THE UNIVERSITY OF MANITOBA

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

by

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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.

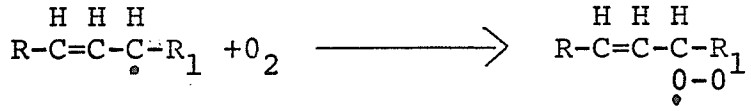
The rate of autoxidation is determined by such factors as the number of double bonds, temperature, light and the presence of pro-oxidants. Examples of the latter include various metals, their salts and metallic soaps (Tsuchiya, 1961).

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).

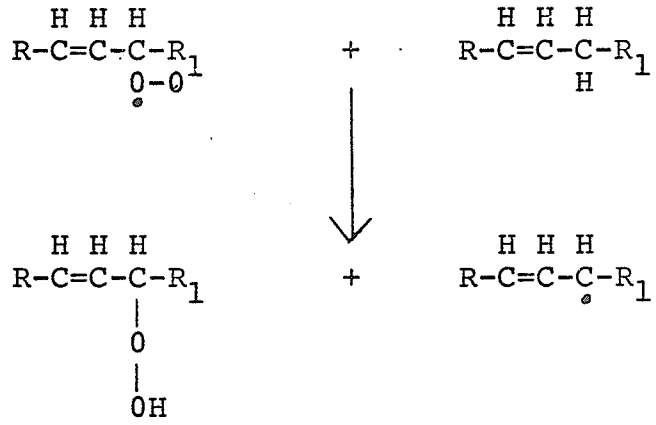
Initiation Step

Hydrocarbon Chain Radical

Propogation Steps



Hydroperoxy Radical



Conjugated Hydroperoxide

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

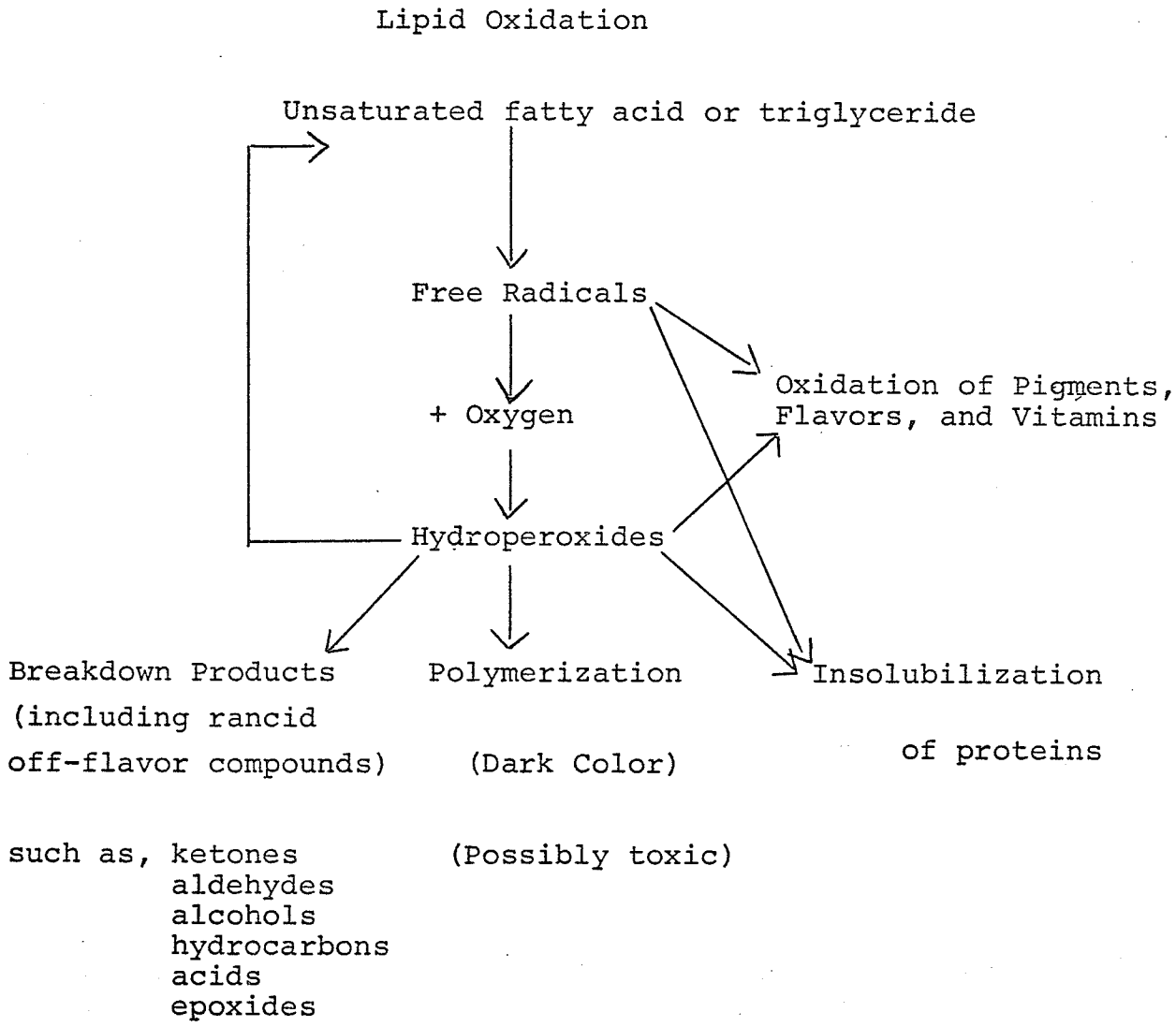


FIGURE 2. Overall Mechanisms of Lipid Oxidation (Labuza, 1971).

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)

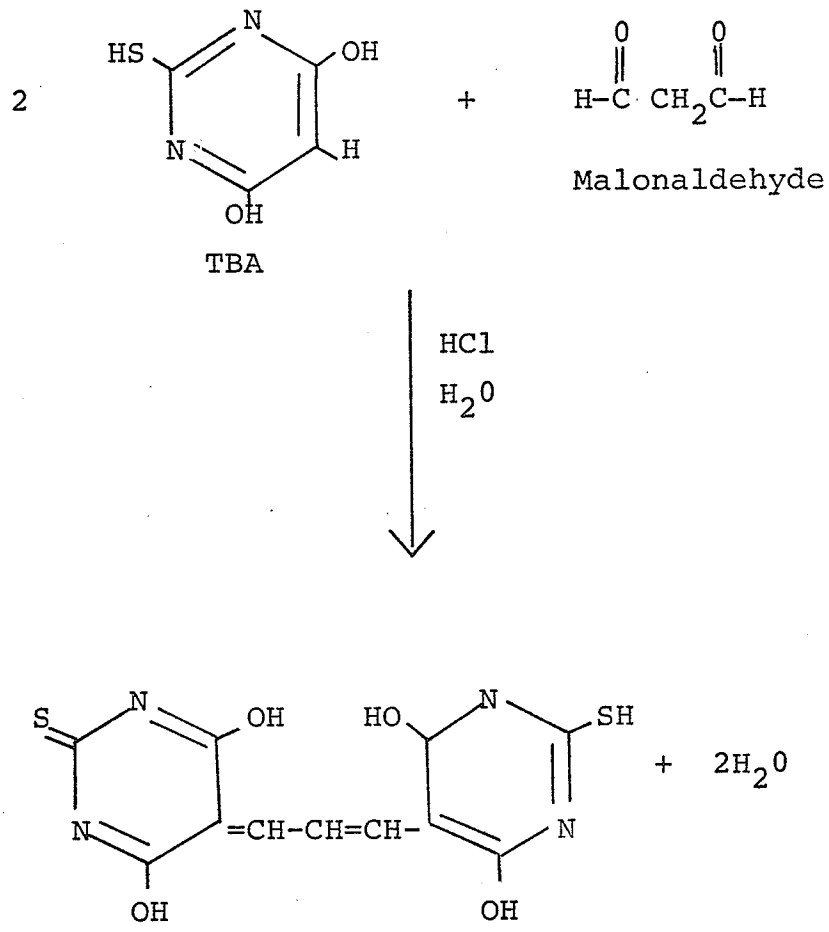


FIGURE 3. Proposed Formation of TBA Pigment from Malonaldehyde (Sinnhuber et al., 1958).

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

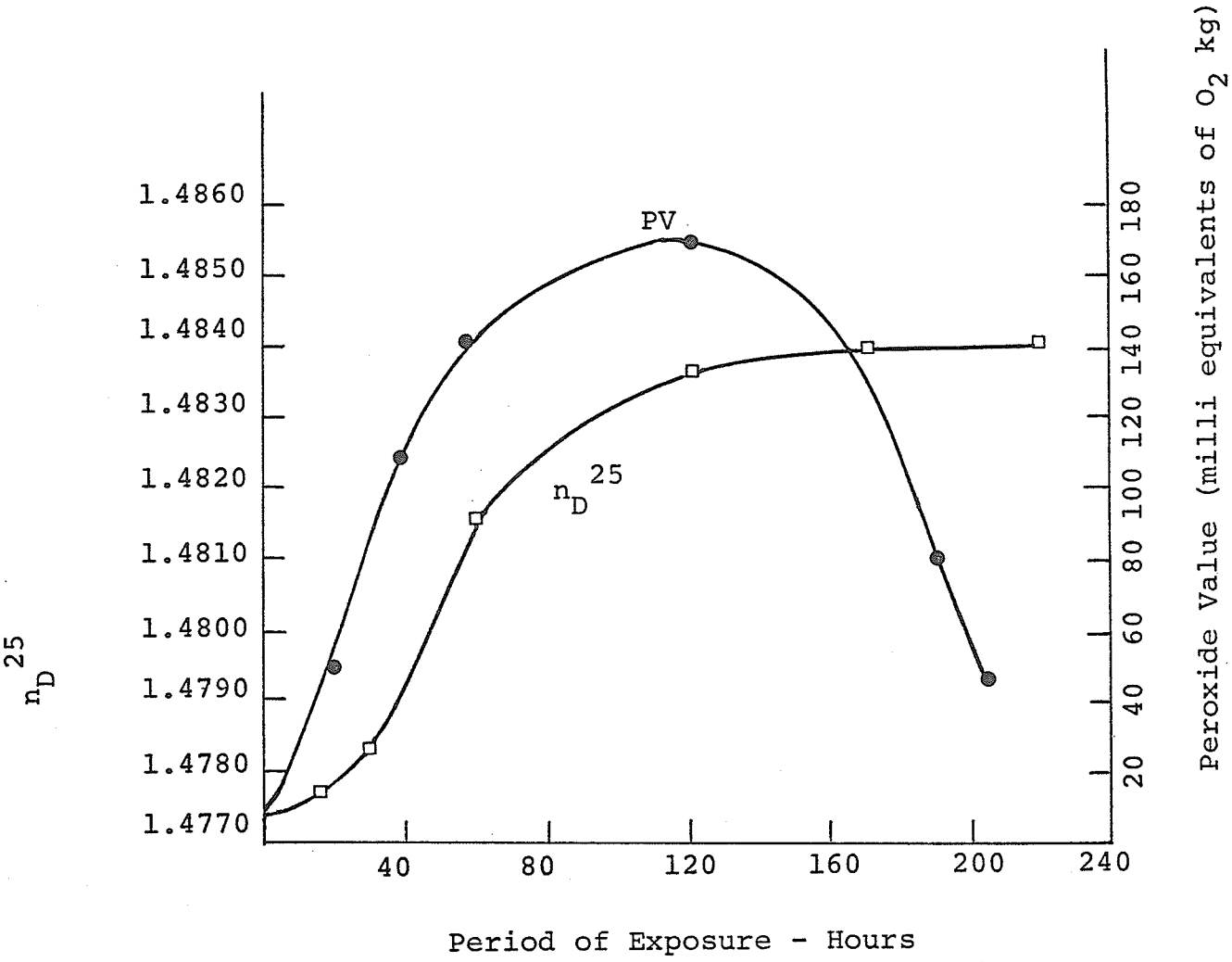


FIGURE 4. Relation Between Peroxide Value and Refractive Index (safflower oil autoxidized at 100 ± 5°C) (Arya et al., 1969).

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

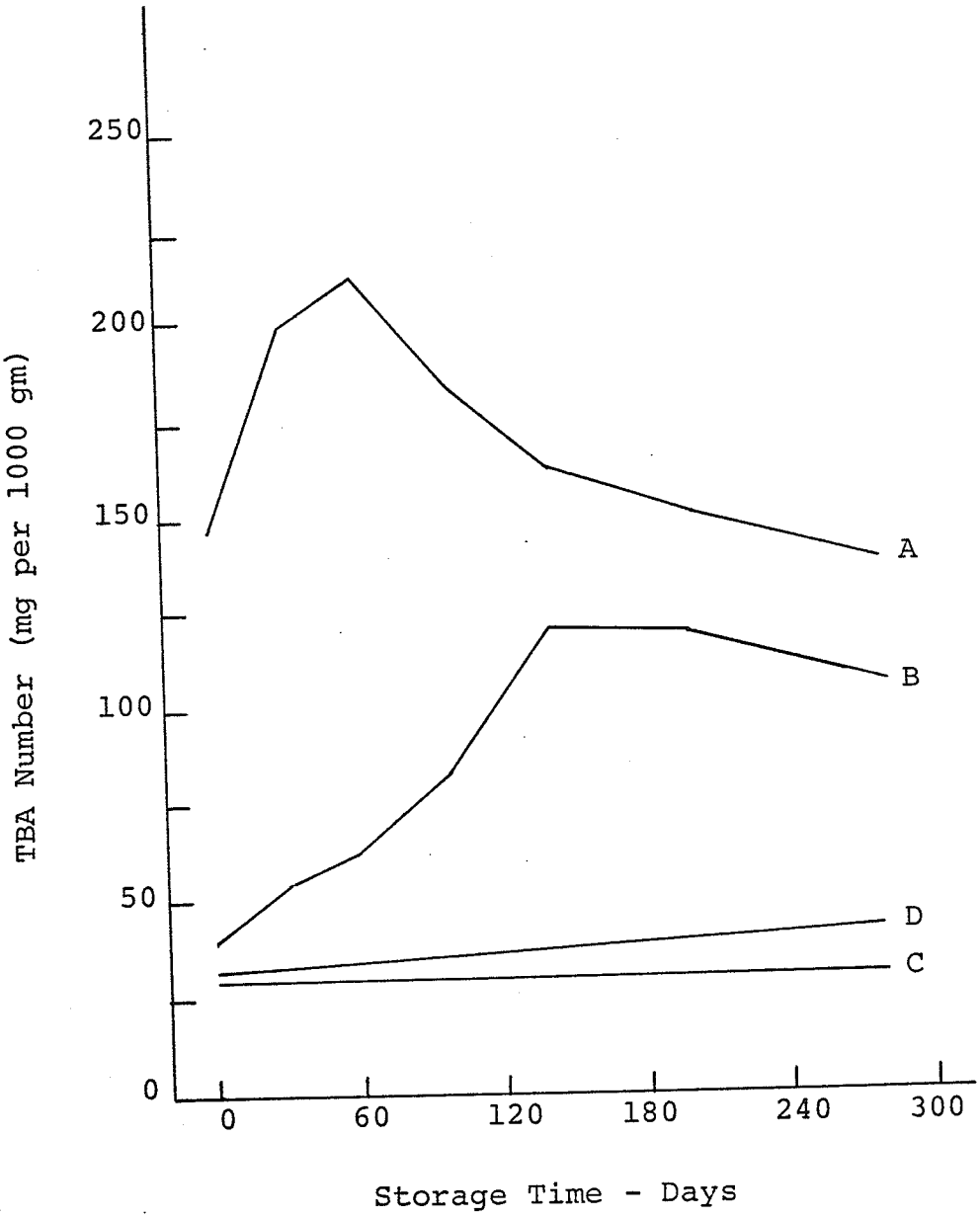


FIGURE 5. The Relation of Storage Time and Treatment of Frozen Tuna Scrap to TBA Number (Sinnhuber and Yu, 1958)

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

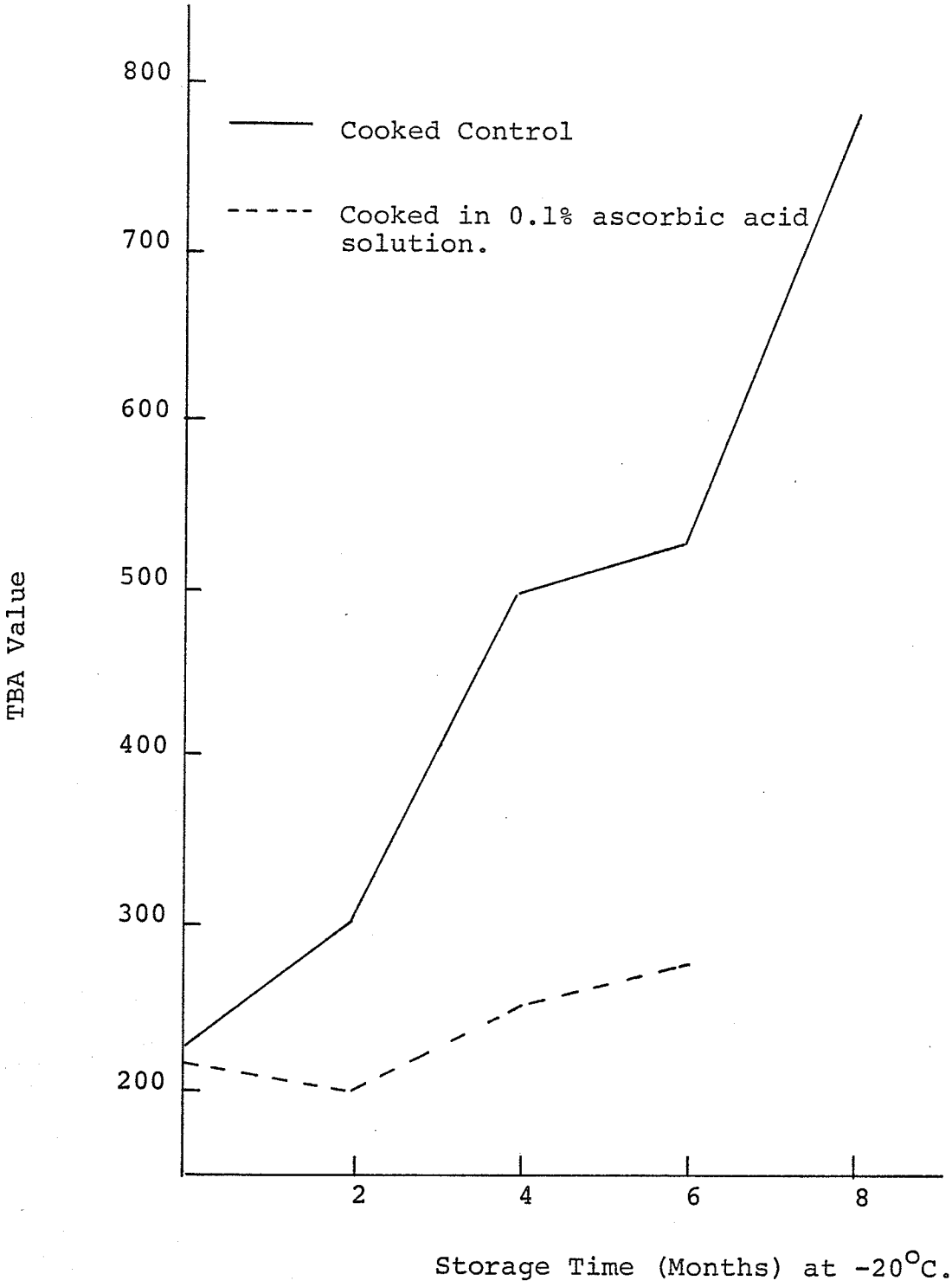


FIGURE 6. Cooked Control versus Oysters in 0.1 percent Ascorbic Acid Solution (Schwartz and Watts, 1959).

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

TABLE I

EFFECT OF ANTIOXIDANTS ON TBA NUMBERS AND SENSORY SCORES OF COOKED MULLET (ZIPSER & WATTS, 1961)

Storage (days)	Treatment	TBA	Score (Av.)*
2	Antioxidant	0.3	5.0
	No antioxidant	6.9	2.7
5	Antioxidant	0.4	5.3
	No antioxidant	9.8	3.0

* 6 = Not detectable
1 = Very strong

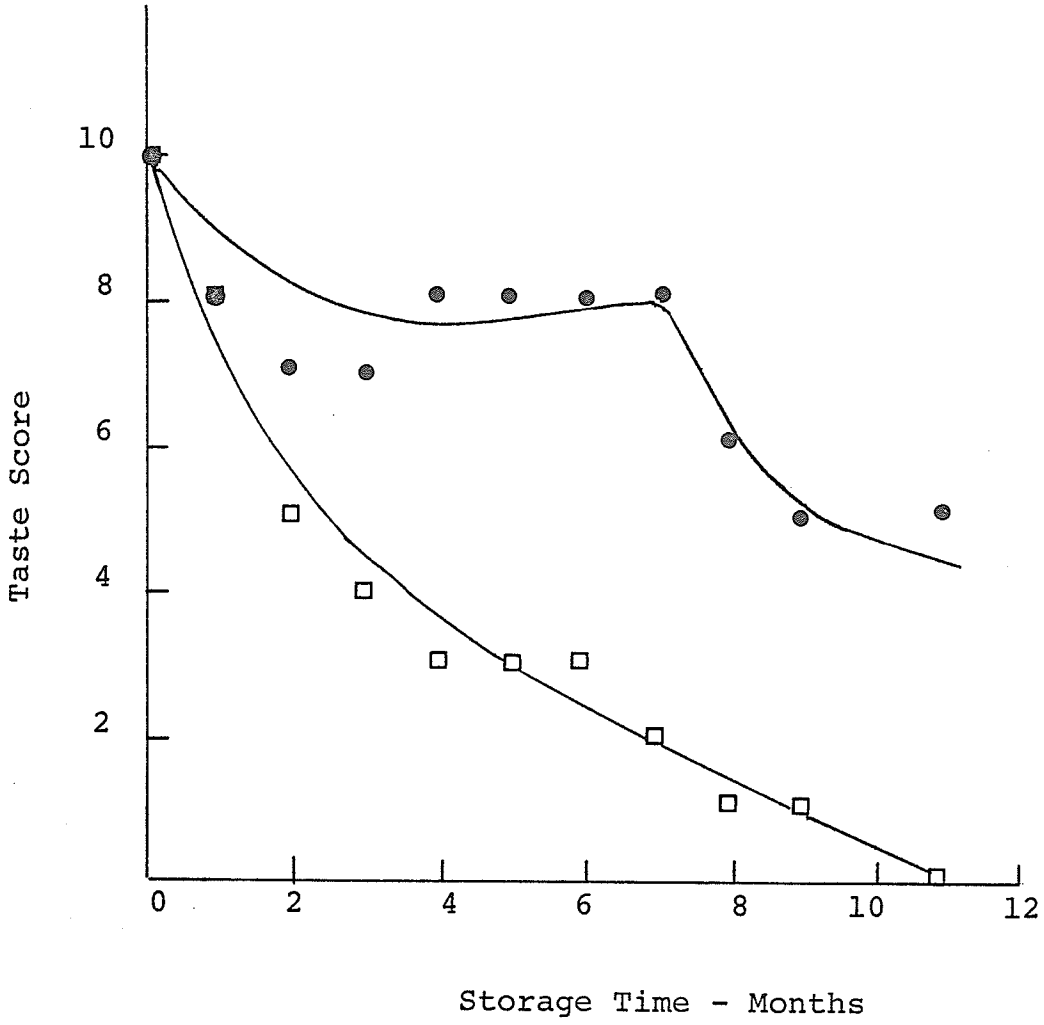


FIGURE 7. Results from the Organoleptic Evaluation of Untreated Herring (□) and Samples Treated with Ascorbic Acid (●) after Different Times of Storage (Andersson and Danielson, 1961).

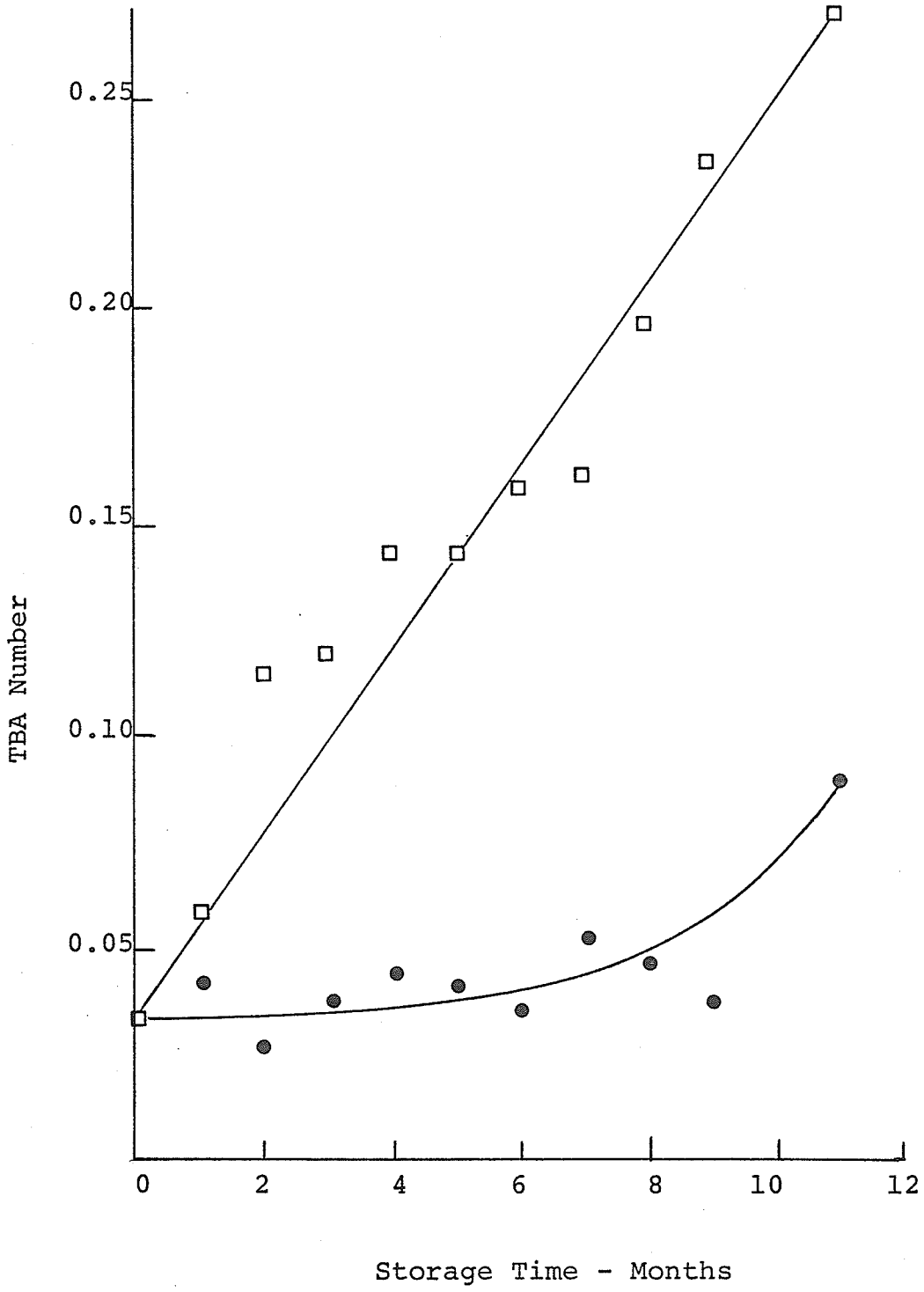


FIGURE 8. TBA Value as a Function of Time of Storage in Frozen Herring, in Untreated (□) Samples and in the Samples Treated with Ascorbic Acid (●) (Andersson and Danielson, 1961).

samples were mixed and portions removed for evaluation of oxidative rancidity by the TBA test and assessment of odor by 7 - 11 panelists selected for their ability to detect rancid odors and duplicate their ratings. In all cases, lipid oxidation decreased with increasing concentrations of polyphosphate used (Table II). Since no significant differences were observed when polyphosphates were added before or after cooking, the results were averaged and appear in Table II.

Greig et al., (1967) used ascorbic acid, monosodium glutamate, sodium tripolyphosphate or propyl gallate in ground cisco (Coregonous artedii) which was stored at -18°C for a period of 14 weeks. TBA results showed that ascorbic acid was the most effective antioxidant in retarding the onset of rancidity.

TABLE II

ADDITION OF POLYPHOSPHATE TO GROUND MULLET (RAMSEY AND WATTS, 1963).

Polyphosphate Conc. (%)	Rancidity after storage			
	2-3 days		8 days	
	TBA No.	Odor score*	TBA No.	Odor score*
0.5	1.8	5.0	10	4.2
0.1	2.2	5.4	14	2.4
0.03	2.6	4.6	20	3.5
0.01	5.8	4.0	25	2.5
0	16.0	3.5	41	3.0

* 6 = Not detectable
  1 = Very strong

HYDROLYTIC RANCIDITY

This process involves the enzymic cleavage of phospholipids and triglycerides by lipases and phospholipases present in the liver and flesh of fish. The free fatty acids released in these reactions affect the flavor in addition to the insolubilization of the fish protein (King et al., 1962)

SPOILAGE INDEX OF HYDROLYTIC RANCIDITY

Free Fatty Acid Value

This test gives an indication of the free fatty acids (FFA) formed by hydrolytic rancidity, and of these the short chain fatty acids are of direct importance to flavor development. The method involves titration with a base, but is subject to error, since it is impossible to distinguish between free fatty acids and other acidic impurities (Sherwin, 1968).

HYDROLYTIC RANCIDITY IN MARINE PRODUCTS

Under conditions of processing, the enzymes involved in hydrolytic rancidity would normally be inactivated, although high thermal stability for phospholipases has been reported in snake venom (Braganca and Quastel, 1953) and bacteria (Hayaishi and Kornberg, 1954). Olley and Lovern (1960) in studies on cod fillets, found that after 90 minutes'cooking with steam and subsequent storage for 4 weeks under chloroform-toluene at $0^{o}C$ an anomolous result of 12.8% FFA was observed (Table III). Since non-enzymic hydrolysis cannot occur under these conditions, these researchers concluded that this might be due to the incomplete inactivation of the enzymes by the heat treatment.

TABLE III.

DEVELOPMENT OF FREE FATTY ACIDS (FFA) IN THE FLESH LIPIDS OF COD PRESERVED IN CHLOROFORM-TOLUENE VAPOR AT 0°C. (FFA as g/100 g lipid, calc. at mean equivalent 300) (Olley and Lovern, 1960).

Storage time (weeks)	Raw Fish	Cooked Fish
2	10.7	5.1
3	22.6	4.5
4	28.5	12.8
5	16.2	4.7
6	13.0	6.7
7	20.6	7.7

The majority of studies reported in the literature are concerned with hydrolytic rancidity in stored frozen fish.

The rate of hydrolytic rancidity varies with the species of fish. Dyer and Fraser (1959) reported that the development of FFA in stored frozen halibut was considerably higher at $-12^{o}C$ than at $-23^{o}C$. In plaice stored at $-12^{o}C$, the production of FFA was more rapid than in halibut stored at $-12^{o}C$, while the FFA content for rosefish remained fairly constant. The increase in FFA appeared to be related to deterioration in quality on storage as indicated by actomyosin denaturation in addition to a decrease in taste panel acceptability scores.

Olley and Lovern (1960) studied the development of FFA in raw cod stored at -14^{o}, -22^{o} and $-29^{o}C$. The rate of hydrolysis at -14^{o} was approximately ten times that at -22^{o}; however, data at -29^{o} were too few for comparison. Wood and Haqq (1962) determined the FFA composition of Pacific gray cod and lingcod during storage at $-12^{o}C$ over a period of 15 weeks. They reported a rapid increase in FFA in Pacific gray cod during the initial 2 or 3 weeks of storage, which became more uniform during subsequent storage. The lingcod however, exhibited a more uniform rate of hydrolysis throughout the storage period (Figure 9).

Frozen storage studies on cod muscle at $-12^{o}C$ over a 9 month period resulted in a rapid increase in the FFA content from 5 mg/100 g tissue to a plateau after 4 months of around 300 mg/100 g tissue (Bligh and Scott, 1966). Awad and co-workers (1969) reported the initial FFA content of frozen freshwater whitefish muscle to be 177 mg/100 g muscle, which

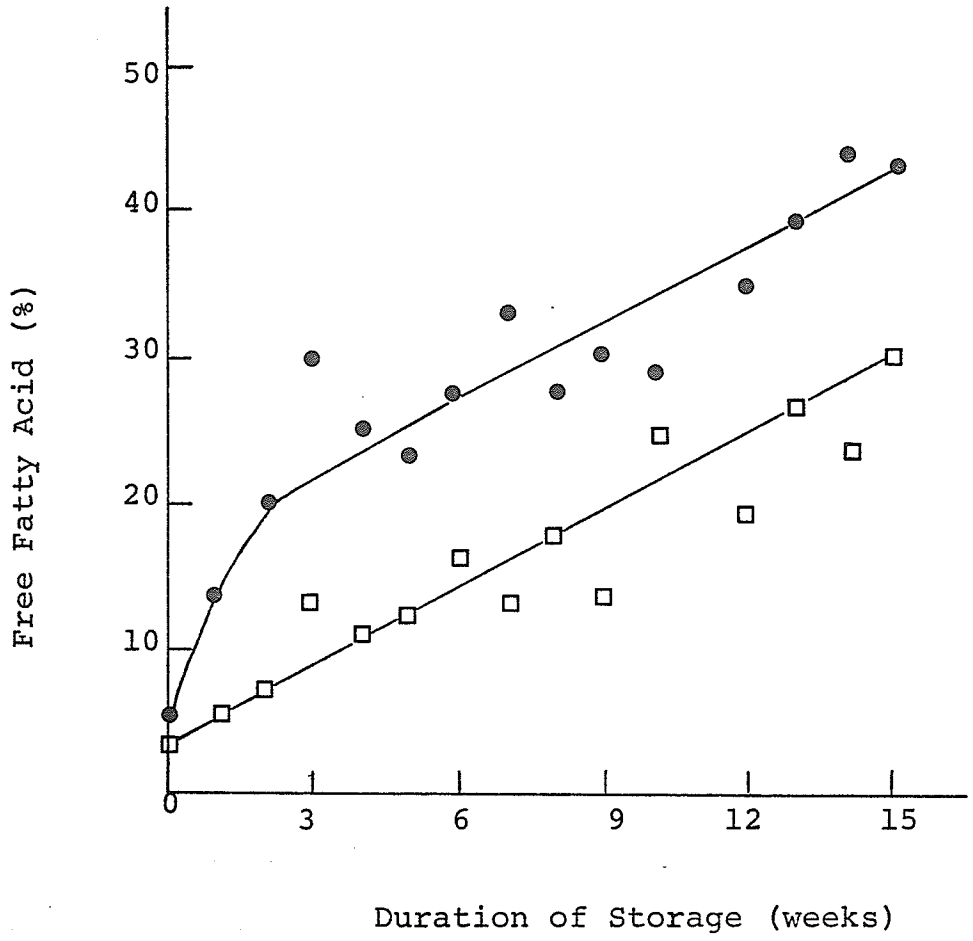


FIGURE 9. The Formation of Free Fatty Acids in Lingcod and Pacific Cod Fillets ●———● Pacific Gray Cod; □———□ Lingcod. Each value is the Mean Value for 10 Fillets (Wood and Haqq, 1962).

increased to 814 mg/100 g muscle when held at -10°C for 16 weeks.

It appears evident that further research is required to determine the development of off-flavors in canned fish products and those antioxidants best suited for controlling these oxidative changes which occur once the tin has been opened.

METHODS

Freshwater whitefish (Coregonous clupeaformis) was canned with and without antioxidants and the flavor evaluated after open refrigerated storage using organoleptic, chemical and physical measurements (Table IV).

Fish used in this study were caught by gill net in May, 1971 in William Lake, Manitoba. The fish were gutted, packed in ice in styrofoam boxes and transported by truck within twelve hours to the Freshwater Institute, Fisheries Research Board of Canada, at the University of Manitoba Campus for canning. The fish were filleted, cut into steaks and canned in 100 gram lots in enamelled cans with a diameter of 6.5 cm. and a depth of 3.2 cm.

In this study, there were three treatments of the white-fish, in which one of the following was added prior to canning:

1) a mixture of 20% BHA (butylated hydroxyanisole), 20% BHT (butylated hydroxytoluene) and 60% vegetable oil carrier[1] - AX 1,
2) a mixture of 10% BHA, 10% BHT, 6% citric acid, 6% propyl gallate, propylene glycol, monoglyceride citrate and a vegetable oil carrier[1] - AX 2,
3) no added antioxidant.

The total amount of antioxidant did not exceed 0.02%

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[1]Griffith's Laboratories

TABLE IV. SUMMARY OF METHODS USED TO EVALUATE CANNED WHITEFISH AFTER OPEN REFRIGERATED STORAGE

Method	Measurement	Open Refrigerated Storage Time	Number of Repeats of Experiment
8-member preliminary panel using the paired comparison method	the sample member of a pair (control of AX-treated) that has the most "off-flavor"	0, 3, 6, 18, 24 hours	3
8-member trained panel using the paired comparison method with degree of difference scale	the degree of "off-flavor" of both AX 1 and AX 2-treated fish in relation to the un-treated sample	0, 1, 2, 3, 4 days	3
hedonic scoring of sandwiches made from whitefish with and without antioxidants and aged 24 hours prior to the test or unaged	consumer acceptance of sandwiches made from canned whitefish	0 and 1 day	-
TBA method - sample homogenized with PG and EDTA	measures the change in optical density	0, 1, 2, 3, 4 days	2
- sample homogenized without PG and EDTA		0, 1, 2, 3, 4 days	2
fatty acid composition of the 3 treatments of canned whitefish using gas-liquid chromatography	measures changes in the percentage of poly-unsaturated fatty acids	0 and 4 days	3
free fatty acid content using titrimetric method	measures hydrolytic rancidity (mg FFA/100 g fish)	0, 2, 4 days	3
refractive index	measures changes in refractive index that corresponds to the end of the induction period in [illegible]	0, 2, 4 days	3

of the fat or oil content of the fish in order to comply with Food and Drug Regulations, 1970. The antioxidants were added to empty gelatin capsules[1] by means of a Winetraub dropper, using gravity alone. The average weight of each drop of the different antioxidant mixtures was determined by weighing drops taken from the Winetraub dropper when almost empty and when full. Thus, to each gelatin capsule, one drop of antioxidant with a weight of 0.0146 ± 0.0002 grams was added. In order to reduce the variability as much as possible, the treatment with no antioxidant received an empty capsule. In addition to the gelatin capsule with or without antioxidants, a salt pellet (2 g.) was added and the cans vacuum sealed and sterilized at 115°C for one hour. The cans were then allowed to cool, labelled and stored at room temperature for a mimimum of 30 days to obtain good flavor and aroma distribution (Anonymous, 1966). After this ageing period, the cans were refrigerated in household-style refrigerators prior to examination.

ORGANOLEPTIC EVALUATION

Three types of panels were used to determine the effectiveness of 2 antioxidants in preventing flavor deterioration after the cans of fish were opened. A small untrained panel was used in preliminary work to determine if a more controlled study would be feasible. In the main study, a minimally-trained panel was used to examine the fish under laboratory conditions

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[1] Lilly empty gelatin capsules No. 3. Eli Lilly and Company, Indianapolis, Ind. 46206.

while a much larger panel of consumers was used to ascertain if some of the effects noted by the trained panel would be obvious in a normal eating situation.

Preliminary Panel

An eight-member panel consisting of graduate students and staff members of the Department of Foods and Nutrition examined fish which had been canned with or without added antioxidants, then opened and aged in a refrigerator for 0, 3, 6, 18 or 24 hours.

Using the paired comparison method, (Figure 10) each panelist compared the two antioxidant treatments with the untreated fish after each of the five ageing periods. Each pair was examined three times. Sample order was balanced within pairs among the judges. The order of pair presentation was randomized for the entire experiment. The judges evaluated 3 pairs of samples at each of 10 sessions in order to limit the sensory fatigue involved in tasting off-flavors (Table V). The judges were provided with an identified sample that had been aged 24 hours which illustrated "off-flavor" in canned whitefish. They were requested to taste this reference sample prior to evaluating the pairs of samples. Unsalted soda crackers and water were provided and the panelists were asked to rinse between pairs, but not between members of the same pair. At the end of each session, panelists were rewarded with lemonade and candy. Sensory testing took place in a small, quiet sensory testing room equipped with five individual booths. Red lights were used to mask color differences observed in the samples (Shaykewich,

Characteristic being examined: "OFF-FLAVOR" IN CANNED WHITEFISH

Name: _____

Date: _____

In the following comparisons, please make a choice. If you feel there was no difference between the samples, that is, you made a guess, note this at the bottom of the page.

Pair (list in the order examined).	Which sample has more of the characteristic	Comments

Figure 10. Questionnaire used by the Preliminary Panel to Evaluate Canned Whitefish.

TABLE V

EXPERIMENTAL DESIGN OF PAIRS EXAMINED BY THE PRELIMINARY PANEL

Session	Pairs[1] Examined	Refrigerated Storage Time After Opening (Hrs).	Session	Pairs Examined	Refrigerated Storage Time After Opening (Hrs).
1	C x AX 2	24	6	C x AX 2	6
	C x AX 1	24		C x AX 2	0
	C x AX 1	3		C x AX 1	18
2	C x AX 1	3	7	C x AX 2	18
	C x AX 2	24		C x AX 1	18
	C x AX 2	3		C x AX 1	0
3	C x AX 2	18	8	C x AX 2	6
	C x AX 2	18		C x AX 2	3
	C x AX 1	3		C x AX 1	18
4	C x AX 1	24	9	C x AX 2	0
	C x AX 1	6		C x AX 1	6
	C x AX 1	24		C x AX 2	24
5	C x AX 1	0	10	C x AX 2	6
	C x AX 2	3		C x AX 1	0
	C x AX 1	6		C x AX 2	0

[1]The order of sample presentation was balanced within each pair so that one-half the panelists tasted a control sample first and the other half tasted an antioxidant-treated sample first.

1971). The color of the whitefish was noted to change from a pink color observed immediately on opening to white or gray after extended storage.

For each pair of fish samples examined over the various time intervals, a tin of each antioxidant treatment and two tins of the control were opened, drained a standard time and placed in separate pyrex bowls. The fish was then fork-flaked to uniformity. The individual bowls were covered with plastic film[1] and the samples aged in a home-style refrigerator at 10°C for the specified time period. After ageing and immediately before serving, the fish were portioned into 21 ml coded plastic containers[2] and covered with cardboard lids. Plastic spoons were provided.

Trained Panel

An eight-member panel of graduate students and staff members of the Department of Foods and Nutrition who were willing to participate in the main study were trained to evaluate the flavor of canned whitefish. Only 2 of the panelists had participated in the preliminary study.

In order to acquaint the judges with the flavor changes which take place in whitefish after cans have been opened, three training sessions were held. During the first session, the judges were presented with four coded samples, one which was freshly opened and others which had been aged for 3, 24 and 48

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[1]Saran Wrap, Dow Chemicals Ltd., Montreal, Quebec.

[2]Lily Brand, Plastic Creamers No.3/4 SP. Lily-Tulip Cup Corp.. New York, N.Y.

hours in a household-style refrigerator. Their task was to rank these samples in order of least to most "off-flavor". In the event that a panelist ranked a sample incorrectly, she was requested to re-taste the sample.

The second training session attempted to simulate actual panel conditions. Each judge was given an example of the questionnaire to be used (Figure 11) and provided with two reference samples, one which was freshly opened and the other which had been aged 24 hours. These served as examples of "fresh" and "off-flavor" in whitefish.

The judges then evaluated two pairs of samples:

1) 24 hour control vs. 24 hour AX 2

2) freshly opened control vs. freshly opened AX 2.

They were required to choose the member of each pair which had more "off-flavor" and indicate the degree of flavor difference between the two members of each pair. The judges were informed of their results and a discussion was held concerning different clues they used for detecting the off-flavor.

In the third session, the panelists were given additional practice using the questionnaire. They assessed the following two pairs of samples:

1) 24 hour control vs. 24 hour AX 2

2) 4 hour control vs. 4 hour AX 2.

They were provided with the two reference samples described previously and after evaluation a discussion followed.

During the course of the 3 replications, 4 of the original panel members were no longer available and so the replacements were given samples of freshly opened whitefish and whitefish

COMPARISON OF FLAVORS IN CANNED WHITEFISH

Name _____ Date _____

You are receiving two samples to compare for "off-flavor".

1. Taste the samples in the order listed below.
2. Circle the code number of the one which has more "off-flavor"
3. Indicate the degree of difference in off flavor between the two samples.

SAMPLE CODES _____ _____

AMOUNT OF DIFFERENCE

Indicate the degree of difference between the two samples by marking the appropriate phrase on this scale:

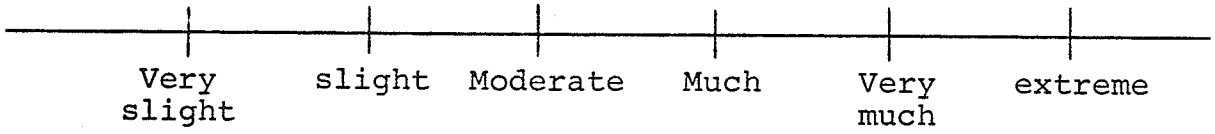

Figure 11. Questionnaire used by the Trained Panel to Evaluate Canned Whitefish.

that had been aged 24 hours. After tasting these two samples, the flavor was discussed with the new panelists on a less formal basis.

For the actual test, the trained panel evaluated opened cans of whitefish which had been subjected to 0, 1, 2, 3 and 4 days of ageing in a household-style refrigerator.

Using the questionnaire from days 2 and 3 of the training session (Figure 11), each panelist compared the 2 antioxidant treatments to the untreated fish after each of the 5 time intervals (Table VI). This procedure was repeated on 3 separate occasions. The order of sample presentation within each pair was arranged so that one-half of the judges received a control sample first and the other half tasted an antioxidant-treated sample first. At each session, the order of pair presentation was randomized. The panelists were supplied with the two reference samples and rinsing materials described earlier. At the end of each session, panelists received a token monetary reward and candy.

All the cans of fish for one replication were opened at the beginning of the storage study. The fish was flaked and stored as described earlier. Duplicate bowls of 6 to 8 tins of fish were kept for each treatment (Figure 12). Samples were taken from each of the 2 bowls on each test day to give duplicates for the TBA test and 4 samples for the sensory evaluation. Sensory samples from both bowls were assigned to the 8 panelists at random.

Consumer Panel

Pairs of sandwiches made from untreated and antioxidant-

TABLE VI.

EXPERIMENTAL DESIGN* OF PAIRS EXAMINED BY TRAINED PANELISTS

Session	Pairs Examined	Refrigerated Storage Time After Opening (Days).
1	C x AX 1 C x AX 2	0
2	C x AX 1 C x AX 2	1
3	C x AX 1 C x AX 2	2
4	C x AX 1 C x AX 2	3
5	C x AX 1 C x AX 2	4

* Repeated 3 times.

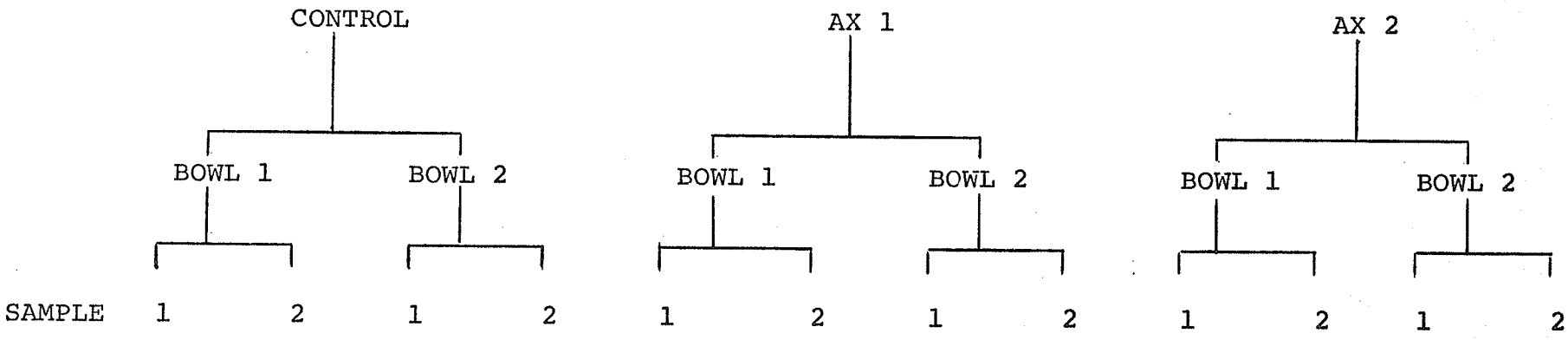

FIGURE 12. Basic Experimental Design[1]

[1]Duplications and time intervals varied as described in the text.

treated canned whitefish were tested for consumer acceptability at Opinion Place[1] in the mall at Polo Park Shopping Centre on three occasions. The variations of the fish sandwiches examined are illustrated in Table VII.

The judges were homemakers of any age who were shopping in the mall and who used either canned tuna or canned salmon for sandwiches. Each was classed as either a tuna or salmon user according to which fish she used more frequently. The interviewer determined how often the panelists made fish sandwiches and who they were usually for. The judges were advised that the test product was in the experimental stage and not yet available on the market. Each judge was asked to taste just one pair of sandwiches. The two samples were presented singly, in alternate order and the judge was asked to rinse with water between samples. After each sandwich the judge was asked to rate the fish on a 9-point V-type hedonic scale (Figure 13). After both sandwiches were evaluated, the judges were asked what they liked most about the whitefish and what they liked least. They were also asked at what price range they would be willing to buy the whitefish and about their impressions of the product quality from just looking at the tins. All forms were completed by the interviewer.

Sandwich spreads were prepared for each treatment from freshly opened cans of fish using this simple recipe:

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[1]Marketing Insights, Ltd. Opinion Place, 66K Polo Park Shopping Centre, 1485 Portage Avenue, Winnipeg, Manitoba

TABLE VII.

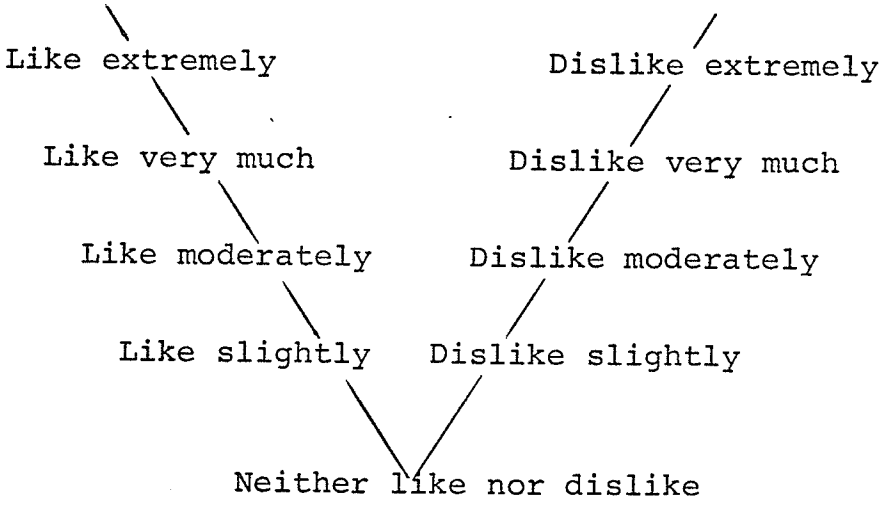
EXPERIMENTAL DESIGN OF PAIRS OF FISH SANDWICHES EXAMINED BY THE CONSUMER PANEL

Session	Number of Judges	Pairs of Fish Sandwiches Examined	Refrigerated Storage Time of the Sandwich Spreads	Interview Form Completed
1	21	C x AX 1	prepared morning of the test day	Appendix 1
2	51	AX 1 x AX 2	Stored 24 hrs prior to making the sandwiches	Appendix 2
3	43	C x AX 1	Stored 24 hrs prior to making the sandwiches	Appendix 2

Date _____

Name _____

Taste the food sample. On the diagram below, circle the phrase that best describes your opinion of the food.



Comments:

Figure 13. Questionnaire used by the Consumer Panel to Evaluate Whitefish Sandwiches.

800 g drained whitefish

2/3 c commercial salad dressing[1]

1/2 t salt

Sandwiches consisted of 1/4 c of spread topped with shredded lettuce between two slices of day-old buttered bread. The sandwiches were prepared at 11:00 a.m. of the test day, covered with plastic film[2] and refrigerated until cut immediately before testing began at 2:00 p.m. Six samples were cut from each prepared sandwich, after the crusts had been removed. During the 1 1/2 hour test period, the sandwiches were held at room temperature.

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[1]Miracle Whip, Kraft Foods Ltd., Montreal, Quebec

[2]Saran Wrap, Dow Chemicals Ltd., Montreal, Quebec

OBJECTIVE EVALUATION

Chemical Tests

Thiobarbituric Acid Test

Thiobarbituric acid (TBA) values were determined according to the method described by Vyncke (1970). Samples containing the contents of six to eight drained tins of whitefish were set up in duplicate for each treatment. TBA values were measured initially and following 1, 2, 3 and 4 days of storage in a household-style refrigerator (Figure 12). Twenty grams of fish sample were homogenized for one minute with 100 ml of 7.5% trichloroacetic acid (TCA) in a Virtis 23 Homogenizer[1] at full speed. The mixture was filtered through Whatman No.1 filter paper using a Buchner funnel. 5 ml of the filtrate were placed in test tubes with screw caps and 5 ml of TBA reagent (0.02N 2-thiobarbituric acid) added. The test tubes were then placed in a boiling water bath for 40 minutes and the absorbance read at 538 mu following cooling using a Coleman Junior Spectrophotometer (Model 6A)[2]. The original TCA solution was used as a blank.

TBA values were also determined on fish samples to which 0.1 g EDTA (ethylene diaminetetra acetic acid) and 0.1 g PG (propyl gallate) had been added prior to homogenizing. The same procedure was followed as described previously.

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[1]Virtis Company, Inc. Gardiner, N.Y. 12525.

[2]Coleman Instruments, Inc. Maywood, Ill. U.S.A.

Total Lipid Extraction

Total lipid was extracted from the fish samples using the method described by Bligh and Dyer (1959). Using the extracted lipid, the fatty acid composition, free fatty acid content and refractive indices were determined. Duplicate bowls each containing 4 cans of fish from each of the 3 treatments were set up. The lipid was extracted immediately on opening and after 2 and 4 days of refrigerated storage. This procedure was repeated on 3 separate occasions.

The lipid of one sample from each bowl was extracted as follows: 50 g of fish were homogenized in a Waring blender for 2 minutes with a mixture of 50 ml chloroform, 100 ml methanol and 2.5 ml water to give a ratio of 1:2:0.8 (v/v/v). To this mixture was then added 50 ml chloroform and following blending for 30 seconds, 50 ml distilled water were then added to give a ratio of 2:2:1.8 (c/m/w) and the mixture blended for a further 2 minutes. The extraction mixture was filtered with slight suction through Whatman No.1 filter paper. The residue was re-homogenized with 50 ml of chloroform to ensure total fat extraction, and the filtrate from the two extractions combined and transferred to a 500 ml separatory funnel and allowed to separate overnight.

On separation, the chloroform layer was collected and the total volume recorded. A 30 ml aliquot was reserved for free fatty acid determinations. The remainder of the chloroform layer was filtered through Whatman No.2 filter paper into a 250 ml tared round bottom flask and evaporated to dryness under vacuum using a Buchler Portable Flash Evaporator

(Model PF-10DN)[1]. The flasks were then flushed with nitrogen and placed in a dessicator over concentrated sulfuric acid to remove any residual traces of moisture. Total lipid was determined gravimetrically and adjusted for total volume.

Preparation of Methyl Esters

A 300 mg aliquot of lipid was removed from the dried sample and transferred to a 50 ml volumetric flask. The methyl esters were prepared according to the method of Metcalfe et al. (1966) and stored under nitrogen in screw-top glass vials in a -10°C freezer prior to gas chromatographic analysis.

Fatty Acid Determination

The fatty acid composition of the canned whitefish was determined initially and following 4 days of refrigerated storage. For each of these 2 time intervals, three fatty acid methyl esters from each of the 3 treatments were analyzed by gas-liquid chromatography using a dual column Aerograph (Model 1740-1)[2] gas chromatograph equipped with flame ionization detectors and helium[3] as a carrier gas. The samples were injected on 2.7 m x 3.2 mm steel columns packed with 10% EGSS-Y on 100/120 mesh CHROMQ[4]. The flow rates were 30 ml/minute for the helium[3], 25 ml/minute for hydrogen[3] and 250 ml/minute for air[3].

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[1]Buchler Instruments, Inc. Fort Lee, New Jersey 07024.

[2]Varian Aerograph, 6358 Viscount Road, Malton, Ontario

[3]Welder's Suppliers, 25 McPhillips Street, Winnipeg 3, Manitoba

[4]Applied Science Lab., Inc., P.O. Box 440 State College, P.A. 16801

The columns were operated isothermally at a temperature of 200°C with injector and detector temperatures maintained at 250°C and 230°C respectively. The gas chromatograph was equipped with Varian Aerograph (Model 20)[1] single pen recorder and a Varian Aerograph (Model 477)[1] Digital Integrator. Individual fatty acid methyl esters were identified by comparing retention times with known fatty acid mixtures.[2]

Free Fatty Acid Determination

The free fatty acid content was determined initially and after 2 and 4 days of refrigerated storage (Figure 12) using a titrimetric method described by Bligh and Scott (1966). There were 12 samples for each treatment at each time interval (2 bowls x duplicates x 3 repeats of the experiment).

Blanks were prepared in duplicate containing 15 ml chloroform and 15 ml methanol. The solution was placed in a beaker on a magnetic stirrer[3] and titrated against 0.033 N sodium methylate to an end point of pH 10.5 using a Radiometer pH meter 26[4] and Titrator 11 with a magnetic valve[4]. The volume of sodium methylate was recorded before and after titrating. Duplicate samples of chloroform extract (10.0 ml) were added to 5 ml chloroform and 15 ml methanol and similarly titrated to an end point of pH 10.5. The volume of titrant for the blank was subtracted from that volume used for the sample and the free

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[1]Varian Aerograph, 6358 Viscount Road, Malton, Ontario

[2]Hormel Institute, Lipids Preparation Laboratory, 801-16th Avenue. N.E. Autin, Minn. 55912.

[3]Mag-Mix, Precision Scientific, Chicago, Ill.

[4]Radiometer A/5, 72 Emdrupuej, Copenhagen, NV, Denmark.

fatty acid content calculated (1 ml of sodium methylate equivalent to 10 mg free fatty acid average molecular weight 300).

Physical Measurements

Refractive Index

Refractive indices were determined on samples of lipid extracted immediately on opening the tin and following 2 and 4 days of refrigerated storage using the method described by Arya et al. (1969). For each time interval there were 6 samples for each treatment (2 bowls x 3 repeats of the experiment).

The vials containing the stored extracted lipid in petroleum ether were removed from the freezer and the petroleum ether evaporated under nitrogen in a warm water bath. The vials were then placed over concentrated sulfuric acid in a dessicator for a period of an hour to remove any remaining traces of petroleum ether. Approximately four drops of extracted oil were placed on the prism of an Abbe-3L Refractometer[1] using Pasteur pipettes. The cell was maintained at a constant temperature of 25°C and the refractive indices (n_D^{25}) recorded.

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[1]Bausch and Lomb, Rochester, N.Y., 14602.

STATISTICAL ANALYSIS

Preliminary Panel

The results of the 3 replicates for the preliminary panel were analyzed individually using a chi-square test as described by Amerine et al., (1965). The effects of the antioxidants at each of the 5 time intervals were examined separately. Because the same 8 judges participated in the tests of all time intervals, chi-square statistics, which demand independent observations, could not be calculated between intervals.

Training Session

Inversion values for the ranking test were calculated and analyzed as described by Amerine et al., (1965). For the paired comparisons evaluated at the training session, the proportions of the panelists who could distinguish between the control sample and the antioxidant-treated sample were simply tabulated.

Trained Panel

Total scores measuring degree of "off-flavor" between the control and each antioxidant treatment at each day of storage were derived following the procedure of Scheffé (1952) Table VIII. These total scores were then analyzed by multiple regression using a model based on Scheffé's analysis. The model was designed to permit a comparison of the effects of both antioxidants in relationship to the control. This modification to the analysis was necessary because all possible comparisons of

TABLE VIII.

DERIVATION OF TOTAL SCORES (y) FOR TRAINED PANELISTS' ASSESSMENTS OF RELATIVE OFF-FLAVOR[1]

Day	Order of Presentation	Frequency of Degree of Difference Assignment by Panelists													Total Score
		- 6	- 5	- 4	- 3	- 2	- 1	+ 1	+ 2	+ 3	+ 4	+ 5	+ 6		
0	C, AX 1					1	1	1	3	4	1				+ 20
	AX 1, C				4	4	1		1		1				- 15
	C, AX 2					1	1	3	5			1			+ 15
	AX 2, C				3	2	1	1	1	3					- 2
1	C, AX 1								2	6	2	2			+ 40
	AX 1, C		2	1	7	1			1						- 35
	C, AX 2								6	4	2				+ 32
	AX 2, C			1	7	2			1	1					- 24
2	C, AX 1				1	1				6	3	1			+ 30
	AX 1, C		5	2	4	1									- 47
	C, AX 2			1			1		2	3	1	3		1	+ 33
	AX 2, C	1	1	3	2	4				1					- 34
3	C, AX 1				2				2	4	2	1			+ 23
	AX 1, C	1	3	4	2				1						- 41
	C, AX 2				1					5	2	3			+ 35
	AX 2, C		4	3	3					1					- 38
4	C, AX 1				1				2	5	2				+ 24
	AX 1, C	1	2	3	2					1		1			- 26
	C, AX 2					1			2	2	2	3			+ 31
	AX 2, C		1	1	5	1				1	1				- 19
	Total	3	18	19	44	19	5	5	29	47	19	15	1		

[1] Used in Multiple Regression Analysis.

the 3 treatments were not examined; that is AX 1 and AX 2 were not compared directly. A further modification was necessary because the same samples were examined on subsequent days of storage, giving a time effect.

The variables, X_1 and X_2 used to measure the treatment effects must be derived (Table IX). Let the variables W_1, W_2, and W_3 represent the three treatments; control, AX 1 and AX 2. A value of +1 was assigned if the treatment was presented to the panelist first, -1 if it was presented second and 0 when it was not included in the comparison. The regression coefficients for these variables sum to zero and this restraint was incorporated in the analysis by forming the variables $X_1 = W_1 - W_3$ and $X_2 = W_2 - W_3$. The regression coefficient for X_1 measured the treatment effect for the control and the coefficient of X_2 measured the treatment effect for AX 1. The treatment effect for AX 2 was obtained by using the above restraint.

The source of variation "deviation from order" determined whether the panelists were consistent in the manner in which they were "ordering" the samples as to the degree of rancidity at each ageing period; that is, if they were rating the three samples differently on the different days of the storage study. The coded values in columns X_3 to X_6 of Table X were assigned to the various days and these variables represent the "deviation from order over days".

TABLE IX

DERIVATION OF 'x' VALUES FOR ALL POSSIBLE PAIRS OF THE 3 TREATMENTS FOR ONE OF THE 5 STORAGE DAYS.

Total Score (y)	Control W_1	AX 1 W_2	AX 2 W_3	X_1 (W_1-W_3)	X_2 $(W_2 - W_3)$
20	1	-1	0	1	-1
-15	-1	1	0	-1	1
15	1	0	-1	2	1
- 2	-1	0	1	-2	-1

TABLE X.

MATRIX USED IN MULTIPLE REGRESSION ANALYSIS OF OFF-FLAVOR DATA.

			Treatments		Deviation from Order over Days				Interaction							
Day	Order of Sample Presentation	Y	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
0	C, AX 1	+20	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1
	AX 1, C	-15	-1	1	-1	-1	-1	-1	1	1	1	1	-1	-1	-1	-1
	C, AX 2	+15	2	1	-1	-1	-1	-1	-2	-2	-2	-2	-1	-1	-1	-1
	AX 2, C	- 2	-2	-1	-1	-1	-1	-1	2	2	2	2	1	1	1	1
1	C, AX 1	+40	1	-1	1	0	0	0	1	0	0	0	-1	0	0	0
	AX 1, C	-35	-1	1	1	0	0	0	-1	0	0	0	1	0	0	0
	C, AX 2	+32	2	1	1	0	0	0	2	0	0	0	1	0	0	0
	AX 2, C	-24	-2	-1	1	0	0	0	-2	0	0	0	-1	0	0	0
2	C, AX 1	+30	1	-1	0	1	0	0	0	1	0	0	0	-1	0	0
	AX 1, C	-47	-1	1	0	1	0	0	0	-1	0	0	0	1	0	0
	C, AX 2	+33	2	1	0	1	0	0	0	2	0	0	0	1	0	0
	AX 2, C	-34	-2	-1	0	1	0	0	0	-2	0	0	0	-1	0	0
3	C, AX 1	+23	1	-1	0	0	1	0	0	0	1	0	0	0	-1	0
	AX 1, C	-41	-1	1	0	0	1	0	0	0	-1	0	0	0	1	0
	C, AX 2	+35	2	1	0	0	1	0	0	0	2	0	0	0	1	0
	AX 2, C	-38	-2	-1	0	0	1	0	0	0	-2	0	0	0	-1	0
4	C, AX 1	+24	1	-1	0	0	0	1	0	0	0	1	0	0	0	-1
	AX 1, C	-26	-1	1	0	0	0	1	0	0	0	-1	0	0	0	1
	C, AX 2	+31	2	1	0	0	0	1	0	0	0	2	0	0	0	1
	AX 2, C	-19	-2	-1	0	0	0	1	0	0	0	-2	0	0	0	-1

To determine if there was a treatment x order interaction, the two treatment effects were multiplied by each of the 4 "deviation from order" effects and these values are found in columns X_7 to X_{14} in Table X.

Consumer Panel

A paired t-test was used to determine if there was a significant difference between the untreated and antioxidant-treated sandwich spreads.

Objective Measurements

Statistical treatment of objective measurements included analyses of variance for TBA values, FFA content and refractive indices. Orthogonal contrasts were used to determine significant differences ($P<0.05$) between treatments and between days (Snedecor and Cochran, 1967).

The quantities of fatty acids having three or more double bonds in their structure were analyzed using a split plot analysis of a completely randomized design. Missing values were calculated according to Steel and Torrie (1960).

RESULTS AND DISCUSSION

Organoleptic and chemical data showed that the canned whitefish deteriorated in flavor fairly rapidly after the tins were opened, even though refrigerated storage was imposed. The two antioxidants had some success in limiting or minimizing these changes.

Preliminary Panel

Preliminary work suggested that fish canned without antioxidants had a stronger flavor after open refrigerated storage than fish that had been canned with antioxidants.

Both antioxidant treatments appeared to protect the fresh fish flavor during the 24 hours of refrigerated storage. However, it seemed that AX 1 afforded greater protection during canning than AX 2, as less "off-flavor" was detected in AX 1-treated fish immediately after opening and after only 3 hours of refrigerated storage. This is illustrated in Table XI and Figure 14.

With AX 2, the protective effect against oxidative flavor deterioration was more apparent after 18 and 24 hours storage. Immediately after opening, no differences could be detected by the panelists between the control and fish canned with AX 2. Initially, it is expected that there should be no difference between the control and antioxidant-treated samples unless there was oxidative deterioration during processing. As the time that the fish remains open increases, it is expected that the antioxidant-treated fish would show less "off-flavor" if the

TABLE XI.

PAIRED FLAVOR COMPARISONS FOR
PRELIMINARY 24-HOUR STUDY

Refrigerated Storage Time (Hours) After Opening		Proportions of Panelists Stating Control had more "Off-Flavor" than A X 1	Proportions of Panelists Stating Control had more "Off-Flavor" than AX 2
0	rep 1	7/8*	1/6
	2	7/8*	2/6
	3	4/7	4/7
	Total	18/23*	7/19
3	rep 1	4/8	8/8
	2	8/9*	0/8
	3	6/8	2/6
	Total	18/25*	10/20
6	rep 1	5/8	3/6
	2	4/6	3/6
	3	5/7	3/7
	Total	14/21	9/19
18	rep 1	5/8	7/8*
	2	3/8	5/8
	3	2/6	6/8
	Total	10/22	18/24*
24	rep 1	6/9	7/9
	2	5/8	4/8
	3	4/8	6/7
	Total	15/25	17/24*

* Chi-square significant at $P < 0.05$ (Amerine et al., 1965)

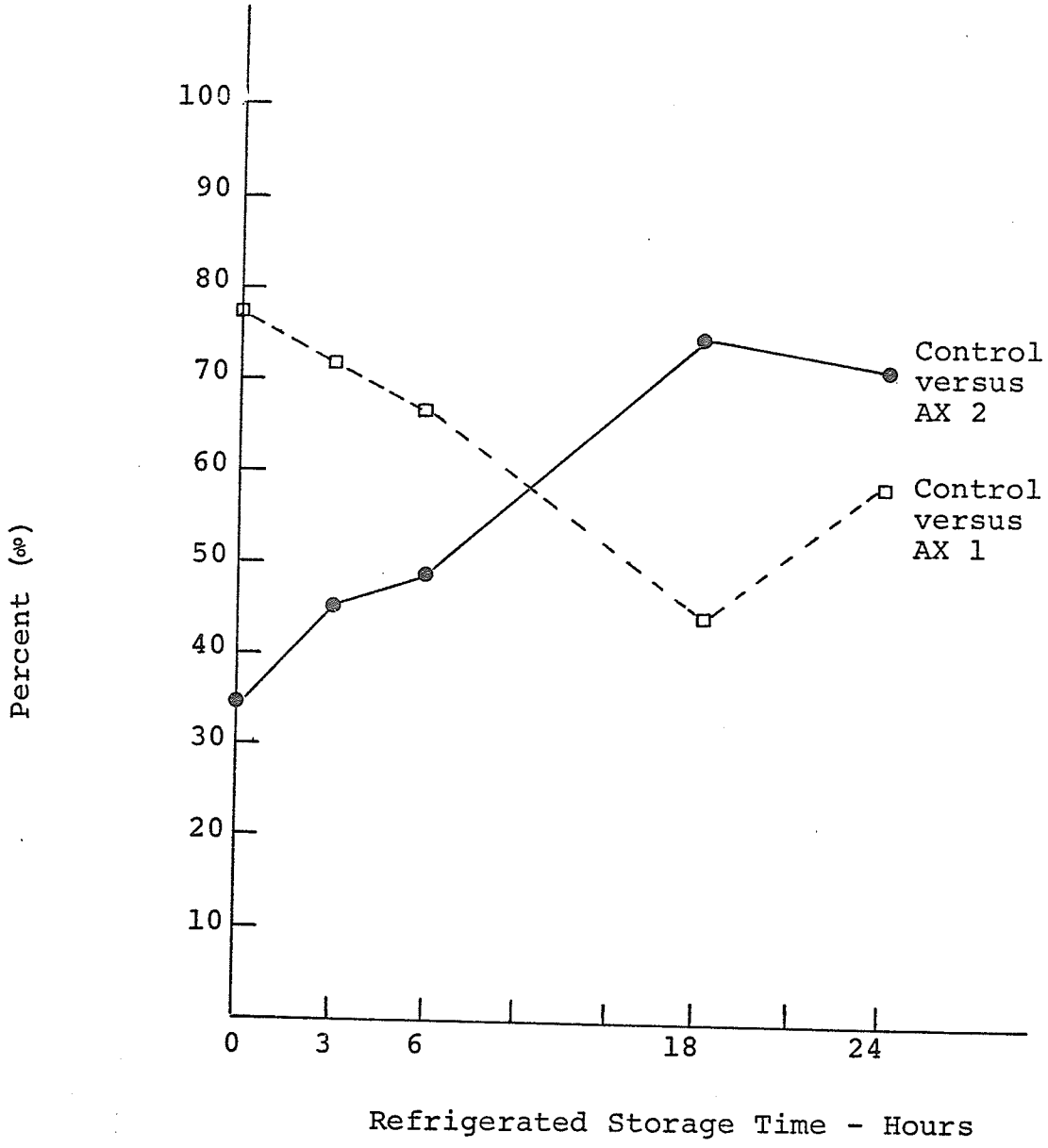

FIGURE 14. Percentage[1] of Paired Comparisons where Control Fish had more "Off-Flavor" than Antioxidant-Treated Fish during 24 Hours' Refrigerated Storage.

[1] Data from Table XI.

antioxidants are protecting against oxidative rancidity.

From these results it was observed that the differences between the control sample and antioxidant-treated fish were marginal, but it was decided to extend the storage time of the fish samples and to train a panel to detect the flavor differences more accurately. The amount of dark flesh varies between cans and Castell and McLean (1964) reported that the darker portions of the fish flesh undergo oxidative deterioration more rapidly than the white flesh. Therefore, it was felt that more reproducible results might be obtained if several tins of fish were mixed and aliquots removed to provide a more representative sample. This would reduce the variation between cans of fish.

Trained Panel

Panelists were minimally trained to detect "off-flavor" in canned whitefish held open at refrigerator temperatures up to 4 days. During the training sessions, only one panelist had difficulty ranking the 4 samples (Table XII). At the second training session, almost half the judges could distinguish between the control and antioxidant-treated sample; while at the third session they could clearly recognize the "off-flavor".

In the actual test, whitefish canned with antioxidants had better flavor over 4 days of open refrigerated storage than fish with no antioxidants. From the F values in the analysis of variance table it can be seen that there was a significant difference between treatments (Table XIII). To determine which of the treatments were different, a t-test was applied to the

TABLE XII

PERFORMANCE OF JUDGES IN TRAINING SESSIONS

		Responses[2] for the Paired Comparisons Examined at the Various Time Intervals			
	Session 1	Session 2		Session 3	
Judge	Inversion Value[1]	0 Hours	24 Hours	4 Hours	24 Hours
1	0	✓	✓	✓	✓
2	0	x	x	✓	✓
3	0	x	x	x	✓
4	1	✓	✓	✓	✓
5	1	x	x	✓	✓
6	1	x	x	✓	✓
7	5	✓	✓	✓	✓
8	-	x	x	✓	✓
Totals	8	3/8	3/8	7/8	8/8

[1] A lower inversion value signifies the panelist is ranking in the expected order. Calculated chi-square was 1.71 while tabulated value was 12.59 at the 5% level with 6 degrees of freedom. Therefore, panelists were ranking in the order expected.

[2] ✓ = panelist chose control as having more "off-flavor" than AX-treated fish

x = panelist chose AX-treated fish as having more "off-flavor" than control

TABLE XIII

ANALYSIS OF VARIANCE FROM MULTIPLE REGRESSION ANALYSIS FOR TRAINED PANELISTS

Source of Variation	df	SS	MS	F
Regression	14	1534		
Treatments	2	1386	693.00	138.47*
"Deviation from Order"	4	30	7.50	1.50
Interaction	8	119	14.88	2.97*
Order	1	18	18.00	3.60
Error	209	1046	5.00	
Total	224	2598		

* Significant at $P < 0.05$

overall mean values for the treatments illustrated in Table XIV. A more positive value is associated with a greater degree of "off-flavor" and a more negative number corresponds to less "off-flavor". From Table XIV it is clear that the control sample exhibited the most "off-flavor" while fish canned with AX 1 had the least "off-flavor".

The first test considered:

$$H_o : \mu_c = \mu_{AX\ 1}$$

$$H_a : \mu_c > \mu_{AX\ 1}$$

A one-tailed test was used since it was expected that the mean score of rancidity would be greater for the control than for fish canned with an antioxidant. The calculated t-value was 15.14 and it was concluded that the mean score for "off-flavor" for the control was significantly greater than that for fish canned with AX 1.

The second hypothesis tested was:

$$H_o : \mu_c = \mu_{AX\ 2}$$

$$H_a : \mu_c > \mu_{AX\ 2}$$

The calculated t-value was 13.27 and therefore it can be said that panelists rated fish with no antioxidant as having a significantly greater degree of "off-flavour" than fish protected with AX 2.

The hypothesis:

$$H_o : \mu_{AX\ 1} = \mu_{AX\ 2}$$

$$H_a : \mu_{AX\ 1} \neq \mu_{AX\ 2}$$

TABLE XIV

VARIANCE AND β-VALUES FOR THE ANTIOXIDANT TREATMENTS AT EACH OF THE REFRIGERATED STORAGE DAYS

Treatment	β-Values						Variance[1]
	Day 0	Day 1	Day 2	Day 3	Day 4	Overall	
Control	0.79	1.82	2.00	2.08	1.67	1.67	0.0069
AX 1	-0.80	-1.31	-1.21	-0.83	-0.83	-1.00	0.0173
AX 2	0.01	-0.51	-0.79	-1.25	-0.84	-0.67	0.0172

[1]Variance used in t-test to determine which of the treatments were different.

Covariance for Control and AX 1 was -0.0035.

was tested and a 2-tailed test was used since there was no prior knowledge that either of the antioxidants were superior. The t-value was 1.32 and was not significant and therefore fish with AX 1 and fish with AX 2 were not significantly different as evaluated by the trained panel.

The regression coefficients for control, AX 1 and AX 2 were calculated for each of the 5 time intervals and are shown in Table XIV. From Figure 15 it is clear that the "off-flavor" scores were always lower for antioxidant-treated fish than for the control.

The "deviation from order" over days was not significant and so overall, the panelists were consistent in the manner in which they were evaluating the sample order.

There was a significant interaction for treatments x "deviation from order". In other words, the difference in responses to the 3 treatments were not of the same magnitude from day to day. Table XIV indicated the individual effects observed for the 3 treatments on each test day. On Day 3 the fish canned with AX 2 was now reported by the panelists to have the least "off-flavor".

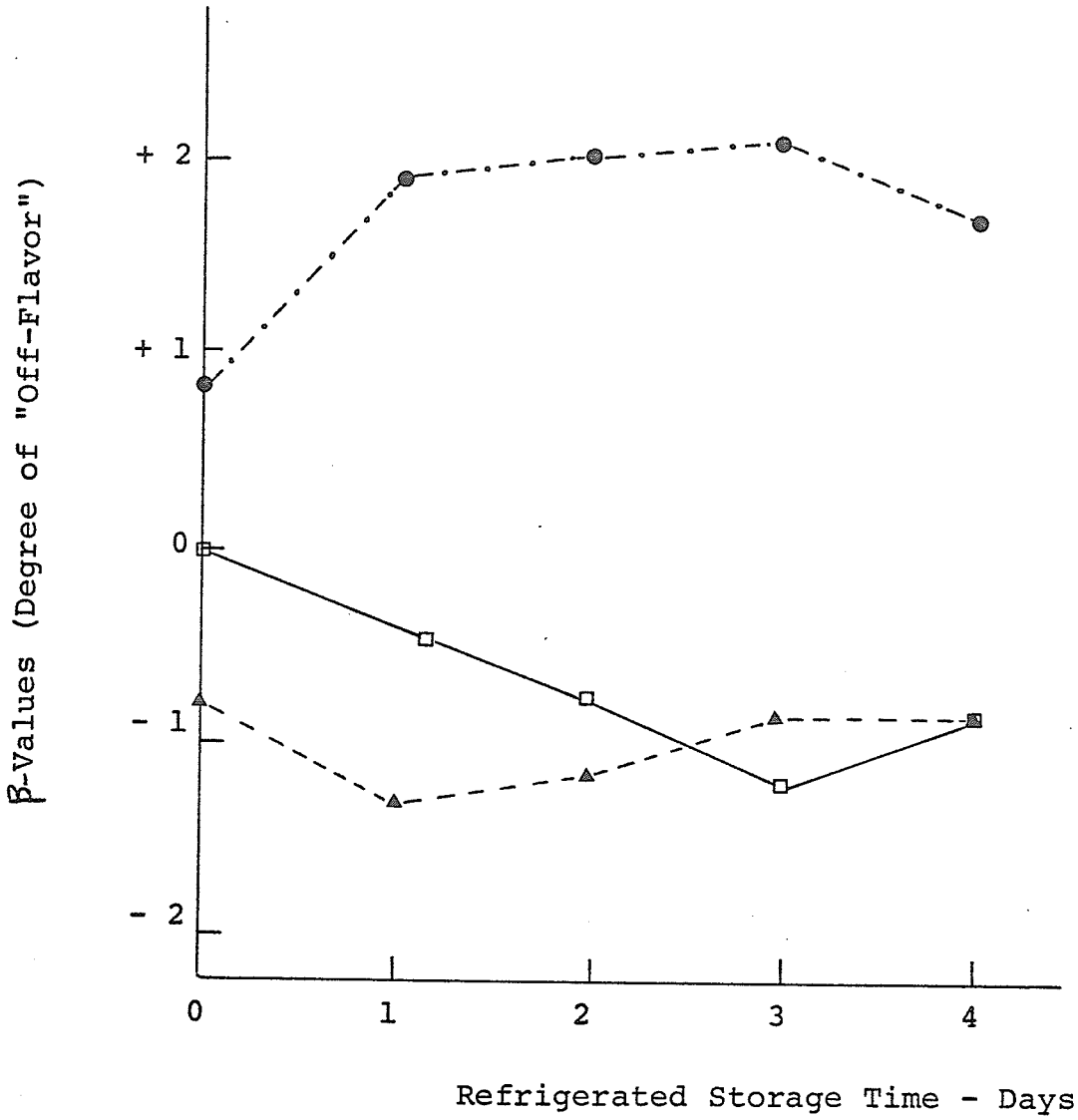

FIGURE 15. β-Values[1] for the Antioxidant Treatments at each of the Refrigerated Storage Days.

Control
AX 1
AX 2

[1] Data from Table XIV.

Consumer Results

The consumer panels found no significant differences among the whitefish sandwiches whether fish was canned with antioxidants or not. It is possible that the salad dressing was masking any flavor differences there may have been.

The mean hedonic scores assigned to the fish sandwiches are summarized in Table XV. The paired t-test was applied to the results of each session and none of the mean scores were statistically different. At Session 1, the untreated fish scored the lowest value; 7.00. It is interesting to note that the antioxidant-treated fish scored almost the same at the first 2 sessions in spite of the fact that the fish spreads tested at the second session had been aged 24 hours prior to preparing the sandwiches. At the third session, the untreated sandwiches scored slightly higher than AX 1-treated fish, although the results were not statistically significant. These results also indicated that the consumers either showed no preference between the 2 sandwiches they examined or that almost equal numbers of panelists showed a preference for both.

When the consumers were further questioned as to why they preferred the antioxidant-treated fish sandwich, some of the replies were:

antioxidant not as fishy, bland, milder (5); control poor flavor (2); and control too fishy (2). On the other hand, of the people who preferred the control to AX 1-treated fish, their comments were:

TABLE XV

MEAN HEDONIC SCORES FOR FISH SANDWICHES AND SUMMARY OF PREFERENCES OF CONSUMER PANELISTS.

	Session 1*		Session 2*		Session 3*	
	Control	AX 1	AX 1	AX 2	Control	AX 1
Mean Score[1]	7.00	7.71	7.75	7.67	7.72	7.51
No. Judgements	21	18	51	51	43	43
Preference	-	-	12	12	19	14
Variance for t-test	3.48		1.07		2.09	

[1] 9 = like extremely
1 = dislike extremely

*Session 1 = sandwich spreads prepared on the morning of the test day.

Sessions 2 and 3 = sandwich spreads prepared 24 hours prior to making sandwiches on the morning of the test day.

control tastes more like fish, more flavor, tastier (6); antioxidant-treated fish is tasteless, bland, flat (5). It appears that some of the consumers preferred a "fishier" or stronger tasting fish product.

The canned whitefish was highly accepted by the consumers; for instance, at Session 2, the fish treated with AX 1 received a mean score of 7.75 which corresponds to "like very much" on the 9-point hedonic scale, while the lowest mean score obtained for the 3 test days was 7.00. A study by Eindhoven and Peryam (1959) on preferences of food combinations reported a rating of 5.88 for fried fish and a value of 5.79 for salmon cakes which were evaluated on a 9-point hedonic scale. Shaykewich (1971) reported flavor hedonic scores for canned whitefish ranging from 5.00 to 6.75 as evaluated by 60 consumer panelists in a laboratory setting. Table XVI shows that the majority of the consumers felt that the whitefish product was "about as nice" as either the canned tuna or salmon that they used. The major criticisms from salmon users concerned the color - they felt that they were "more used to salmon", "red sockeye has richer flavor" and the whitefish "color is not as nice for sandwiches".

The consumers were also questioned as to the price they would be willing to pay for the canned whitefish. The results are summarized in Table XVII. The majority said they would be willing to pay .50¢ - .70¢ because this is the price range for tuna and salmon.

It seems that the canned whitefish prepared as sandwiches is highly acceptable and that flavor deterioration was not

TABLE XVI

COMPARISON OF THE FLAVOR OF CANNED WHITEFISH TO CANNED TUNA AND CANNED SALMON AS REPORTED BY THE CONSUMER PANEL

Compared to Canned Tuna or Salmon do you think Whitefish tastes:	Session 1		Session 2		Session 3	
	Tuna	Salmon	Tuna	Salmon	Tuna	Salmon
Much nicer	2	0	2	1	0	5
A little nicer	0	0	5	2	5	4
About as nice	5	8	7	19	5	11
Not quite as nice	2	3	4	8	2	9
Definitely not as nice	0	1	2	1	0	1

TABLE XVII

PRICE RANGES AT WHICH CONSUMERS[1] WOULD BE WILLING TO BUY CANNED WHITEFISH

Price	Tuna Users	Salmon Users
$1.25 - 1.00	0	0
$1.00 - .80	1	1
.70 - .50	3	9
.50 - .30	5	2

[1]Consumers interviewed at Session 1.

obvious in fish blended with salad dressing.

TBA Test

Of the objective measurements carried out, the TBA values showed the best relationship to the sensory assessments of flavor deterioration in the canned whitefish.

The results from the TBA test are summarized in Figure 16. A higher TBA value is indicative of a greater degree of oxidative deterioration. Analysis of variance of the data showed significant differences between treatments and between ageing periods (Table XVIII).

The sums of squares for treatments were partitioned into 5 orthogonal contrasts. The first contrast looked at the effect of the addition of antioxidants on the TBA number. The calculated F value was highly significant and from Figure 16, it is clear that there is a great difference in oxidative deterioration between the control and the 2 antioxidant treatments. The antioxidants are definitely providing protection against oxidative rancidity. Sinnhuber and Yu (1958) and Zipser and Watts (1961) also demonstrated dramatic differences in TBA values between those fish samples with added antioxidants compared to those without antioxidants.

The second comparison investigated the effectiveness of antioxidant mixture AX 1 on the TBA value as opposed to antioxidant mixture AX 2. There was a significant difference between the 2 antioxidant mixtures and it was concluded that AX 1 and AX 2 had different effects on whitefish oxidation. It can be seen in Figure 16 that the TBA values for whitefish

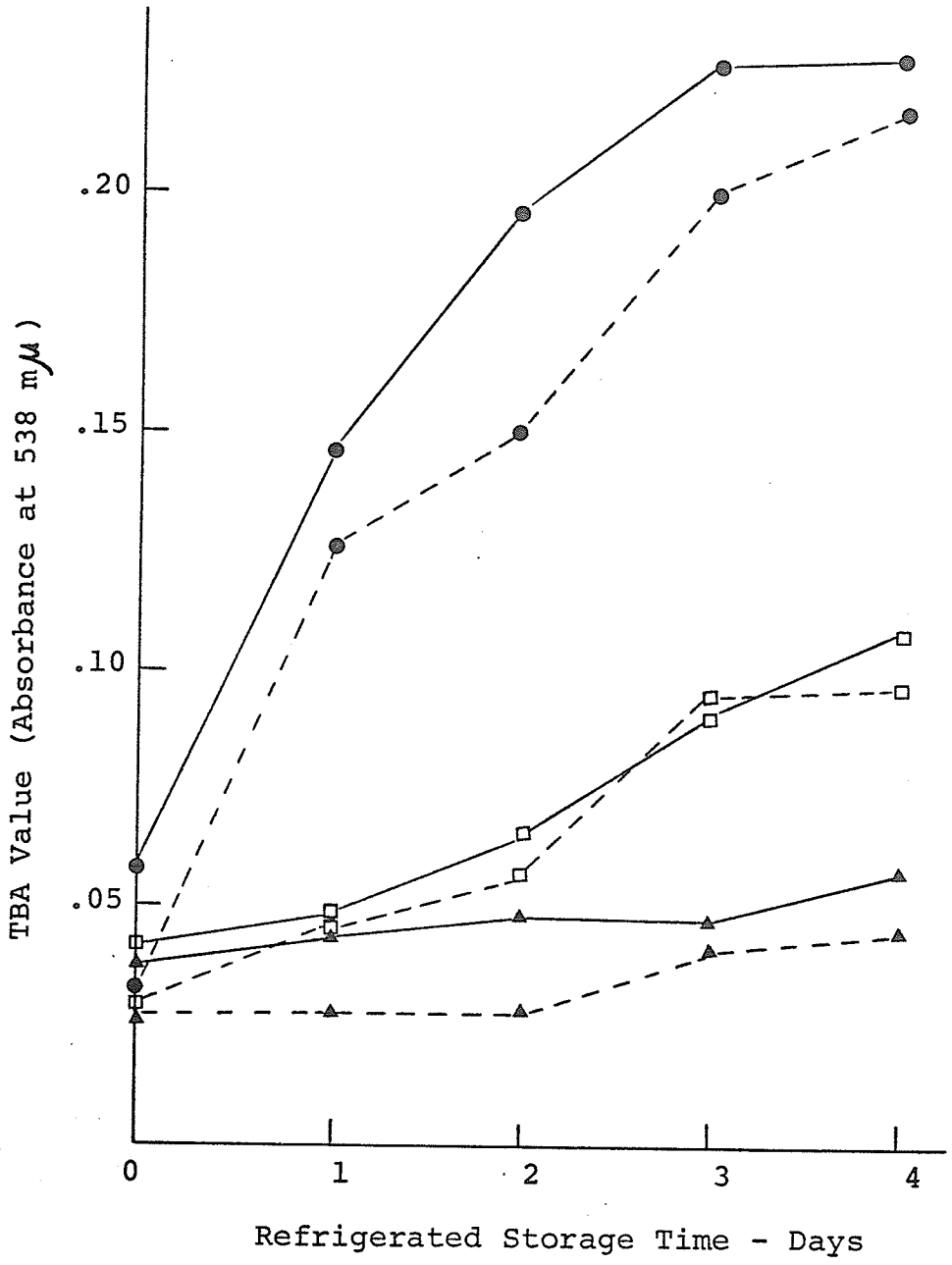

FIGURE 16. Mean TBA Values for the 3 Treatments of Stored Whitefish Homogenized with and without PG and EDTA.

Control — Control plus PG and EDTA
AX 1 — AX 1 plus PG and EDTA
AX 2 — AX 2 plus PG and EDTA

TABLE XVIII

ANALYSIS OF VARIANCE FOR TBA VALUES

Source of Variation	df	SS	MS	F
Treatments[1]	5	0.636702	0.127340	115.55*
L_1	1	0.589471	0.589471	534.91*
L_2	1	0.029735	0.029735	26.98*
L_3	1	0.013485	0.013485	13.24*
L_4	1	0.003870	0.003870	3.51
L_5	1	0.000146	0.000146	0.13
Bowls within Treatments	18	0.019838	0.001102	
Days	4	0.243531	0.060883	41.79*
Linear	1	0.234083	0.234083	125.23*
Quadratic	1	0.007641	0.007641	4.08*
Cubic	1	0.000001	0.000001	0.001
Quartic	1	0.001805	0.001805	0.96
Days x Treatments	20	0.029132	0.001457	0.78
Duplicates	120	0.023525	0.000196	
Experimental Error	72	0.135031	0.001875	
Total	239	1.087759		

* Significant at $P < 0.05$.

[1] L_1 Control vs. Antioxidants.

L_2 AX 1 vs. AX 2.

L_3 PG and EDTA vs. no PG and EDTA.

L_4 Control with and without PG and EDTA vs. Antioxidants with and without PG and EDTA.

L_5 AX 1 with and without PG and EDTA vs. AX 2 with and without PG and EDTA.

canned with AX 2 are higher than the TBA values for fish canned with AX 1. It can therefore be concluded that under the conditions of this storage study, AX 1 is more effective in controlling oxidative rancidity than AX 2.

The third comparison looked at the effect of adding PG and EDTA prior to homogenizing the fish samples. The calculated F value was significant and it was concluded that it does make a difference whether or not PG and EDTA are present when performing the test. It is evident from Figure 16 that the TBA values were lower over the 4 days of refrigerated storage for the fish samples treated with PG and EDTA prior to homogenization. The higher TBA values associated with the samples without PG and EDTA indicate that oxidative deterioration is taking place during homogenizing. These results are in agreement with those of Vyncke (1970) who reported that the initial values of the extracts without PG and EDTA were higher although the average increase in absorbance was practically the same for both extracts.

The fourth comparison investigated the difference between the control with and without the added PG and EDTA and the 2 antioxidant treatments with and without these substances. The differences were not significant. The final contrast compared the effect of AX 1 versus AX 1 with PG and EDTA and AX 2 versus AX 2 with PG and EDTA and the calculated F value was not significant. These results indicate that the presence of PG and EDTA during homogenization did not significantly affect the samples with added antioxidants because the anti-

oxidants are probably providing protection against further oxidative deterioration during the test.

TBA values for the different treatments increased as refrigerated storage time extended. A significant difference was observed for the effect of days and therefore, the sums of squares for days were sub-divided into linear, quadratic, cubic and quartic components (Table XVIII). The linear component was significant and it was concluded that the TBA values increased in a linear fashion over the 4 day storage period (Figure 16). The quadratic component was also significant, and while the relationship between TBA values and length of storage was almost entirely linear, on the fourth day of storage, the TBA values for the control (homogenized without PG and EDTA) and for fish canned with AX 2 (homogenized with PG and EDTA) appeared to have reached a plateau and began to level off.

Fatty Acid Analysis

In the course of oxidative deterioration, poly-unsaturated fatty acids are broken down into the various breakdown products such as aldehydes, ketones, hydrocarbons and expoxides (Labuza, 1971). A preliminary examination was made of the fatty acid composition of whitefish canned with and without antioxidants and subsequently held open under refrigerated storage up to 4 days.

Analysis of variance was performed on the quantities of fatty acids containing 3 or more double bonds in their structure. Only C22:6(ω3) (docosahexanoic acid) showed a significant difference between treatments (Table XIX). The control sample had an average of 9.04% of C22:6(ω3) initially and

TABLE XIX

ANALYSIS OF VARIANCE FOR THE PERCENTAGES OF C22:6(ω 3) IN WHITEFISH

Source of Variation	df	SS	MS	F
Treatments	2	28.97	14.49	7.69*
Error (a)	6	11.31	1.88	
Days	1	13.01	13.01	2.56
Days x Treatments	2	32.26	16.13	3.22
Error (b)	6	30.06	5.01	
Total	17	115.61		

* Significant at $P < 0.05$

after 4 days of refrigerated storage, only 4.24% C22:6(ω3). The percentage of C22:6(ω3) for the 2 antioxidant treatments remained reasonably constant over the 4 day storage period (Table XX). These results, although somewhat preliminary, showed that the method of analysis was not sensitive enough to determine changes in the other poly-unsaturated fatty acids. However, the fatty acid composition of the canned freshwater whitefish was determined (Table XXI)and it was found to compare favorably with the composition of fresh whitefish as reported by Gruger et al. (1964).

FFA Content

Hydrolytic rancidity is indicated by an increase in FFA content but did not occur in the canned whitefish stored open at refrigerator temperatures up to 4 days. Table XXII summarizes the results from the FFA analysis. Analysis of variance was carried out and no significant differences were apparent between the 3 treatments (Table XXIII). However, there was a significant difference for the effect of days and so the sums of squares for days were sub-divided into linear and quadratic components. The calculated F value for the linear component was not significant, while the quadratic component had a calculated F value of 4.43 and was significant.

It can be seen from Table XXII that the control sample showed little change in FFA content over the 4 day storage period. Fish canned with AX 1 showed a slight increase in FFA content after 2 days and then decreased on the fourth day of storage. Fish that had been canned with AX 2 contained

TABLE XX

PERCENT C22:6(ω3) IN THE 3 TREATMENTS OF STORED WHITEFISH

Open Refrigerated Storage Time (Days)	Control	AX 1	AX 2
0	9.04	10.54	8.23
4	4.24	8.50	9.97

TABLE XXI

COMPARISON OF THE PERCENT FATTY ACID COMPOSITION OF CANNED WHITEFISH (CONTROL) USED IN THIS STUDY WITH THAT REPORTED FOR RAW LAKE WHITEFISH BY GRUGER *et al.*, (1964).

Carbon No. and Double Bonds	Canned Whitefish (Control)	Raw Whitefish (Gruger *et al.* 1964)
C14:0	1.8	2.2
C14:1	0.8	-
C15:0	0.9	0.3
C15:1	-	0.3
C16 iso	0.5	-
C16:0	16.0	12.1
C16:1 (ω 6)	12.8	10.5
C16:2	1.9	1.2
C17:0	1.5	1.1
C18:0	5.1	4.0
C18:1 (ω 9)	29.9	27.2
C18:2 (ω 6)	3.0	5.5
C18:3 (ω 6)	1.0	3.7
C18:3 (ω 3)	1.0	-
C18:4	-	1.0
C20:1	3.0	2.1
C20:2	-	0.8
C20:3	-	0.6
C20:4 (ω 9)	5.4	3.9
C20:5 (ω 3)	5.2	6.4
C22:1	-	1.1
C22:4	-	1.1
C22:5 (ω 3)	0.9	3.3
C22:6 (ω 3)	9.0	8.8
C24:1	-	1.2

TABLE XXII

MEAN FREE FATTY ACID CONTENT[1] (mg/100g flesh)
FOR STORED WHITEFISH

Open Refrigerated Storage Time (Days)	Control	AX 1	AX 2
0	87.05	89.69	97.75
2	86.01	92.73	106.83
4	83.48	85.66	86.45

[1] each value is the mean of 12 individual results.

TABLE XXIII

ANALYSIS OF VARIANCE FOR FREE FATTY ACID CONTENT OF THE STORED WHITEFISH

Source of Variation	df	SS	MS	F
Treatments	2	2736.4	1368.2	3.38
Bowls within Treatments	15	6073.2	404.9	
Days	2	2053.8	1026.9	3.5*
Linear	1	754.1	754.1	2.57
Quadratic	1	1299.7	1299.7	4.43*
Days x Treatments	4	1428.1	357.03	1.22
Duplicates	54	1907.4	35.32	0.12
Experimental Error	30	8801.9	293.40	
Total	107	23000.8		

* Significant at $P < 0.05$

significantly more free fatty acids initially than the other 2 treatments, but showed the same pattern over storage as fish with AX 1. The FFA data suggests that some hydrolytic rancidity may have taken place during the canning process, which may account for the slightly higher FFA content of fish canned with AX 2.

The method used for the determination of FFA is subject to error, since when titrating with base, it is impossible to distinguish between fatty acids and other acidic impurities (Sherwin, 1968). Hydrolytic rancidity as observed in stored frozen fish did not occur in these processed samples and it can be concluded that the enzymes responsible for hydrolytic rancidity were completely inactivated during the processing of the canned whitefish product.

Refractive Index

Refractive indices of whitefish lipids showed no pattern that related to the sensory observations and no difference was observed between the 3 treatments stored up to 4 days at refrigerator temperatures.

The results are summarized in Table XXIV. The analysis of variance that was carried out (Table XXV) indicated no significant differences between treatments or between days. Arya et al. (1969) from their work with edible oils reported an increase of the order of $0.001 \pm 0.0003 (n_D^{25})$ at the stage of development of perceptible rancid odor. They concluded that the refractive index curve indicated the end of the induction period of oxidation more precisely than the peroxide value

TABLE XXIV

MEAN REFRACTIVE INDICES[1] (n_D^{25}) FOR LIPIDS FROM STORED WHITEFISH

Open Refrigerated Storage Time (Days)	Control	AX 1	AX 2
0	1.4780	1.4787	1.4778
2	1.4778	1.4771	1.4772
4	1.4773	1.4781	1.4773

[1] each value is the mean of 6 individual results.

TABLE XXV

ANALYSIS OF VARIANCE FOR REFRACTIVE INDICES

Source of Variation	df	SS	MS	F
Treatments	2	0.00000301	0.00000150	0.09
Bowls within Treatments	15	0.00024989	0.00001665	
Days	2	0.00000639	0.00000319	1.02
Days x Treatments	4	0.00000410	0.00000102	0.33
Experimental Error	30	0.00009385	0.00000312	
Total	53	0.00035724		

curve and the refractive index was subject to less error than the peroxide value. As can be seen from Table XXIV, no clear-cut pattern was evident for any of the 3 treatments. The results appear somewhat erratic; decreasing after 4 days of refrigerated storage rather than increasing. However, it should be pointed out that when Arya and co-workers performed their experiments, the oils were heated at 100± 5°C. for lengths of time ranging from 4 to 268 hours. Therefore, it was concluded that the method used was not sensitive enough to detect differences in oxidative rancidity under the conditions of this experiment.

IMPLICATIONS

These investigations showed that the flavor of canned whitefish treated with antioxidants was better after open refrigerated storage than fish that had no added antioxidants. The present concern by the consumer for food products of high nutritional value and free from toxicants, imposes greater responsibility on the food industry to meet these demands. The use of antioxidants in this study prevented the production of intermediates related to off-flavor development. It is possible that some of these intermediates may have toxic properties or interfere with the nutritional value of the protein (Labuza, 1971; Osner and Johnson, 1968). While flavor changes may not be evident in some food products, as was observed in the case of the whitefish sandwiches evaluated by the consumer groups in this study, the addition of antioxidants does ensure the maintenance of superior food products which will meet consumer standards.

SUMMARY AND CONCLUSIONS

The flavor of freshwater whitefish was evaluated using organoleptic, chemical and physical measurements. Evaluation of oxidative rancidity using the TBA method and trained panels showed the control sample to be associated with the greatest degree of oxidative deterioration during the 4 days of open refrigerated storage. While AX 2 did provide protection against "off-flavor" development, AX 1 appeared to afford better protection. In general, the degree of "off-flavor" reported by the panelists increased on storage with the TBA values increasing linearly over the 4 day refrigerated storage period.

No significant differences were evident for the amount of free fatty acids (FFA) produced among the 3 treatments after 4 days of open refrigerated storage. This indicated the inactivation of the enzymes responsible for hydrolytic rancidity during the processing of the canned whitefish.

Gas chromatographic analysis of the extracted whitefish lipid was carried out to determine the quantities of the unsaturated fatty acids containing 3 or more double bonds. Analysis of variance indicated a significant difference ($P < 0.05$) for C22:6 ($\omega 3$) between treatments. Since these results were only preliminary, further investigation is required to confirm this observation.

Refractive index measurements did not show any significant

differences ($P < 0.05$) among the 3 treatments or for the effect of days. It appeared evident that this method was not sufficiently sensitive to detect the oxidative changes occurring.

Consumer studies were carried out to evaluate whitefish sandwiches prepared with salad dressing. Three independent sessions comprising 21 to 51 homemakers were set up to evaluate pairs of treated and untreated whitefish sandwiches which were rated on a 9-point hedonic scale. No significant flavor differences ($P < 0.05$) were apparent between pairs of sandwiches which were scored over a range of 7.00 to 7.75.

It can therefore be concluded that the 2 antioxidants used in this study provided protection against oxidative deterioration. The TBA values and sensory results suggest that these 2 antioxidants differed in the degree of protection they provided.

APPENDIX

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APPENDIX 1

EXAMPLE OF QUESTIONNAIRE USED BY CONSUMER PANEL AT SESSION 1

FISH SANDWICH TEST

1. Do you ever buy canned tuna? YES ( ) NO ( )
   canned salmon? YES ( ) NO ( )

(IF BUY NEITHER, TERMINATE)

2. Do you ever make sandwiches with
   canned tuna? YES ( ) NO ( )
   canned salmon? YES ( ) NO ( )

(IF NO TO BOTH, TERMINATE - IF YES TO EITHER, DIRECT TO DESK)

DECIDE IF SUBJECT IS A TUNA OR SALMON USER - TUNA ( )
SALMON ( )

3. About how often do you make sandwiches as a rule:

FROM CANNED TUNA		or	FROM CANNED SALMON	
Every week	( )		Every week	( )
Twice a month	( )		Twice a month	( )
Once a month	( )		Once a month	( )
Once in a while	( )		Once in a while	( )

4. When you make sandwiches are they usually for:

FROM TUNA		or	FROM SALMON	
Self	( )		Self	( )
Husband	( )		Husband	( )
Children	( )		Children	( )
Some combination	( )		Some combination	( )

5. Today we have samples of sandwiches made with canned whitefish which is a product not sold in the supermarkets yet. Would you taste and tell us how much you like them?

TASTE FISH

(INTERVIEWER: GIVE X, WATER, THEN O. ALTERNATE SAMPLE SERVING ORDER. IDENTIFY EACH V-SHEET IMMEDIATELY AFTER TASTING).

6. (a) What do you like most about this whitefish?

   (b) Why do you say that? (PROBE)

7. (a) What do you like least about this whitefish?

   (b) Why do you say that? (PROBE)

8. (a) Compared to canned tuna or salmon (CIRCLE WHICH FISH) do you think the whitefish tastes:

   Much Nicer ( )
   A Little Nicer ( )
   About as Nice ( )
   Not quite as Nice ( )
   Definitely not as Nice ( )

   (b) Why is that? (PROBE)

9. (a) At about what price range do you feel you would buy this canned whitefish?

   $1.25 - $1.00
   1.00 - .80
   .70 - .50
   .50 - .30

   (b) Why is that? (PROBE)

10. (a) Just looking at this package, what impression do you get of the kind of product which might be in it? What about the quality: Would it be:

    Very High Quality ( )
    Fairly High Quality ( )
    All Right ( )
    Rather Poor ( )
    Very Poor ( )

    (b) Why do you say that? (PROBE)

APPENDIX 2

EXAMPLE OF QUESTIONNAIRE USED BY CONSUMER PANEL AT SESSIONS 2 and 3

FISH SANDWICH TEST

1. Do you ever buy canned tuna? YES ( ) NO ( )
   canned salmon? YES ( ) NO ( )

(IF BUY NEITHER, TERMINATE)

2. Do you ever make sandwiches with
   canned tuna? YES ( ) NO ( )
   canned salmon? YES ( ) NO ( )

(IF NO TO BOTH, TERMINATE - IF YES TO EITHER, DIRECT TO DESK).

DECIDE IF SUBJECT IS A TUNA OR SALMON USER - TUNA ( )
SALMON ( )

3. About how often do you make sandwiches as a rule:

FROM CANNED TUNA		or	FROM CANNED SALMON	
Every week	( )		Every week	( )
Twice a month	( )		Twice a month	( )
Once a month	( )		Once a month	( )
Once in a while	( )		Once in a while	( )

4. When you make sandwiches are they usually for:

FROM TUNA		or	FROM SALMON	
Self	( )		Self	( )
Husband	( )		Husband	( )
Children	( )		Children	( )
Some combination	( )		Some combination	( )

5. Today we have samples of sandwiches made with canned white-fish which is a product not sold in the supermarkets yet. Would you taste these and tell us how much you like them.

TASTE FISH

(INTERVIEWER: GIVE X, WATER, THEN O. ALTERNATE SAMPLE SERVING ORDER. IDENTIFY EACH V-SHEET IMMEDIATELY AFTER TASTING).

If you found the two sandwiches different, why do you like _____ better than _____? (PROBE)

6. (a) Compared to canned tuna or salmon (CIRCLE WHICH FISH) do you think the whitefish tastes:

Much Nicer	( )
A Little Nicer	( )
About as Nice	( )
Not quite as Nice	( )
Definitely not as Nice	( )

(b) Why is that? (PROBE)

APPENDIX 3

SPECIFIC RESULTS FROM CONSUMER PANELS

Session 1

Total number of housewives interviewed	21
Number of tuna users	9
Number of salmon users	12

Survey Questions

"About how often do you make sandwiches as a rule?"

	Tuna Users	Salmon Users
Every week	5	5
Twice a month	1	4
Once a month	2	1
Once in a while	1	2
	9	12

"When you make sandwiches are they usually for:"

	Tuna Users	Salmon Users
Self	2	2
Husband	0	0
Children	1	1
Some combination	6	8
	9	11

"What do you like most about this whitefish?"

- flavor; satisfying; palatable	7
- not as strong a flavor	5
- children would prefer it to salmon or tuna	3
- tastes like tuna	2
- tastes like salmon	2
- like all freshwater fish	2
- tastes like crab	1
- smoothness	1
- easily digested	1
- good for dieting	1
- not afraid to serve to company	1
- drier; doesn't soak to bread as much, therefore sandwiches would stay fresher longer	1

"What do you like least about this whitefish?"

- nothing	11
- lacking in taste	2
- salmon more smoked taste	1
- not salty enough	1
- funny taste	1
- don't like bones	1
- could be enhanced by celery	1

"Compared to canned tuna or salmon do you think the Whitefish tastes:"

	Tuna Users	Salmon Users
Much nicer	2	0
A little nicer	0	0
About as nice	5	8
Not quite as nice	2	3
Definitely not as nice	0	1
	0	12

"Why do you think whitefish tastes much nicer than:"

Tuna		Salmon
- not as strong	1	—
- like freshwater fish	1	
- like the white color	1	

"Why do you think Whitefish tastes a little nicer than:"

Tuna	Salmon
—	—

"Why do you think Whitefish tastes about as nice as:"

Tuna		Salmon	
- almost thought it was tuna	3	- enjoy both equally	3
- tuna not as fishy/ more delicate	2	- similar to salmon	2
- depends on the brand	1	- white color richer	1
- she's no gourmet	1	- not as fishy/strong as salmon	1
		- like fish any way	1
		- quite a nice taste	1

"Why do you think Whitefish tastes not quite as nice as:"

Tuna		Salmon	
- flavor not quite as full	2	- less distinctive flavor	3
- tuna drier	1	- color not as nice for sandwiches	1
- not as flaky	1		

"Why do you think Whitefish tastes definitely not as nice as:"

Tuna	Salmon	
-	- salmon has a more definite smoked flavor	1

"At about what price range do you feel you would buy this Whitefish?"

	Tuna Users	Salmon Users
$1.25 - $1.00	0	0
$1.00 - .80	1	1
.70 - .50	3	9
.50 - .30	5	2
	9	12

"Why would you pay $1.00 - .80¢?"

Tuna Users		Salmon Users	
- really like whitefish	1	- you pay for what you get	1

"Why would you pay 70¢ - 50¢?"

Tuna Users		Salmon Users	
- not too oily	1	- salmon, tuna & crab sell at that price range	3
- about like tuna & pay the same for tuna	1	- looks like white salmon	1

"Why would you pay 50¢ to 30¢?"

Tuna Users		Salmon Users	
- on a pension	1	- should be less than salmon	1
- too expensive & doesn't go far enough	1	- don't pay more than I have to	1
- whitefish doesn't cost as much	1		

The women were then shown an unopened tin of Artic Whitefish (7 3/4 oz) and asked: "Just looking at this package, what impression do you get of the kind of product which might be in it? What about the quality; would it be:"

very high quality	6
fairly high quality	9
alright	4
rather poor	0
very poor	1
	20

"Why do you think it would be very high"?

- way portrayed/recipe/pattie shells/appetizing 6
- type of paper 1
- both languages 1
- trademark should mean something or wouldn't be on the label 1
- Home Ec wouldn't advertise low quality product 1

"Why do you think it would be fairly high?"

- nice appearance/picture/pattie shells/suggests gourmet food 4
- had good impression of sandwich 2
- recipe looks good/moist/whipped 1
- Canadian product 1
- word "Arctic" - healthy - no contamination 1

"Why do you think it would be alright?"

- mediocre - no color 1
- doesn't say fancy quality - label lacks information 1
- impressive - the way it can be served 1
- background of tin too dark 1

"Why do you think it would be very poor?"

- not a bright-looking tin 1
- looks larger than salmon tin 1

Session 2

Total number of housewives interviewed	51
Number of salmon users	30
Number of tuna users	21

The judges were then asked:

"If you found the two sandwiches different, why do you like _____ better than _____?"

"Why do you like AX 2 better than AX 1?"

AX 1 is:	too strong/fishy	4
	not fishy enough	3
	too dry	1
	too tinny	1
	too salty	1
AX 2 is:	stronger/more flavor	3
	more tasty	2
	not as salty	1
	not too fishy	1

"Why do you like AX 1 better than AX 2?"

AX 1 is:	more tarter/more flavor	5
	milder in taste	1
	more salty	1
AX 2 is:	musher, softer, too watery	3
	stronger/fishier	1
	more flat/weaker	1

"Compared to canned tuna or salmon do you think whitefish tastes:"

	Tuna Users	Salmon Users
Much nicer	2	1
A little nicer	5	2
About as nice	7	19
Not quite as nice	4	8
Definitely not as nice	2	1
	20	31

"Why do you think whitefish is about as nice as:"

Tuna		Salmon	
- much the same flavor	2	- different flavor	6
- lighter/blander than tuna	2	- tastes a lot like salmon	4
- tuna fishier	1	- very pleasant	2
- prepared so good	1	- like both equally	2
- tasty	1	- not fishy	
		- tastes just as good	1
		- nice for a change	1

"Why do you think whitefish is not quite as nice as:"

Tuna		Salmon	
- whitefish too fishy	1	- salmon has more flavor	5
- whitefish tastier, more flavorful	1	- whitefish more like tuna	2
- more used to tuna	1	- whitefish bland	2
- not much difference	1	- more used to salmon	1
		- color	1
		- don't like appearance of whitefish as much as salmon	1

"Why do you think whitefish is definitely not as nice as:"

Tuna		Salmon	
- whitefish fishier	1	- red sockeye has richer flavor	1
- tuna has more taste than whitefish	1	- not too fussy on whitefish	1

Session 3

Total number of housewives interviewed	43
Number of tuna users	12
Number of salmon users	31

Survey Questions

"About how often do you make sandwiches as a rule?"

	Tuna Users	Salmon Users
Every week	9	19
Twice a month	2	4
Once a month	1	4
Once in a while	0	4
	12	31

"When you make sandwiches are they usually for:"

	Tuna Users	Salmon Users
Self	2	5
Husband	1	3
Children	0	2
Some combination	9	21
	12	31

The judges were then asked:

"If you found the two sandwiches different, why do you like _____ better than _____?"

"Why do you like Control better than AX 1?"

- control tastes more like fish; more flavor, tastier 6
- AX 1 tasteless; bland; flat 5
- Control not as fishy 1
- Control smoother 1
- AX is more fishy 1

Why do you like AX 1 better than Control?"

- AX tastier 5
- AX not as fishy; bland; milder 5
- Control poor flavor 2
- Control too fishy 2
- prefer stronger flavor of AX 1

"Compared to canned tuna or salmon do you think whitefish tastes:"

	Tuna Users	Salmon Users
Much nicer	0	5
A little nicer	5	4
About as nice	5	11
Not quite as nice	2	9
Definitely not as nice	0	1
	12	30

"Why do you think whitefish is much nicer than:"

Tuna	Salmon	
—	- smoother tasting; creamier, not as dry	2
	- not as fishy; milder	2
	- more like fresh fish, hope it is soon on the market	1

"Why do you think whitefish is a little nicer than:"

Tuna		Salmon	
- Whitefish not too fishy; milder	3	- bland flavor; not as fishy or as strong	4
- creamier; tuna a little drier	2	- smoother	1
- tastes different; better	1		

"Why do you think Whitefish is about as nice as:"

Tuna		Salmon	
- very similar to salmon or tuna; comparable	2	- blander; not as strong as tuna or salmon	4
- smooth	1	- similar to salmon; as tasty	3
- not as dry as tuna	1	- smoother	2
- like it as much as any other canned fish		- not as oily	1
	1	- salmon stronger	1
		- nice flavor; family would like it.	1

"Why do you think Whitefish is not quite as nice as:"

Tuna		Salmon	
- more bland	1	- like salmon better; better flavor; like salty flavor	
- texture smoother than usual	1	of salmon	5
		- too bland, not as tasty	3
		- appearance - colorless	1
		- if great difference in price would buy whitefish	1

"Why do you think Whitefish is definitely not as nice as:"

Tuna	Salmon	
—	- different taste than salmon	1