THE UNIVERSITY OF MANITOBA

CHEMICAL AND SENSORY STUDIES OF THE OXIDATIVE DETERIORATION OF FLAVOR IN CANNED WHITEFISH

by

CANDICE ANNE SCHWARTZ

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ABSTRACT

Freshwater whitefish (Coregonous clupeaformis) was canned with one of two antioxidant mixtures; AX 1 (20% butylated hydroxyanisole, 20% butylated hydroxytoluene) or AX 2 (10% butylated hydroxyanisole, 10% butylated hydroxytoluene, 6% citric acid and 6% propyl gallate) and compared to untreated control samples. The flavor of the fish was examined at intervals during 4 days of open refrigerated storage using sensory and objective methods. Both thiobarbituric acid (TBA) values and the results of paired flavor comparisons by an 8-member trained sensory panel demonstrated that the untreated fish was always associated with the greatest degree of oxidative deterioration, while AX 1-treated fish exhibited the least amount of "off-flavor" over the storage period. The degree of "off-flavor" as evaluated by the trained panelists tended to increase on storage; TBA values showed a linear relationship with time. Free fatty acid content did not show any significant differences ($P < 0.05$) among treatments indicating that the enzymes responsible for hydrolytic rancidity were inactivated during the canning process. Preliminary work on fatty acid composition of the fish oils using gas chromatography indicated a difference in the amount of C22:6(ω 3) among the 3 treatments. Refractive indices of whitefish lipid did not show any significant differences ($P < 0.05$) among treatments or days. Consumer studies involved three sessions with 21 to 51 homemakers who evaluated pairs of antioxidant-treated and untreated whitefish sandwiches prepared with salad dressing. Each session varied

in the ageing conditions. These consumers were unable to detect any significant flavor differences ($P < 0.05$) between the pairs of sandwiches.

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INTRODUCTION

Lake whitefish (Coregonous clupeaformis) is an important commercial species of freshwater fish in Canada, and is found in all suitable lakes from the Maritimes to the North West Territories. The fat content varies from 1.7 to 3.9% in fish from Cedar Lake or Lac la Rouge to 16.3% in fish caught in Lake Huron (Bligh, 1971). Since whitefish is a highly perishable product, attempts to extend its storage life have been reported including freezing (Osterhaug, 1956; Awad et al., 1969); irradiation (Ostovar et al., 1967) and smoking (Slusar and Vaisey, 1970). Canning the fish, however, appears to be the best method of extending the storage life since it excludes air, inactivates enzymes and destroys spoilage organisms.

The canned whitefish product has a characteristic flavor which has been described as "chickeny". This quality, however, appears to be unstable and changes within minutes of opening the can to a "fishy" taste which is thought to be associated with fat oxidation (Shaykewich, 1971). In a report to the Manitoba Department of Industry and Commerce, Arthur D. Little, Inc., reported that canned whitefish could compete cost-wise with canned salmon and canned tuna (Anonymous, 1965). Since canned fish is an acceptable product, the potential market for canned whitefish appears promising, as long as the product is of sufficiently high quality.

The purpose of the present study was to extend the time

that canned whitefish flavor remains highly acceptable through the addition of antioxidants, and to obtain consumer opinions on the acceptability of the canned whitefish product.

REVIEW OF LITERATURE

Introduction

Rancidity is a serious problem associated with fishery products resulting in deteriorative changes in flavor. Such products are rejected by the consumer and can lead to serious economic losses in the fishing industry. Lea (1952) defined rancidity as any "off-odor or flavor" which developed in an oil or fat as a result of deterioration or storage. Two types of deteriorative changes are generally recognized in fish oils, hydrolytic and oxidative rancidity. The former represents reactions catalyzed by enzymes which are unaffected by antioxidants, and can only be controlled through inactivation of the enzymes involved. Oxidative rancidity, however, is an autoxidative process catalyzed by metal ions which can be controlled through the addition of antioxidants. The products formed from these reactions can be toxic as well as having a deleterious effect on protein quality (Carpenter et al., 1962; Lea et al., 1958).

Concern for prolonging the storage life of fishery products has stimulated research using antioxidants to control some of these reactions. While considerable research has been reported on fresh fish, little has been published on canned fish products.

This review will attempt to summarize those investigations concerned with controlling rancidity in fish products as well as the chemical and physical methods available to detect these changes.

OXIDATIVE RANCIDITY

This process is autocatalytic and is primarily responsible for the oxidative deterioration of fish oils. The reaction appears to proceed through the following stages:

1) the induction period

2) propagation.

The first stage involves the formation of free radicals (Swern, 1961) which are responsible for the rapid conversion of oxygen to hydroperoxides. These compounds being unstable, readily break down to produce more free radicals, thereby initiating a chain reaction, as illustrated in Figure 1. The overall result is the production of rancid off-flavors together with other reactions affecting both shelf life and nutritional value (Labuza, 1971). A general picture of the pathways involved in lipid oxidation is shown in Figure 2.

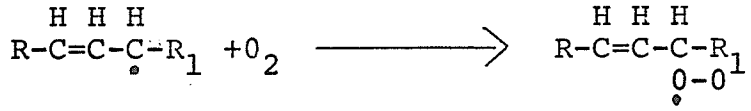
The rate of autoxidation is determined by such factors as the number of double bonds, temperature, light and the presence of pro-oxidants. Examples of the latter include various metals, their salts and metallic soaps (Tsuchiya, 1961).

In order to maintain high quality and good shelf life in food products, modern processing methods require the addition of certain chemicals. Antioxidants are an example of such chemicals, since they facilitate control of oxidative rancidity in foods. An antioxidant can be defined as a substance, when present in an oxidizable substrate in relatively low concentrations, will markedly inhibit the rate of reaction with oxygen (Olcott, 1967).

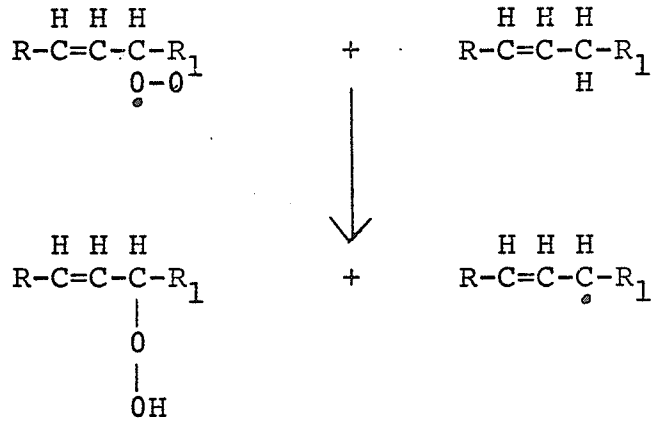
Initiation Step

Hydrocarbon Chain Radical

Propogation Steps



Hydroperoxy Radical



Conjugated Hydroperoxide

FIGURE 1. Mechanism of Autoxidation (Olcott, 1967).

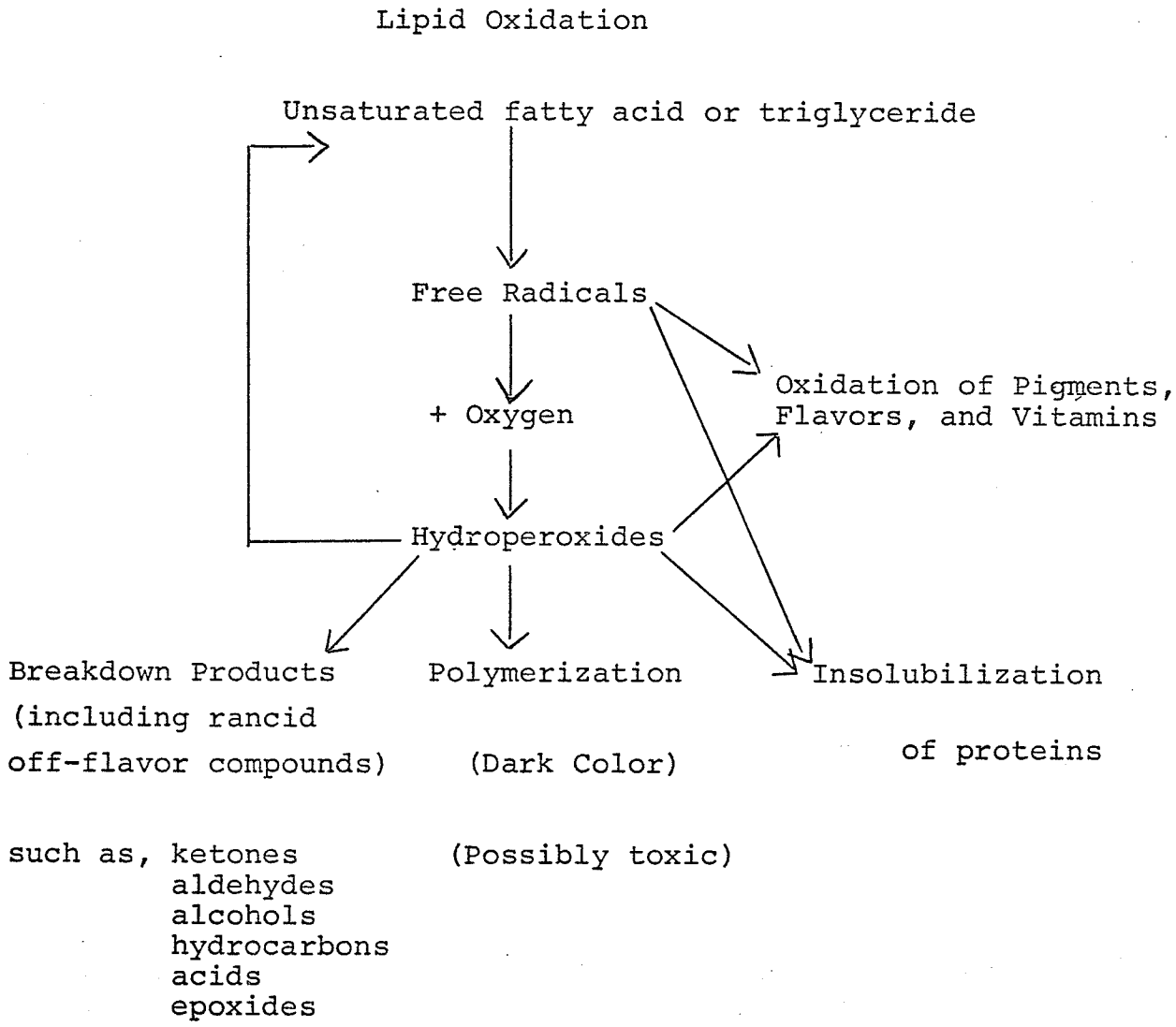


FIGURE 2. Overall Mechanisms of Lipid Oxidation (Labuza, 1971).

Scott (1965) classified antioxidants into three main types.

Type I Free Radical Chain Stoppers

These antioxidants are phenolic compounds capable of donating a hydrogen molecule to the free radical and include BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate), tocopherol and gum guaiac.

Type II Free Radical Production Preventors in Foods

Included in this group are ascorbic acid, citric acid and EDTA (ethylene diamine tetra acetic acid), which act by chelating or tying up metal catalysts.

Type III Environmental Factors

These include oxygen and moisture. Oxidative rancidity can be controlled either by lowering the oxygen partial pressure in the packaged food product or by storing the dehydrated food product at a critical moisture level.

Since the antioxidants utilized in this study were of Type I, the following discussion will include, wherever possible, this group of antioxidants.

Several criteria must be met in order to use antioxidants in foods.

1) there must be no harmful physiological effect,
2) no objectionable odor, flavor or color,
3) they should be effective in retarding rancidity,
4) their effectiveness should carry through to foods made with the fat,
5) they should be sufficiently fat soluble so they can be incorporated with ease, and
6) they should be readily available and inexpensive (Kraybill et al., 1949).

Johnson (1971) reported that the levels of phenolic antioxidants permitted by present-day legislation present no evidence to suggest any hazard to the consumer through use of BHA, BHT or gallate esters. He further commented that the use of these substances has yet to reach their full potential in the food industry.

SPOILAGE INDICES OF OXIDATIVE RANCIDITY

A number of objective methods are now available for detecting oxidative rancidity including peroxide number, carbonyl production, TBA number and refractive index. Henick et al., (1954) pointed out the apparent need for a suitable objective measurement which would correlate well with flavor changes. Organoleptic methods while important in judging the quality of the fat, provide no information regarding the cause of the inferior taste (Holm et al., 1957).

Peroxide Test

The basis of the peroxide test is the ability of peroxides quantitatively to liberate iodine from potassium iodide. One principal limitation is based on the instability of the peroxides that are formed as intermediate products which lead to the formation of the true products of rancidity such as carbonyl compounds. As a result of this transitory nature of the peroxides, the level of peroxide in a given fat or oil may not serve as a true indication of the actual state of oxidative rancidity (Sherwin, 1968).

Carbonyl Production

Carbonyl tests based on the formation of a derivative such as 2,4-dinitrophenyl-hydrazones, are valuable in research but have not found wide acceptance for routine evaluation of flavor (Jacobson, 1961). Attempts were made to correlate aldehyde values with organoleptic results of three fats and one oil stored at 60°C. Good correlations were obtained with beef and turkey fat, only a fair correlation existed with chicken fat, while no correlation was evident with soybean oil (Jacobson, 1961). One of the problems associated with this test is the development of certain aldehydes including 2, 4-decadienal during frying, which are associated with the pleasant flavor of the fried food products.

THIOBARBITURIC ACID (TBA) METHOD

In recent years, malonaldehyde has been recognized as a major degradation product in rancid foods, and its measurment forms the basis of the thiobarbituric acid test. This method is based on the reaction of one molecule of malonaldehyde with two molecules of TBA to produce a red pigment (Tarladgis *et al.*, 1962) as illustrated in Figure 3. The versatility of the TBA test is demonstrated by its use in evaluating rancidity in a wide variety of food products including dairy products (Patton *et al.*, 1951), frozen pork (Turner *et al.*, 1954), bakery products (Caldwell & Grogg,1955) and cooked oysters (Schwartz and Watts, 1957).

Vyncke (1970) investigated the possibility of a direct determination of the TBA value in trichloroacetic acid (TCA)

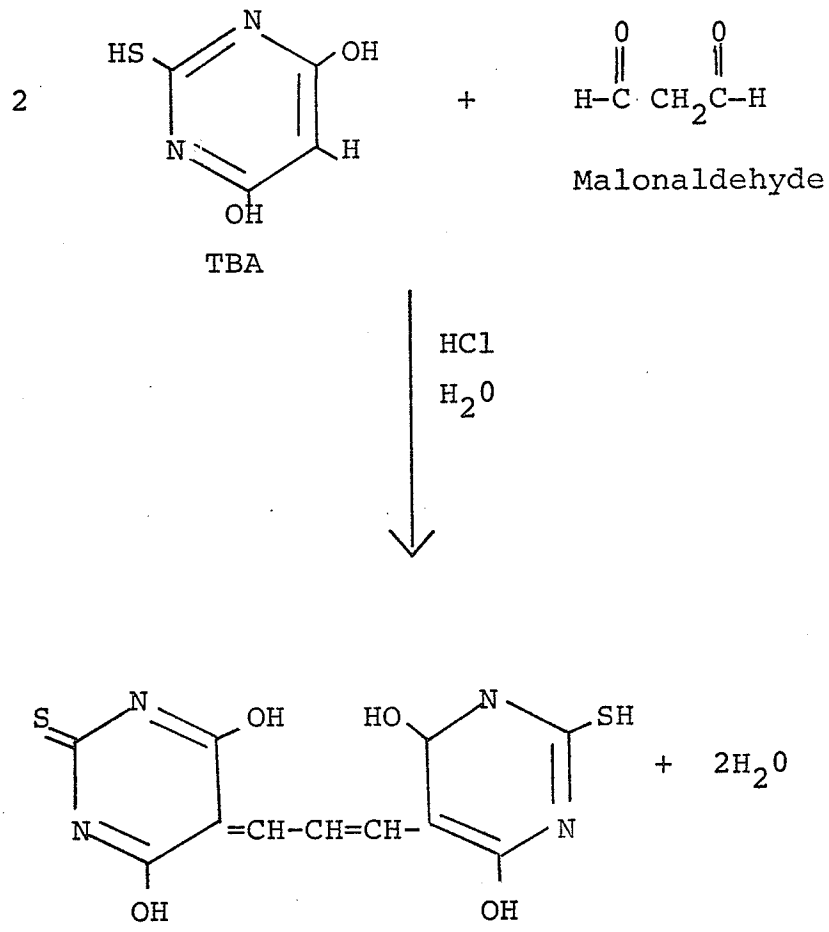


FIGURE 3. Proposed Formation of TBA Pigment from Malonaldehyde (Sinnhuber et al., 1958).

extracts of the fish muscle to replace the conventional use of distillates (Tarladgis et al., 1960). Tests were carried out on several species of fish varying in the degree of oxidation and results showed the teleost species gave 95.3% recovery but only 75.2% in the clasmobranch spurdog, probably due to the presence of urea. However, with the distillation process, recoveries were lower and averaged 66.1% for the teleosts and 43.0% for spurdog. Vyncke (1970) also suggested that an anti-oxidant (PG) and a chelating agent (EDTA) should be added to avoid erroniously formed malonaldehyde or other TBA reactive substances during blending and filtering of the sample.

Wyatt and Day (1965) used 12 trained judges to detect oxidized flavor in milk to which varying concentrations of stored vegetable oils had been added to give a perceptible oxidized flavor. Peroxide values, TBA numbers, and free carbonyl production were determined on the vegetable oils at 2-week intervals up to a period of 16 weeks. A high correlation was observed between the flavor evaluation procedure and the objective tests. While the sensory evaluation is an extremely sensitive tool, it is generally agreed that it sometimes lacks precision and reproducibility. However, the high correlations were believed to be a result of the flavor threshold testing procedure.

Refractive Index

Arya et al., (1969) measured peroxide values and refractive index in several common vegetable oils exposed to sunlight, ultra violet light and heat. The flavor of each sample was

assessed by a panel of 6 judges to determine the exact point of termination of the induction period. They reported a definite relationship between refractive index and peroxide values (Figure 4). At the cessation of the induction period, the refractive index exhibited a sharp increase, while the peroxide value did not show this change. During the secondary stage of peroxide formation, the refractive index roughly paralleled the increase in peroxide value. When decomposition of peroxide occurred, a rapid drop in peroxide value was evident while the refractive index continued at a steady rate. This method was reported to have definite advantages over the peroxide method, since only a small amount of sample is required and the end of the induction period is clearly defined.

Gas Chromatography

A simple gas chromatographic procedure was developed for measuring the degree of rancidity in vegetable oils (Scholz and Ptak, 1966). The procedure employed an internal standard for quantitating the amount of n-pentane in the oil samples and related this quantity to organoleptic tests. The precision was good and correlated well with the taste panels. The procedure appeared very sensitive to detection and quantitation of oxidative changes in oils; minimizing manipulation of the sample and avoiding alteration of rancidity products or the state of oxidation.

In 1962, Nawar and Fagerson investigated the relative sensitivity of olfaction versus gas chromatographic detection and found that when samples of fresh ground beef were held at

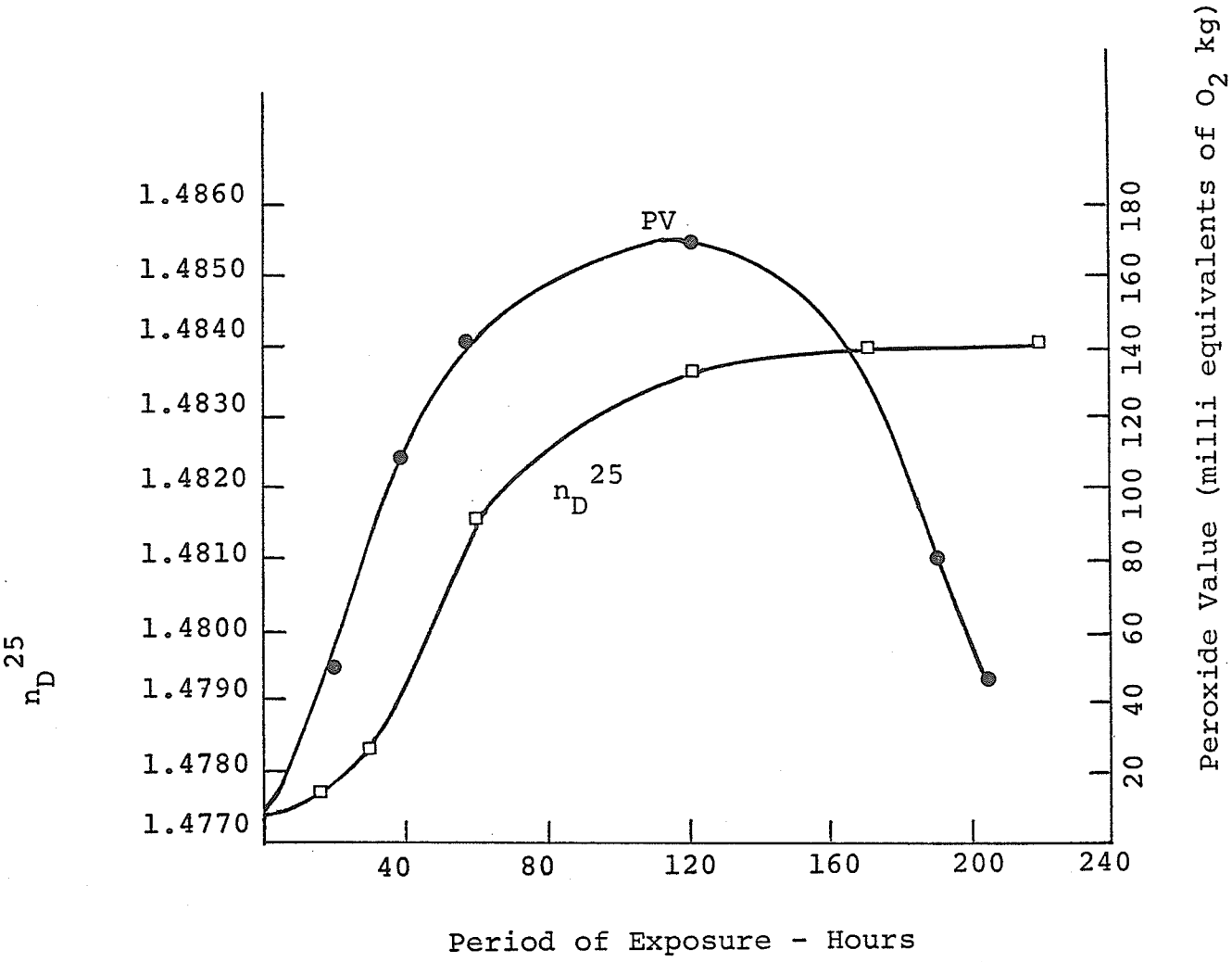


FIGURE 4. Relation Between Peroxide Value and Refractive Index (safflower oil autoxidized at 100 ± 5°C) (Arya et al., 1969).

room temperature and analyzed at 2 hour intervals both organoleptically and by direct gas chromatographic analysis, an odor difference was apparent in 4 hours, although the first clear cut chromatographic difference was not evident until after 10 hours.

THE APPLICATION OF ANTIOXIDANTS IN MARINE PRODUCTS

The application of antioxidants to control rancidity in fishery products has been carried out with varying degrees of success. Tarr (1945) investigated the effectiveness of various antioxidants on salmon fillets, using two different methods of application. One method involved dipping the frozen fillets in a watery solution of the antioxidant (usually 0.25 to 0.5%) for 1 minute, while the second method involved mincing the fillets with a 1% aqueous solution of the antioxidant and then blending with an electric mixer. Of the antioxidants studied, ethyl gallate proved to be the most effective. Tarr (1946) used red spring salmon steaks to study the effect of glazing in the development of rancidity as measured by the peroxide value. It was found that a glaze of 0.5% ascorbic acid or 0.5% sodium ascorbate entirely prevented fat oxidation during the frozen storage period. In 1947, Tarr examined the effectiveness of several of the gallates in retarding the development of rancidity in frozen fish. He reported that ethyl, n-propyl, n-butyl and hexyl gallate, when used in concentrations from 0.01 to 0.05%, considerably retarded the onset of rancidity, while sodium and ethanol

ammonium gallate (0.02%) proved ineffective.

Sinnhuber and Yu (1958) in studies on ground tuna scrap (T. germo) retained one portion as the unheated control (A) while the remainder was heated at 121°C for 30 minutes in an autoclave. The processed tuna scrap was further divided into three lots: B was retained as the processed control, C contained 0.05% N, N′-diphenyl-p-phenylenediamine (DPPD) and D was treated with 0.05% Tenox IV (20% BHA, 20% BHT, and 60% vegetable oil). The samples were stored in tightly sealed glass jars at -24°C until analyzed by the TBA method. They reported that sample A became rancid after one month of storage, while sample B, the processed control, required 100 days before rancidity was detected. Throughout the experiment samples C and D showed little change in odor or TBA number (Figure 5).

Schwartz and Watts (1959) cooked oysters with and without a 0.1% solution of ascorbic acid and stored them at -20°C up to 8 months. As can be seen in Figure 6, oysters treated with ascorbic acid showed a negligible increase in TBA value, and even after storage for 6 months, still retained an "oyster" odor. In contrast, a slight "rancid fish" odor was detected in the untreated oysters after only 2 months of storage, which during subsequent storage, developed in intensity and was accompanied by increased TBA values.

Zipser and Watts (1961) investigated the effect of adding 0.5% sodium tripolyphosphate and 0.22% sodium ascorbate to mullet (Mugil cephalus). The antioxidant-treated and untreated samples were canned and cooked to an internal

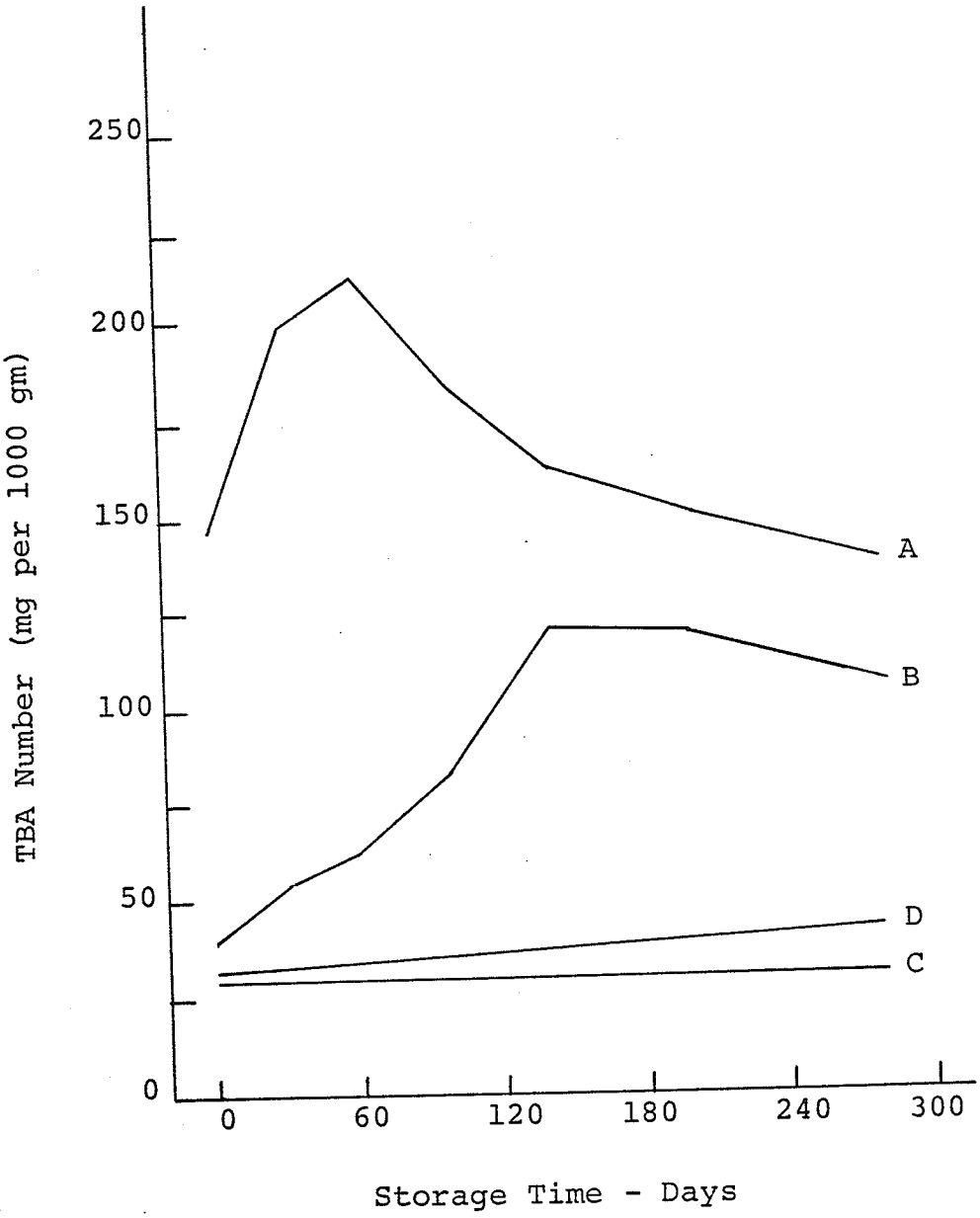


FIGURE 5. The Relation of Storage Time and Treatment of Frozen Tuna Scrap to TBA Number (Sinnhuber and Yu, 1958)

A. Unheated control
B. Heated control
C. Heated, 0.05 percent DPPD
D. Heated, 0.05 percent Tenox IV

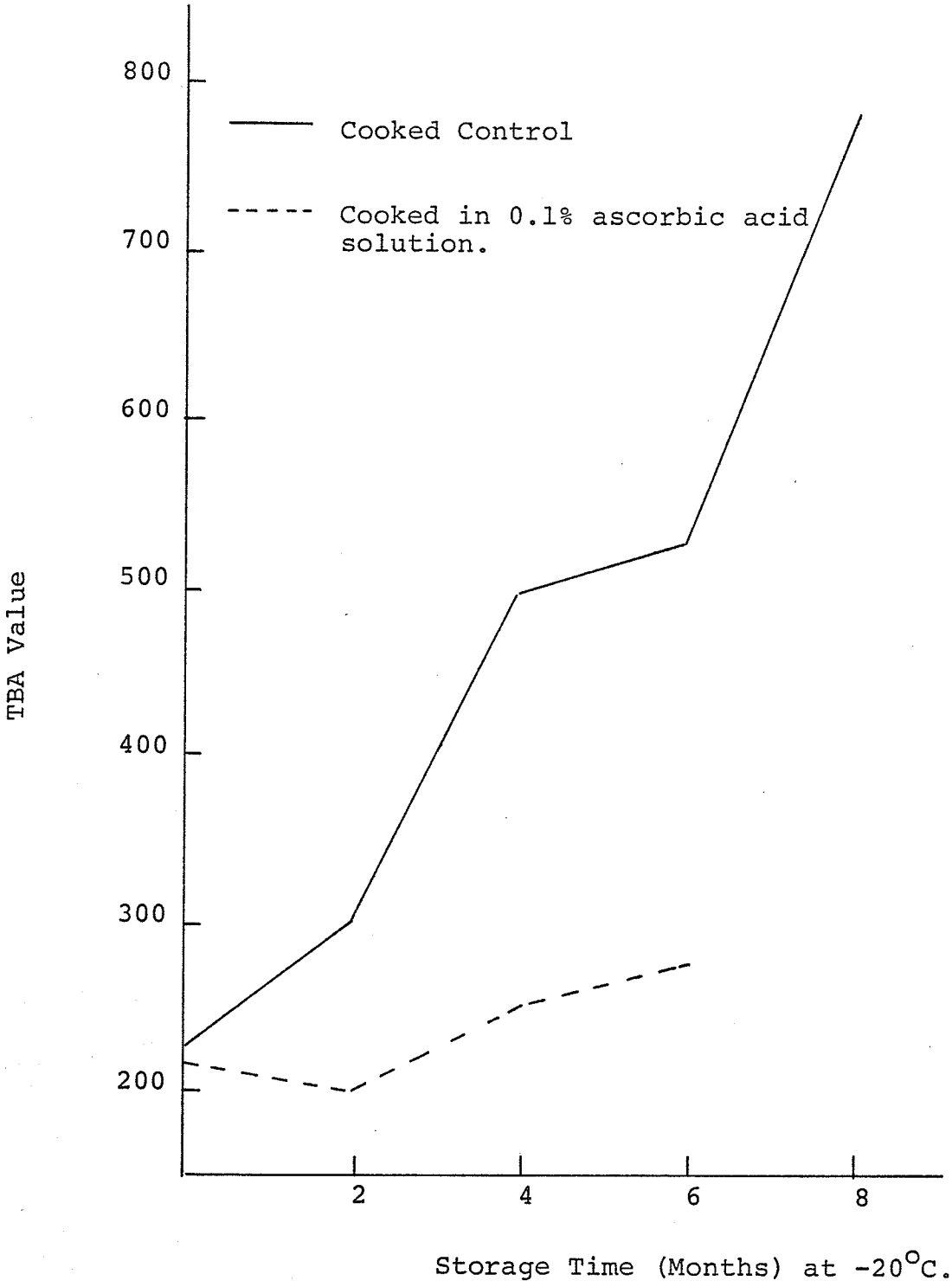


FIGURE 6. Cooked Control versus Oysters in 0.1 percent Ascorbic Acid Solution (Schwartz and Watts, 1959).

temperature of 70°C. The contents of the cans were placed in bowls, covered with aluminum foil and stored at refrigerator temperatures (5-7°C). After 2 and 5 days of storage, the TBA numbers were determined and the samples were organoleptically evaluated for a rancid odor by a panel of 12 judges. The antioxidant combination was found to have a definite protective effect against the off-odors and high TBA numbers normally associated with oxidative rancidity. The differences in average sensory scores were highly significant for both storage periods (Table I).

Andersson and Danielson (1961) examined frozen herring fillets, untreated and treated with 0.5% ascorbic acid. The assessment was performed at monthly intervals using the TBA method together with organoleptic evaluations. The chemical changes observed using the TBA method exhibited good agreement with the organoleptic observations; however, since only 2 judges were used in the sensory analyses, the significance of these results are questionable. The untreated samples, however, became rancid after two months of storage, while the treated samples remained palatable for up to 11 months (Figures 7 and 8).

Ramsey and Watts (1963) added granular polyphosphate to ground mullet in concentrations ranging from 0.01 to 0.5%. The samples were sealed in cans and heated to an internal temperature of 70°C. Portions of the cooked mullet were placed in containers, covered with Aluminum foil and held at refrigerator temperatures. At specified time intervals, the