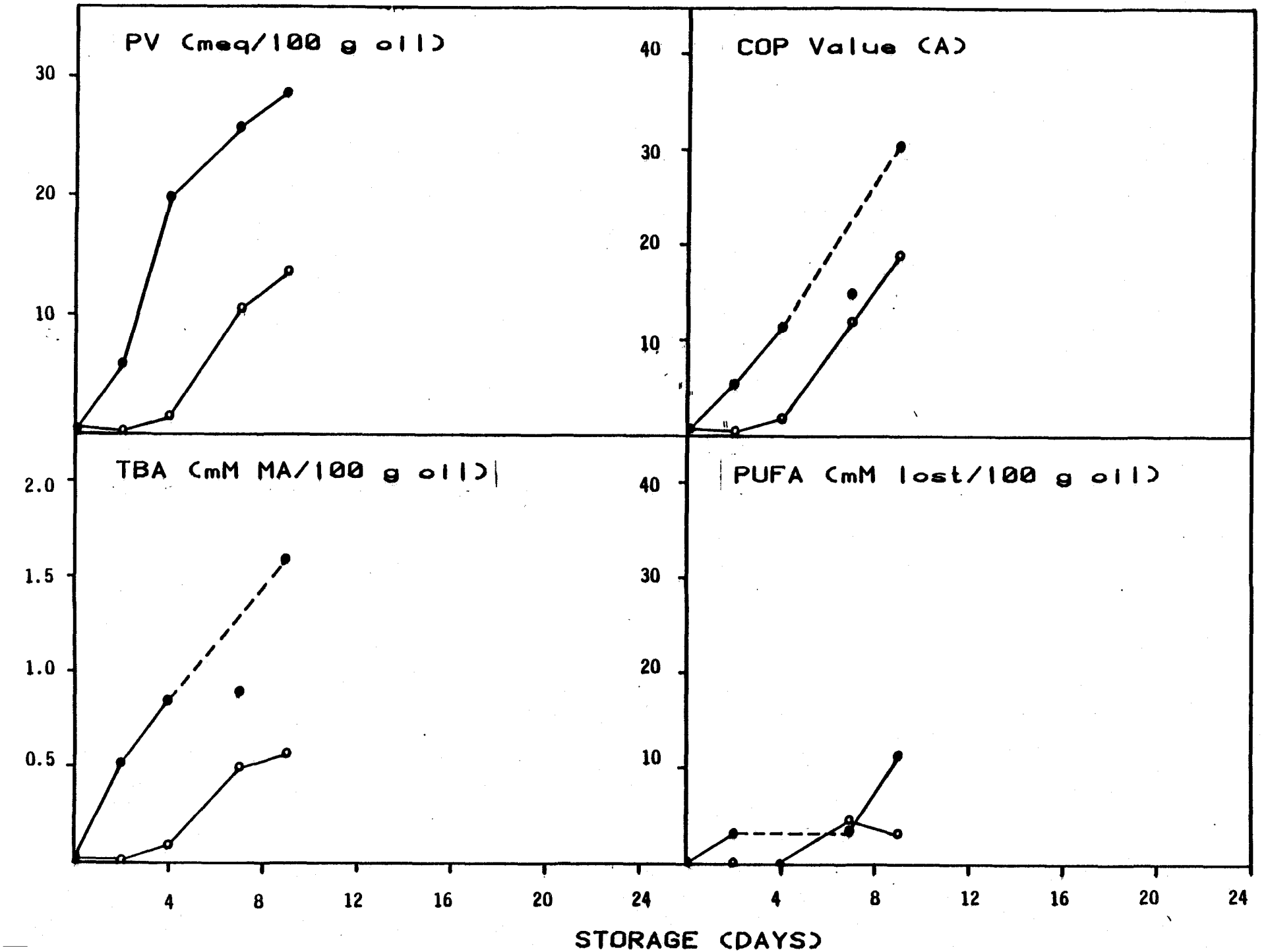
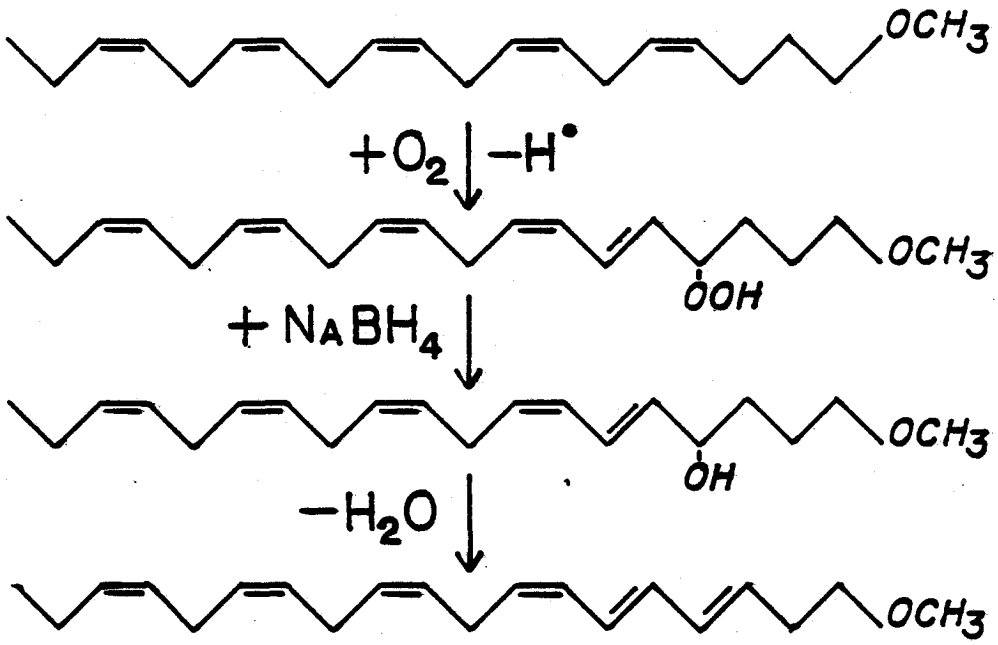

PV (meq/100 g oil)
COP Value (A)
TBA (mM MA/100 g oil)
PUFA (mM lost/100 g oil)
30
20
10
40
30
20
10
2.0
1.5
1.0
0.5
40
30
20
10
4
8
12
16
20
24
4
8
12
16
20
24
STORAGE (DAYS)

Fig. 16. Proposed mechanism, based upon preferential peroxidation site, for the source of conjugated triene and tetraene absorption in reduced and dehydrated marine lipids, using methyl eicosapentaenoate as a model compound. A., terminal olefinic bond autoxidation: B., central olefinic bond autoxidation.

A.


OCH3
+O2
-H•
OCH3
OOH
+ NABH4
OCH3
OH
-H2O
OCH3

B.

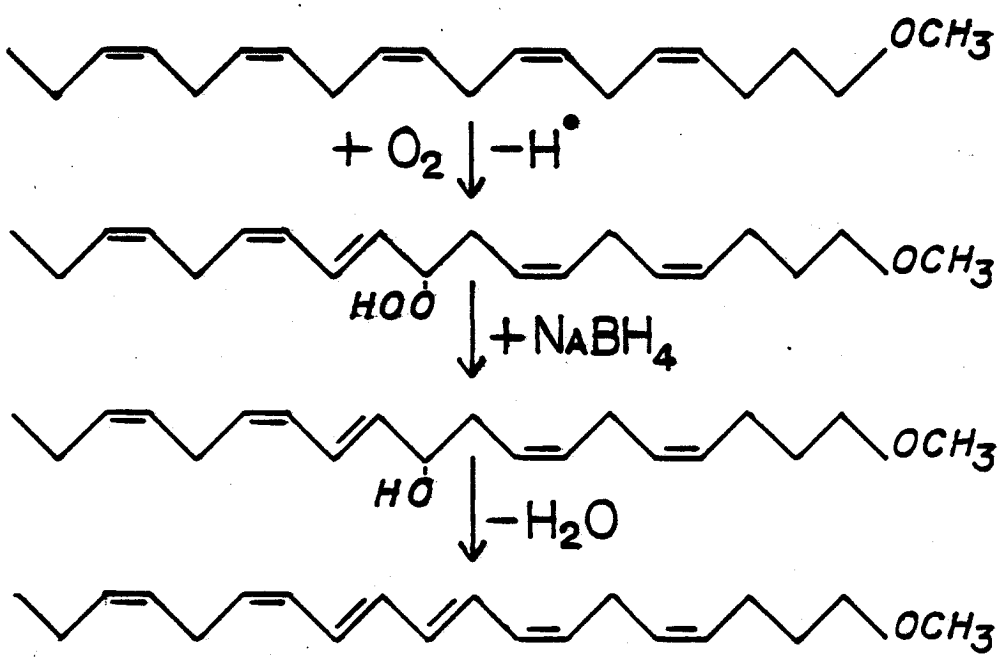
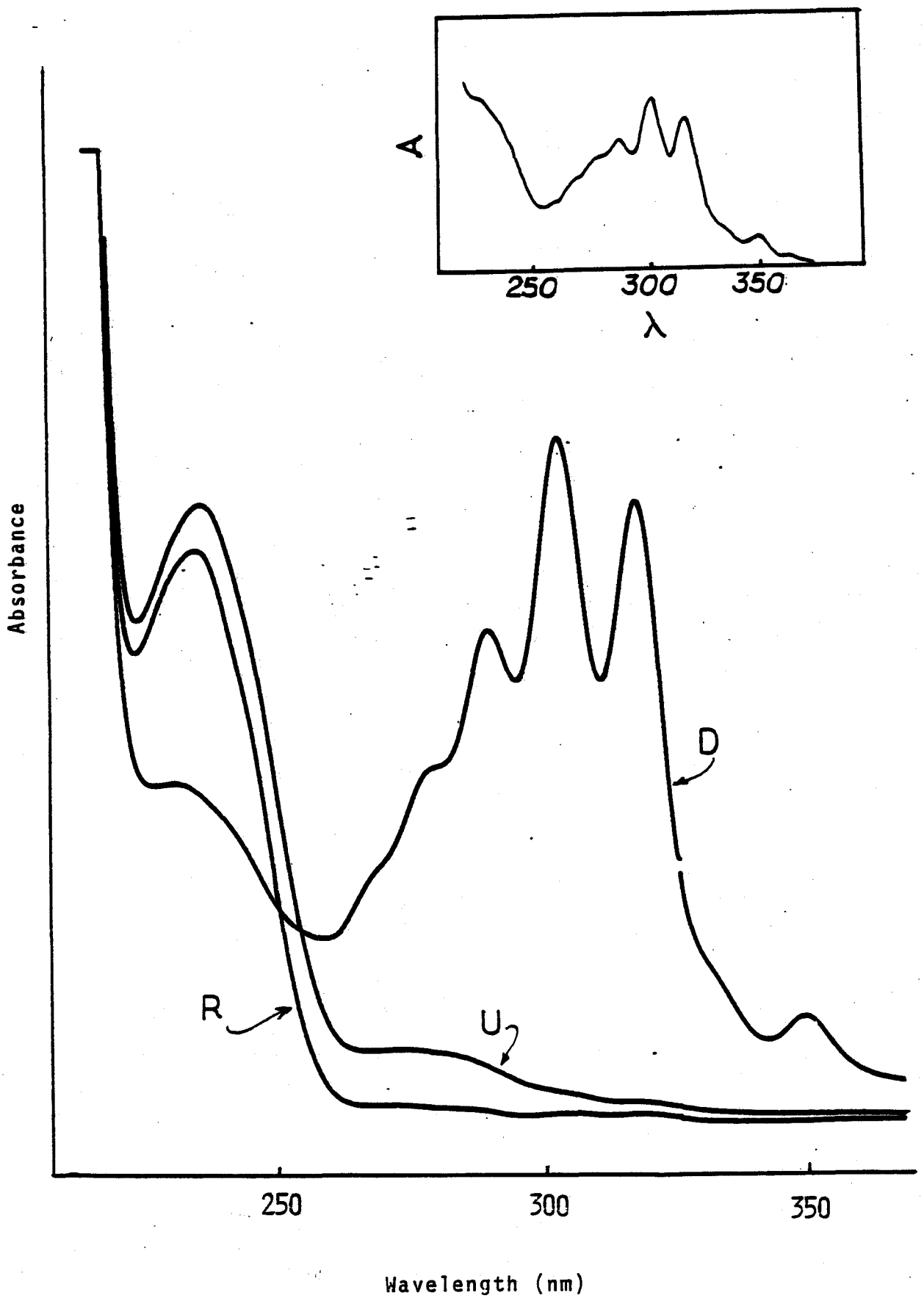

OCH3
+ O2
-H•
OCH3
HOO
+NABH4
OCH3
HO
-H2O
OCH3

Fig. 17. Ultraviolet COP absorbance spectra of unreacted (U), reduced (R) and reduced/dehydrated (D) oxidized methyl docosahexaenoate. Inset, of oxidized herring oil, taken from Parr and Swoboda (1976).


A
250
300
350
λ
Absorbance
D
R
U
250
300
350
Wavelength (nm)