AN ACCELERATED OXIDATION METHOD FOR THE ESTIMATION OF THE STORAGE LIFE OF FROZEN SEAFOODS

by

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DEDICATION

This thesis is dedicated to the memory of my father,

Mr. Oral Edwin Palmateer

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INTRODUCTION

The highly competative nature of todays food industry coupled with the discriminating tastes of the consumer emphasize that only those products maintaining strict adherence to high quality standards will receive favorable acceptance. It is to this end that much research in food science and technology is dedicated.

Deteriorative changes in quality resulting from bacterial, chemical, physical and enzymatic action may begin at the time of harvest or start at some point during handling, processing or storage. This change of quality may seem insignificant at the time, but unless corrective measures are taken the ultimate result is spoilage and economic loss. Many treatments are employed to prolong the period of product acceptability by inhibiting these deteriorative changes. These treatments may include blanching, smoking, salting, freezing, packaging, dehydration and irradiation to name but a few. The variable composition of food products often requires the application of several treatments to achieve an extended shelf life. It must be remembered, however, that those treatments only offer a postponement of eventual unacceptability.

Although great advances have been made in the frozen seafood industry since the first fish was commercially frozen in 1865, rancidity is still one of the major problems. In its broadest sense, rancidity may be defined as the development of disagreeable odors or flavors in

fats and oils and the foods containing them. Lea (38, p. 200) lists five causes for rancidity of fats and oils: (1) absorption of odors, (2) action of tissue lipase, (3) action of micro-organisms, (4) atmospheric oxidation, and (5) "reversion" and "fishiness". The most important form of rancidity occuring in frozen seafoods is that produced by the action of the oxygen of the air on fats and oils. Atmospheric oxidation (autoxidation) occurs spontaneously when any material containing unsaturated fat is exposed to air, though the rate of change varies enormously with the type of fat and the conditions of storage (38, p. 206). This reaction, also known as oxidative rancidity, is characterized by the formation of hydroperoxides. The decomposition of these hydroperoxides yield carbonyls which are the compounds responsible for the off-flavors and odors that taste panels classify as "painty", "fishy", or rancid.

Chemical, as distinct from biological, spoilage of foods is mainly due to two types of reactions: oxidative rancidity of fats and oils and the so-called "browning" reaction. There are, however, many causes of chemical deterioration which lead to degrees of deterioration which scarcely qualify as spoilage, since the result is a small loss in palatability, appearance, and nutritive value with comparatively little effect on wholesomeness and edibility (3, p. 199). These mild spoilage factors may be noted as the discoloration of meat by the oxidation of myoglobin to metmyoglobin, the loss of vitamins during storage or processing due to oxidation or thermal decomposition, and storage induced flavor changes (3, p. 199). Preliminary stages of

oxidative rancidity may also be considered under this classification.

Much improvement has been noted in the quality of frozen seafood products but deleterious changes in the product still occur. These changes may result from prolonged freezer storage, fluctuating temperature conditions and the use of oxygen-permeable packaging materials. The degree of oxidative rancidity resulting from these variables is very difficult to evaluate with the present methods available; partially because of the difficulty of achieving representative random samplings, and the lengthy storage periods required for complete quality assessments. Because of these sampling and storage problems, the evaluation of antioxidants and synergists is extremely difficult.

Seafood products are very susceptible to oxidative rancidity because of their high unsaturated fat content. This liability is not reduced to any great extent by the present methods of handling, processing and storage.

Several chemical and sensory methods have been suggested for the evaluation of oxidative rancidity in frozen seafood products but none have proven entirely satisfactory. It was not until the 2-thiobarbituric acid (TBA) method of Yu and Sinnhuber (71, p. 104-108) was developed, that reproduceable results on intact samples were achieved for fisheries products.

This thesis is concerned with the development of an accelerated autoxidation procedure for the estimation of the storage life in frozen seafood products. The effects of packaging materials, temperature, and antioxidants on the rate of autoxidation were studied as

well as the existance of a possible correlation between chemical and sensory evaluations. This proposed accelerated procedure should permit the rapid evaluation of a processing variable in a matter of days or weeks instead of months or years.

REVIEW OF LITERATURE

The quality of fish and fish products, like other foods, depends upon such factors as nutritive value, palatability, flavor, odor, and general appearance. It is at its best, as far as the fisherman and consumer are concerned, at the time the fish is removed from the water. Little can be done to improve the original quality of the product, but much can be done to retain it. Before we can intelligently approach the problems involved with quality retention, we must first understand the factors which cause these deleterious changes.

I. Autoxidation of Fats

Oxidation by atmospheric oxygen, described as autoxidation, is of great importance in both the development of rancidity in edible fats and in the drying of paint oils. Oxidative reactions in edible fats are undesirable and considerable investigation has been directed toward retarding or eliminating them. The reverse is true in paint oils where oxidative reactions are necessary to achieve proper hardening of the finished surface (29, p. 113; 36, p. 79). Early studies revealed the complexity of the reaction.

In a very comprehensive review of early theories pertaining to the mechanism of autoxidation, Lea (36, p. 86-92) points out that the autoxidation of an organic substance with a carbon to carbon double bond was recognized by Schönbein in the case of turpentine as early as 1858. He also indicates that modern theories as to the mechanism

of reactions of this type date from the work of Bach and of Engler and Wild in 1897.

A. Radical Chain Theory.

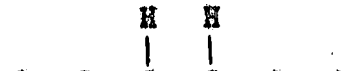
The radical chain theory is the currently accepted theory of autoxidation (29, p. 115-116; 35, p. 48-49; 37, p. 1303; 42, p. 126; 48, p. 238-240). According to this theory oxidation takes place not at the double bond itself, but at the reactive methylene group adjacent to a double bond with production of a hydroperoxide which still retains the original unsaturation. At the same time a resonance system set up by the free radical leads to the production of conjugated isomers. Since geometric isomerization of a considerable proportion of the double bonds from the cis to the trans configuration also occurs, the product of this first stage of autoxidation is a complex mixture of hydroperoxides with a high content of conjugated and trans unsaturation (37, p. 1303). The fundamental concepts leading to the acceptance of the radical chain theory have been substantiated by the development of new or improved techniques in low-temperature crystallizations, chromatography, counter-current distribution, polarography, and spectrophotometry in the ultra-violet and infrared ranges (42, p. 126).

Farmer (24, p. 86-93) stated that the formation of free radicals from fat molecules is dependent on hydrogen lability; the latter determined largely by the type of unsaturation pattern and also by the presence or absence of substitution on the ethylenic carbon atoms. To illustrate this, Farmer (24, p. 86-93) groups the four types of

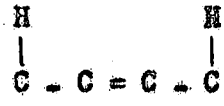
unsaturated systems on the basis of hydrogen lability as follows:

Group I

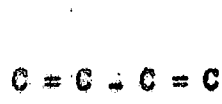
Group II



Group III



Group IV



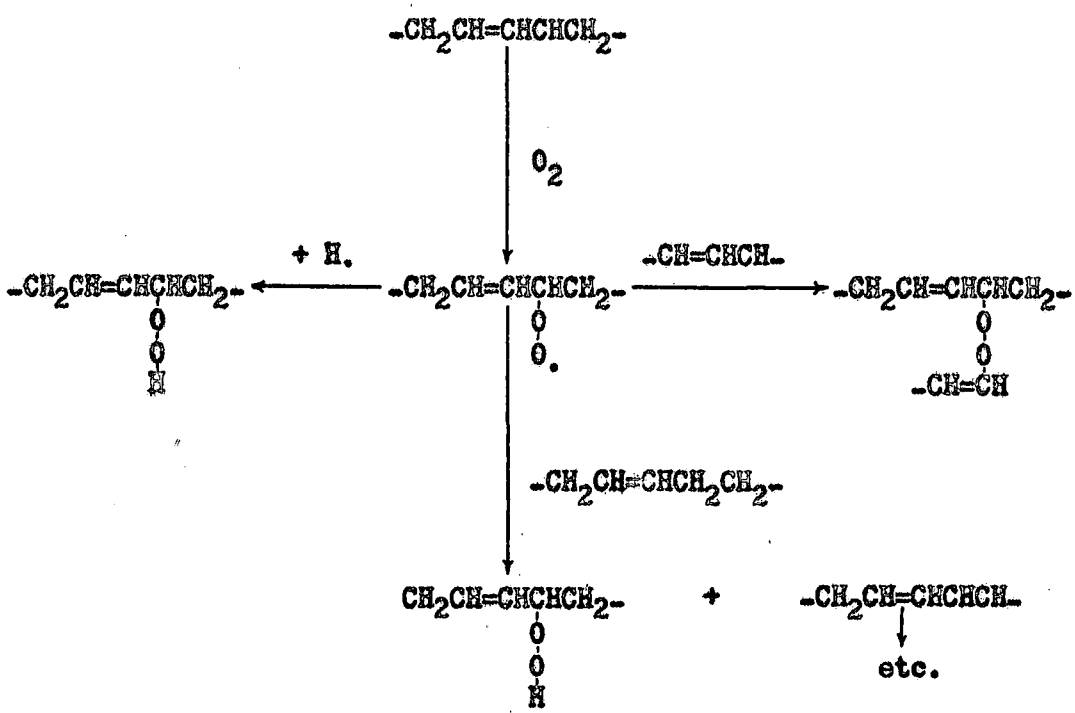
Group I shows the highest degree of hydrogen lability since the alpha-methylene group is flanked by adjacent double bonds. Group II shows less lability because the influence of the two double bonds is divided between the two methylene groups. Group III shows still less hydrogen lability, and Group IV, being a conjugated olefin has lost most of its hydrogen lability (42, p. 127).

B. Reactions of Free Radicals.

According to Koch (35, p. 49), the energy for free radical production stems from the fact that the double bond is capable of capturing outside sources of energy, such as light and heat energy which puts the electrons in a higher energy or excited state. When enough energy has been absorbed so that the electrons reach a critical excitation level, the excess energy is dissipated by the electron breaking away from the rest of the molecule and taking a proton with it. Both the fatty acid molecule remaining and the released hydrogen atom are referred to as free radicals since they possess unpaired electrons.

Free radicals are extremely unstable substances and will tend

to seek another electron to complete the stable electron structure. Some of the methods by which the fatty ester free radical can obtain an electron are shown below.

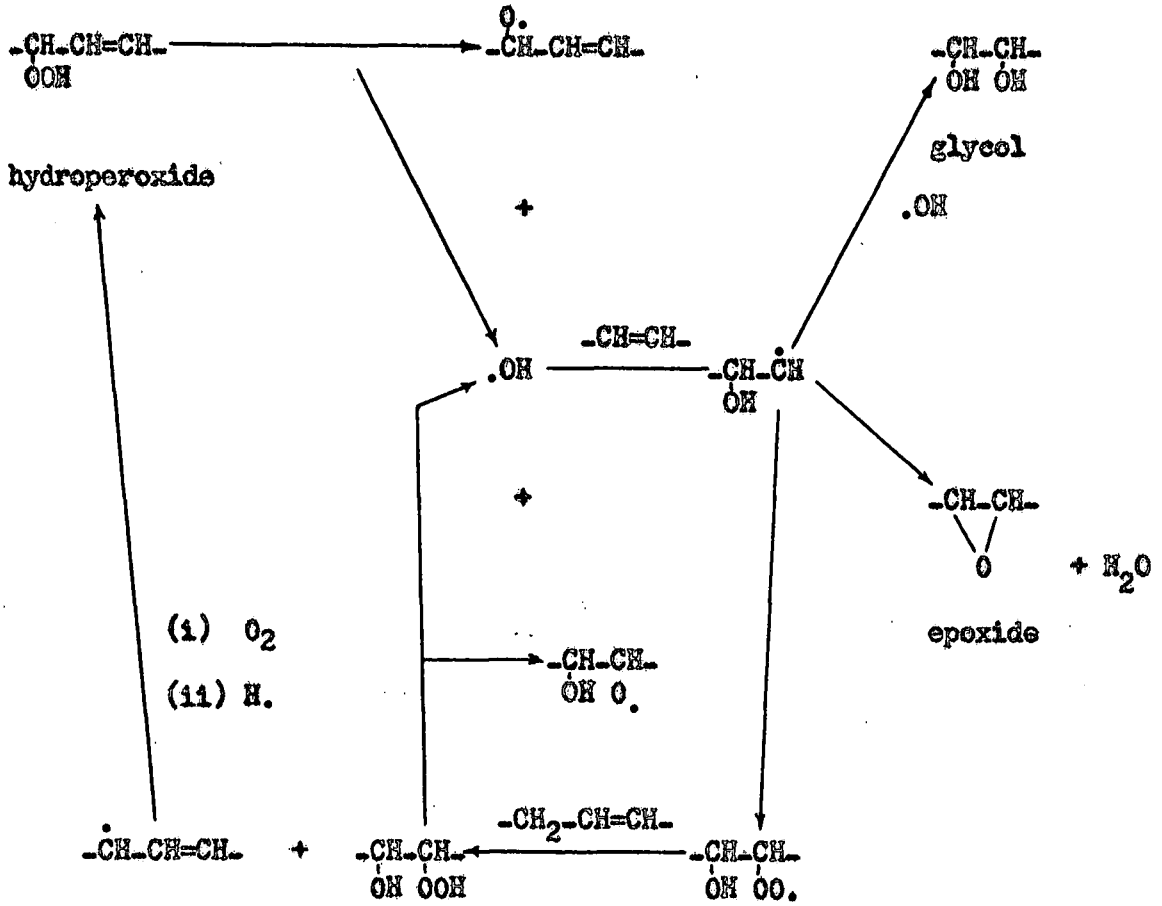


The free radical has a high affinity for oxygen which is believed to add as molecular oxygen to produce a peroxide. This peroxide may react with a free hydrogen atom to form a hydroperoxide and thereby terminate the reaction, or with another molecule of oleic ester which would start a chain reaction that could only be terminated by reaction with another free radical or with an antioxidant.

The oxidation of polyunsaturated fatty esters, such as linoleate, follows the same steps except that the hydrogen which escapes is believed to come from the active methylene group (35, p. 49-51).

C. Secondary Products of Autoxidation.

The hydroperoxides which form the primary autoxidation product are labile compounds readily undergoing further reaction, accounting for the decrease in peroxide values and iodine numbers during the latter stages of autoxidation (29, p. 119). Some of the secondary reactions of hydroperoxides are as follows:



D. Factors Affecting Autoxidation.

It should be noted that factors affecting autoxidation may be either catalytic or inhibitory in nature. Many factors are known to

accelerate or catalyze autoxidative reactions. The proxidant effect of metals, either in metallic form or in solution, on the rate of autoxidation has been shown by many authors (15, p. 241-244; 36, p. 154-161; 38, p. 214; 41, p. 119; and 66, p. 32-33). Other investigators (8, p. 27-31; 56, p. 24-26) show that there is a direct correlation between the hematin-compound content of the various species of fish and the catalytic effect on the linoleate oxidation. Coe (15, p. 241-244) states that the primary factors which accelerate oxidation in fats and oils are metals and light. That light has a definite proxidant effect is well substantiated (36, p. 139-152; 38, p. 213; 68, p. 36-37). This applies not only to photosensitization by pigments but also to photoexcited electron transfer due to the presence of trace metals. Air, moisture, and temperature may be considered as contributory factors (15, p. 241-244). Temperature, as a proxidant in thermal oxidation reactions is noted in the work of Bishov, Henick and Koch (7, p. 174-182) and also in connection with the well known Active Oxygen Method (47, p. 394-398). The effect of fluctuating temperatures on the autoxidation reaction has been reported by numerous workers (44, p. 200-205; 46, p. 19-27; 68, p. 37-38; 69, p. 311-318).

Some of the factors having an inhibitory effect on autoxidation include chemical antioxidants, natural antioxidants, low temperatures, smoke, spices, chelating agents, and packaging materials (18, p. 123-124; 38, p. 216-222; 48, p. 251-268; 65, p. 321-322; 66, p. 33-34; 68, p. 34-42). The effect of these factors will be reviewed in greater detail later.

II. Effect of Temperature on the Quality of Frozen Seafoods.

Much has been written on the freezing method of preserving fishery products. As Young (70, p. 447) points out, "The subject has been covered from substantially every aspect of interest to the technologist, so that if knowledge in itself were the key to high quality, one would expect that nothing but excellent frozen fish would be marketed in America today". Since this is not the case, it is deemed important to review some of the aspects of temperature and its effect on the quality of frozen seafoods. Tressler and Evers (65, p. 747), in their very comprehensive study on the freezing preservation of foods, point out that much of the early experimental work on freezing was carried out on fish. Quick freezing of fish was a common commercial practice for a decade prior to its adoption for the freezing of other foods.

A. Changes Occurring With Storage.

When fish which have been frozen and held in cold storage for an extended period of time are thawed and examined, a number of changes may be found to have taken place which may differentiate them from unfrozen fresh fish. The flesh of the thawed fish will consist of two phases, the solid flesh plus a fluid known as "drip" which was not reabsorbed by the flesh after thawing. Also, the texture of the thawed fish may be soft and the surface may have

become desiccated by the loss of moisture. After cooking, the fish may be found to have acquired an off-flavor or the flesh may be tough and fibrous. These changes from the original condition of the unfrozen fish are sometimes caused by the freezing process. Actually, almost all of such changes are caused, not by the freezing of the fish, but rather by the subsequent cold storage of the frozen product (57, p. 2).

One of the earliest changes taking place in fish occurs shortly after they are caught. This change is known as rigor mortis and is characteristic of perfectly fresh fish. Rigor mortis is caused by the coagulation of the cell proteins and is recognized by a general body rigidity or stiffness. This condition comes about sooner and lasts a shorter time at higher temperatures than at lower temperatures (64, p. 329). Although low temperature storage helps to prevent or retard enzymatic autolysis and bacterial invasion (64, p. 334-335), care must be taken to prevent other changes taking place in the frozen product because of the storage conditions (57, p. 17-27; 64, p. 335; 65, p. 317-328). These changes must be clearly recognized and avoided as far as possible. As indicated by Stansby, Pottinger and Miyauchi (57, p. 2, 17-27), the changes may be recognized as desiccation, off-flavor development, "drip", and color changes. They suggest that careful control of humidity, air circulation, temperature, and packaging may be very helpful in minimizing these changes.

B. Effect of Fluctuating Temperature.

In many articles dealing with the storage of frozen foods,

considerable emphasis has been placed on the need for keeping foods at a constant storage temperature at all times. It is obvious that this condition is almost impossible to maintain. Some fluctuation always occurs during the on-off cycle of the refrigerator compressors as well as when the freezer doors are opened or when large quantities of warmer fish are deposited in the cold storage rooms. Pottinger (46, p. 19) lists numerous items that can cause fluctuations in temperatures. These may include power failure, equipment breakdown, and transportation of the frozen product from the producer to the consumer. At one time it was thought that fluctuations in temperature were harmful to the quality of the frozen fish by causing large ice crystals to form within the tissue which might result in alteration in texture. Pottinger (46, p. 19-27) has shown that this theory does not hold. According to Stansby et al. (57, p. 21), fluctuating temperatures may accelerate oxidative changes and bring about an increase in the frost formation within packages where air pockets are present.

Nikkila and Linko (44, p. 200-205); Seagran (52, 106-109) and Stansby, et al. (57, p. 23-24) suggest that the alterations in the texture of fishery products are due, at least in part, to protein denaturation caused by fluctuations in temperature.

III. Effect of Antioxidants and Synergists On the Quality of Frozen Seafoods.

It has long been known that the reaction between an autoxidizable substance and oxygen can be delayed for long periods of time by the presence of very small concentrations of additives variously termed inhibitors or antioxidants.

Jacobs (32, Vol. III, p. 1937) points out that the following criteria for a chemical additive, as set by H. E. Barnard in 1911 still holds:

1. It must not under any reasonable condition injure the health of the consumer.
2. It must not allow the utilization of unfit raw material.
3. Its use must not make possible the employment of careless and imperfect methods of manufacture.
4. It must be non-irritant.
5. It must be efficient in its action.
6. It must not retard the action of digestive enzymes.
7. It must have no tendency to decompose within the body into substances which have a greater toxicity than that of the preservative itself.
8. It should lend itself to simple methods of determination and thus simplify the control problem.

A. Types of Antioxidants.

One of the biggest problems in the preservation of fats and

fatty foods is rancidity caused by oxidation of unsaturated fats, producing aldehydes and ketones that have strong and undesirable odors and tastes. Antioxidants applicable for stabilization of unsaturated fats are of two general types (59, p. 73-74): (1) The phenolics such as butylated hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA), propyl gallate (PG), and the tocopherols which inhibit fat oxidation by reacting with free radical intermediates of the oxidizing fats; and (2) the synergists, or metal deactivators which have no direct effect upon fat oxidation but supplement the primary phenolic antioxidants, usually by forming stable chelates with trace metal contaminants. Citric acid and ascorbic acid may be classified as good synergists.

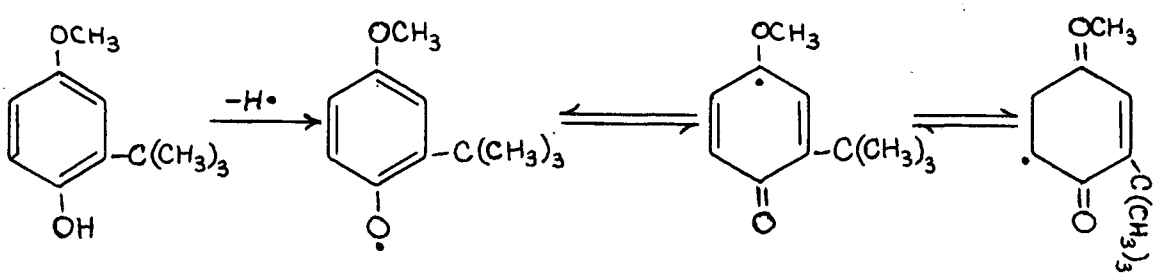
B. Effectiveness of Antioxidants.

The effectiveness of the above compounds, either alone or as synergistic mixtures, has been demonstrated by many authors for a wide variety of fats and fat-containing foods: seafoods (5, 254-260; 20, p. 371-383; 51, p. 1-6; 61, p. 137-154; 62, p. 237-247); cereals (58, p. 585-587); turkey (34, p. 308-311); irradiated cooked pork (60, p. 635-639); and fats (19, p. 457-460; 27, p. 386-390). According to Lew and Tappel (39, p. 285-289), a synergistic mixture of NDGA, BHA and ascorbic acid was very effective in retarding rancidity in fat containing products catalyzed by hematin compounds. It is strange that with all this evidence as to the effectiveness of antioxidants and synergists, little or no application of their use is noted in present-day commercial fisheries products. It may

be as Stansby, et al. (57, p. 31) point out, that although antioxidants are effective in retarding autoxidation of oils, when the oils are present in the tissue, as in the case of frozen fish, the effectiveness of the antioxidant is greatly diminished because of the moisture present.

C. Action of Antioxidants.

The action of antioxidants according to Koch (35, p. 52), lends support to the free radical chain theory. An antioxidant has an affinity for free radicals and will give up a hydrogen atom to the free radical more readily than will a fatty acid molecule. The action of the free radical is stopped when it accepts the hydrogen atom and consequently the chain reaction is terminated. The antioxidant becomes a free radical when it gives up the hydrogen atom but it forms a semi-quinone structure which is much more stable than a free radical of the fatty acid type. The reason for this stability is that the electron is capable of a movement through the ring structure as illustrated for the structures written for the semi-quinone.



This is known as resonance and imparts stability to the structure. This resonating structure is important since it is not strong enough to propagate a chain reaction by taking a hydrogen atom from an alpha-

methylene group of a fatty acid. However, if the semi-quinone should encounter a free hydrogen atom the original antioxidant would be restored (35, p. 52).

IV. Packaging

Packaging is an important factor in the retention of quality of frozen foods because efficient packages, among other things, prevent drying out (desiccation), contact with air (oxidation), and loss of the natural, fresh flavor.

Ball (2, p. 703-704) suggests that the appraisal of a container be based on two series of factors: (1) basic factors such as price to packager, sanitary qualities, resistance to impact injury, efficacy of interior surface, and absence of handling problems; and (2) consumer acceptance factors such as size, ease of opening, reseal features, pouring qualities, light protection, transparency, tamper-proofness, and ease of disposal. A method of evaluation is suggested using the above factors, accompanied by a very detailed explanation of each factor and how it fits into the overall picture (2, p. 704-732).

Browne (9, p. 1992-2017) gives a very comprehensive coverage of the functions of packaging and the materials used in their manufacture. He points out that packaging of food, for the most part, is strictly utilitarian; and that the primary purpose of the food package is to preserve the flavor and keep the product in good condition until it reaches the consumer. The functions which packaging perform

are protection, convenience, economy, and appeal. Many different materials are used for packaging containers: paper, plastics, fibers, metals, coatings and laminations consisting of a variety of substances. Each type of food product must be evaluated as to the particular type of packaging best suited to give it maximum protection.

Various protective coatings are used to protect fishery products during frozen storage. Probably the first "package" that came into general use was an ice glaze. Not until the introduction of quick frozen foods in prepared form was any serious attempt made to improve upon protective covering for frozen fishery products (57, p. 41). Stansby, et al. (57, p. 41-63) list many types of protective coverings used for frozen seafood products and explain the functions of each type. These may include: (1) glazes, which include ice glazes, pectinate films, gelatine-base coatings, and thermoplastic waxes; (2) films and wraps such as cellophane, polyethylene, aluminum foil, waxed paper, and plastic-paper laminants; (3) packages and cartons that are generally made out of paperboard; and (4) metal containers such as the hermetically sealed tin can and the semi-rigid aluminum pan package.

As one can readily see, the package plays an important role in the preservation of frozen seafoods. Undesirable changes in quality due to the action of air, light, and moisture can be eliminated or at least retarded by reasonable care in the selection of the proper packaging container for the product to be frozen.

V. Acceleration Reactions

Many acceleration reactions have been proposed and evaluated as a means of evaluating various treatments, additives, and relative stabilities of fats and oils (7, p. 174-182; 16, p. 47-72, 98-101; 33, p. 105-109; 43, p. 191-194; 45, p. 161-162). Gearhart, Stuckey and Austin (28, p. 427-430) compared the conventional Active Oxygen Method, the Schaal Oven Method, and a modified ASTM Oxygen Bomb Method for determining the stability of fats and oils, and of fatty or oily foods. They found that the ASTM bomb data correlated well with both the other methods and has the advantage of speed, precision, and simplicity of operation. Filer, Mattil and Longenecker (25, p. 196-201), using a modified Swift Stability Test, studied the rates of change of peroxide and iodine values, saponification equivalents, and the amounts of "linoleic and linolenic acids" during the accelerated rancidification of hydrogenated vegetable oil shortenings and refined cottonseed oil. These acceleration reactions may be dependent upon heat, light, or a supporting media such as sand or cellulose to achieve the desired results.

VI. Evaluation of Quality

The majority of the present day methods for determining quality generally fall into one or more of four categories: sensory, chemical, microbiological, and physical. Of these, the chemical tests for various indices seem to predominate.

A. Chemical.

As was stated above, chemical determinations as indicators of quality in food products seem to predominate. Various indices are used, these include trimethylamine and trimethylamine oxide (10, p. 301-304; 21, p. 23-25; 22, p. 292-294); succinic acid (31, p. 842-847); and volatile acids and bases (30, p. 763-776; 53, p. 227-231; 63, p. 614-617). Good correlation is shown between the content of volatile reducing substances and organoleptic judgements of freshness in a variety of fish and fish products (23, p. 677-680). The thiobarbituric acid (TBA) test has been applied to a variety of foods as a measure of quality as affected by oxidative rancidity. These include: butterfat (6, p. 138-145); oat cereals, oatmeal cookies and soda crackers (12, p. 185-186); fish (26, p. 1-35; 71, p. 104-108); cooked oysters (50, p. 76-82); and fats (54, p. 603-606). A quantitative method for the determination of malonaldehyde (the red pigment developed by the TBA test) is presented by Sinnhuber and Yu (55, p. 9-12); the term, TBA number or milligrams of malonaldehyde per 1000 gram of sample, is suggested.

EXPERIMENTAL METHODS

I. Acceleration Reaction

A. Procurement of Sample.

Fresh rockfish fillets (Sebastodes sp.) were purchased locally as needed. Purchases were made on the same day the fish were delivered thereby assuring relatively fresh samples for each treatment.

B. Preparation of Sample.

Approximately two pounds of the fresh fillet were passed through a hand-operated meat grinder which had been modified to prevent iron contamination by chrome plating the internal parts and installing a Formica face plate. After being ground, the sample was well mixed, quartered, and the alternate quarters discarded. This was repeated three times after which a 10 - 20 gram sample was removed.

C. Purification of Surfactant.

A diatomaceous silica, Celite Analytical Filter-Aid, was used as the support media for this acceleration procedure. The surfactant, a product of the Celite Division of the Johns-Manville Company, was selected because it is commercially available and uniform in quality. Preliminary investigations indicated that this product does not interfere in any way with the TBA method employed for chemical determination of oxidative rancidity. Manufacturer's specifications of this and similar diatomaceous earth products point out their large surface area per unit weight (i.e., from four to six square meters per

gram). Chemical analysis for the presence of trace metals which would influence the oxidation reaction revealed appreciable amounts of iron present. Purification by removal of the iron was accomplished by reacting the surfactant with aqua regia for 48 hours, after which the solution was transferred to a large percolator and washed continuously with distilled water, using suction, until a negative chloride test was obtained with silver nitrate. Excess water was removed by suction and drying then completed at 212°F.

D. Method of Mixing Surfactant With Seafoods.

A random sample (10.00 g.) of the seafood to be tested was obtained as previously described and mixed with purified Celite Analytical Filter-Aid (30.00 g.) by thoroughly grinding the sample in a number six mortar with a small amount of surfactant. The remainder of the purified surfactant was added after a homogenous mixture was achieved and blended into the original to yield a final proportion of 1:3 (fish to surfactant). Previous investigation by the author indicated that a homogenous mixture was achieved after mixing for 20 minutes in 35°F room. Standardization of the mixing procedure as to time and temperature is important to achieve uniformity between treatments. The mixture was next passed through a 20 mesh nylon screen, to eliminate any caking that might occur, and placed in suitable containers for storage at the designated temperatures.

E. Evaluation of Oxidative Rancidity.

The 2-thiobarbituric acid (TBA) method of Yu and Sinnhuber (71, p. 104-108), was used for the chemical evaluation of oxidative

rancidity.

F. Treatments.

The following treatments were designed to show the effect of certain variables on the accelerated autoxidation reaction of seafoods. The results of the TBA reaction for the determination of oxidative rancidity, expressed as TBA number, were plotted against time in days.

1. Effect of Purification: The following method was used to determine the need for purification of surfactant and the effect of metal (expressed as percent ferric oxide) on the accelerated autoxidation test. A random sample of fresh, ground rockfish fillet was divided into three portions and individually mixed with the three selected diatomaceous silica products in a mortar using the prescribed procedure. The products used were: Celite 319; Celite Analytical Filter-Aid; and the purified Celite Analytical Filter-Aid. The latter material was purified, as previously described by treatment with acid. The iron content (expressed as percent ferric oxide) of Celite 319 and Celite Analytical Filter-Aid, as stated in the manufacturer's specifications, was 1.0 - 1.5 percent and 0.2 percent respectively.

The samples were placed in one ounce, screw-cap glass jars, after being sifted through a 20 mesh nylon screen, and stored at 0°F. for subsequent TBA determinations.

2. Effect of Temperature: A random sample of fresh, ground rockfish fillet was prepared and mixed with purified surfactant as

previously described. After sifting, fish-surfactant samples were placed in one ounce, screw-cap, glass jars and stored at 0°, 10° and 20°F. Each jar contained approximately 1.5 grams of sample and served as an individual sample for chemical determination of oxidative rancidity by the TBA method.

3. Effect of Fluctuating Temperature: The following experiment was used to determine the effect of fluctuating temperatures on the extent and rate of oxidative rancidity in fresh frozen rockfish using the accelerated procedure described. Thirty-six, one pound cartons of commercially packaged, fresh-frozen rockfish fillets were purchased from a commercial packer. Initial TBA determinations were made and a similar sample was presented to a trained taste panel for sensory evaluation. The results, in each case, indicated a relatively fresh sample. The remaining cartons were evenly divided into four lots (0x, 1x, 2x, 3x). Lot 0x (control) was held at 0°F. Lots 1x, 2x and 3x were placed in a 35°F. cool room and held for 12 hours, during which time, the internal temperature of all the fillets uniformly increased to a maximum of 29°F. as recorded by a electronic recording potentiometer, using a copper-constantin thermocouple. To assure equilibration of temperature, the samples were rapidly cooled to 0°F. and held at that temperature for 24 hours between each fluctuation period. Lot 1x was held at 0°F., lots 2x and 3x were returned to the cool room and subjected to another 12 hour fluctuation in temperature. After equilibration at 0°F., lot 3x was given a third and final treatment. After this third fluctuation period, randomly

selected fillets from two cartons per lot were ground and mixed with the purified surfactant in a ratio of 1:3 as previously described. After sifting to establish uniform particle size, each lot was stored in an eight ounce, screw-cap, glass jar and held at 0°F. storage for subsequent chemical determination of this accelerated autoxidation procedure. For purposes of comparison, the remaining cartons of fillets from each lot were stored, as such, at 0°F. and evaluated by TBA method at the same storage intervals as the ground, accelerated samples.

To assure that uniform temperatures existed in the samples during the fluctuation cycle and the equilibration period, the samples were stored individually on shelves, with free circulation about each package, and without benefit of protection from mass packaging in a carton.

4. Effect of Antioxidants: Fresh silver salmon steaks, (Oncorhynchus kisutch), were prepared in the same manner as the ground rockfish fillets, after carefully removing the skin and bones. The sample was divided, 100 grams thoroughly mixed with 70 milligrams of propylene glycol to serve as the control, the other half carefully mixed with 70 milligrams of a nordihydroguaiaretic acid (NDGA) antioxidant solution containing 10 percent NDGA, 20 percent butylated hydroxyanisole (BHA) and six percent citric acid; the resulting mixture yielding a synergistic concentration of 0.007 percent NDGA, 0.014 percent BHA and 0.0042 percent citric acid on a final weight basis.

A 1:4 ratio of fish to surfactant was used in this treatment. Mixing was done in a mortar, as described previously, under standardized conditions of time and temperature.

The samples were stored in one ounce, screw-cap, glass jars and held at 0°F. for chemical determination.

VI. Effect of Packaging on Autoxidation of Frozen Seafoods as Determined by Chemical and Sensory Evaluations.

A. Procurement of Sample.

To insure the use of the freshest possible samples, 225 pounds of fresh rockfish fillets were acquired from a commercial packing house in Astoria, Oregon, within two hours from the time they had been removed from the fishing boat. The samples were less than four days old and had all been caught just off the Washington coast at approximately 46° 30' NL (along Loran Line 3700). The fish had been well iced and were in excellent condition at the time of purchase. The sample consisted of a random assortment of the following species:

Sebastodes pinninger *Sebastodes rubavictus*	red rockfish
Sebastodes flavidus *Sebastodes melanops*	black rockfish

The fillets had all received a sodium benzoate-benzoic acid dip to retard bacterial action.

B. Preparation of Sample.

The total sample was divided three ways: 125 pounds of fillets were held for organoleptic evaluation, 50 pounds of fillets were held for chemical evaluation, and the remaining 50 pounds were ground by passing the fillets through a Fitzpatrick Comminuting Machine, Model D. fitted with a number three sieve.

C. Treatment of Samples.

1. Packaging: Three representative types of packaging containers were used: (1) a commercial carton with overwrap; (2) a heat-sealable plastic pouch; and (3), a "C" enameled tin can. The commercial carton, produced by Fiberboard, Portland, Oregon was a 0.015 inch solid, bleached sulfite with a cold wax finish; the overwrap was a plain, SS bleached sulfite, glasseal product of Western Waxside Division of Crown Zellerback, Portland, Oregon. The plastic pouch, 1087 Durafilm, was produced by the Dobeckmun Company, a division of the Dow Chemical Company. This film, a tri-laminant of Mylar/Saran/polyethylene, is very strong, easily hand sealed and suitable for use in boiling water baths. A 211 x 400, "C" enameled tin can manufactured by Continental Can Company was used as the third type of container.

Since none of the packages were vacuum packed, care was exercised while filling the packages to eliminate any air pockets and to keep the head space at a minimum. A damp sponge was used during the sealing operation of the plastic pouches to force out as much air as possible.

The grinding and packaging operation was done at the Oregon State

College Seafood Laboratory and the entire operation was completed within 16 hours from the time the samples were acquired. The packaged samples were held at 0°F. prior to shipment under dry ice conditions to Oregon State College.

2. Storage: The samples were held at 0° ± 2°F. Observations for chemical and sensory evaluations were made on random samples from each packaging treatment at monthly intervals over a nine month storage period.

D. Evaluations.

1. Chemical: The TBA method of Yu and Sinnhuber (71, p. 104-108) was used to determine the extent of oxidative rancidity developing in the samples presented to the taste panel as well as the samples designated for chemical evaluation. To ascertain the possible existence of a correlation between chemical and sensory evaluations, the results of the chemical determination were compared with the degree of rancidity in the samples as described by the tasters.

Colorimetric evaluation of the results was accomplished using a Beckman spectrophotometer, model DU. The results were expressed as TBA number (mg. of malonaldehyde per 1000 g. of sample) by multiplying the $E\ {}^{1\%}_{1\ cm.}$ at 535 mμ by the constant 46 (55, p. 9-12).

2. Sensory: The sensory evaluations were made by a trained, staff panel of 14 tasters who were present throughout the test. The samples to be evaluated were removed from 0°F. storage 24 hours prior to the time of serving and thawed at refrigerator temperature. The

fillets were cut into approximately 1-1/2 inch squares and prepared for deep-fat frying by first coating them with an egg batter followed by a coating of cracker crumbs. The samples were deep-fat fried in Vream, a hydrogenated vegetable oil shortening manufactured by Swift and Company. Experimentation indicated that a well cooked sample resulted after cooking for three minutes at 350°F. Each packaging treatment was statistically evaluated, as indicated below, on the basis of two replications. These replications were presented to the panel on the first Tuesday and Thursday of each month throughout the storage period. The samples were served in randomly coded paper cups and tasting was carried out in individual booths designed for that purpose. No seasoning was added to any sample.

A facsimile of the evaluation sheet used for taste panel judgements is shown on page 30. A seven-point line ballot was used for scoring rancidity and tenderness with "1" being extremely tough and extremely rancid, and "7" being very tender and no rancidity. Samples were also scored on a seven-point hedonic preference scale with "1" as very undesirable and "7" as very desirable.

The judgements of the tasters were statistically evaluated by analysis of variance (40, p. 309-324) and the means plotted against storage time. It was understood that a taste panel of fourteen judges would not give a preference score indicative of a consumer population, but emphasis was placed on using the same judges throughout the test and therefore the large student panel could not be utilized.

Facsimile of the Evaluation Sheet Used for Taste Panel Judgements.

Date: _____ Name _____

S E A F O O D

TENDERNESS	RANCIDITY	OVERALL DESIRABILITY
Very Tender	None	Very Desirable
Moderately Tender	Very Slight	Moderately Desirable
Slightly Tender	Slight	Slightly Desirable
Slightly Tough	Moderately	Neutral
Moderately Tough	Very Moderately	Slightly Undesirable
Very Tough	Extremely	Moderately Undesirable
Extremely Tough	Very Extreme	Very Undesirable

RESULTS AND DISCUSSION

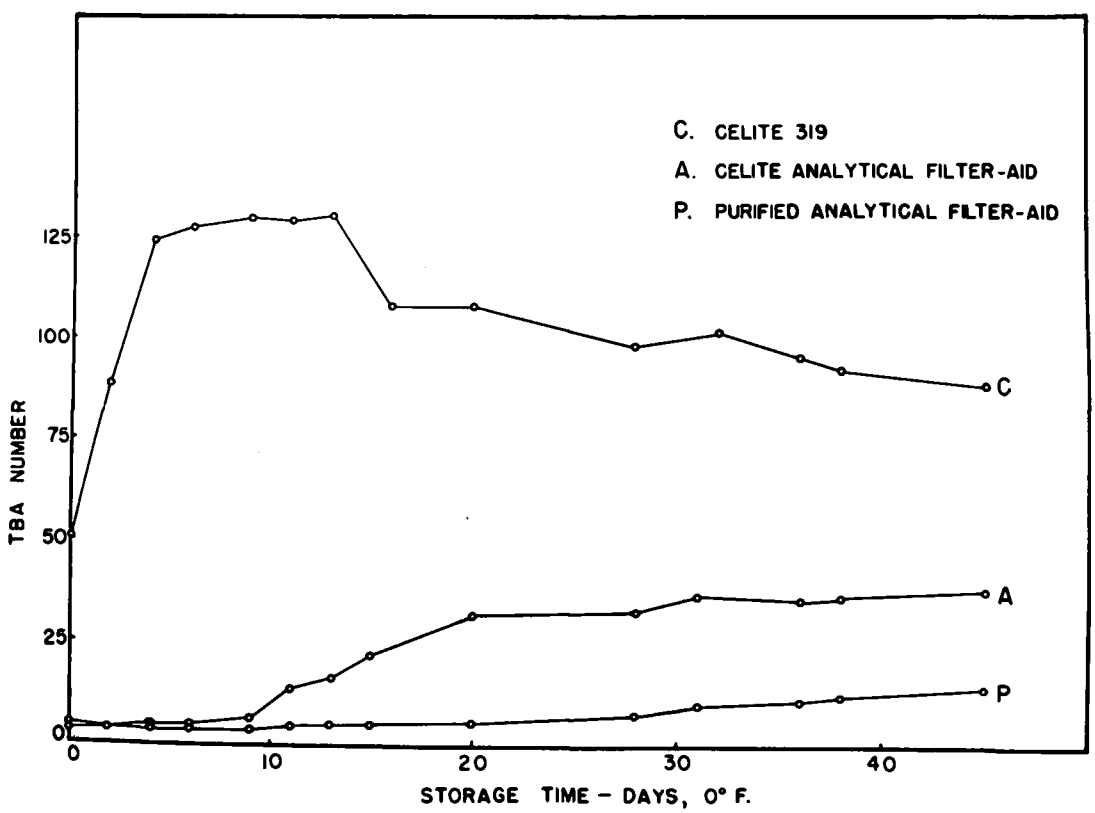
I. Accelerated Oxidation Method

A. Effect of Purification of Surfactant on the Accelerated Autoxidation of Frozen Fish.

The catalytic effect of iron and iron containing compounds on oxidative reactions has been shown by a number of investigators (8, p. 27-31; 36, p. 154-164; 48, p. 240-242). The relationship between the removal of iron by purification and the degree of catalyzed autoxidation is shown in Figure 1. As indicated by TBA method (71, p. 104-108), purification of the surfactant by removal of metal catalysts (expressed as ferric oxide) greatly decreases the rate of autoxidation of the frozen seafood sample. As stated previously, Celite 319 and Celite Analytical Filter-Aid contain 1.0 - 1.5 percent and 0.2 percent ferric oxide respectively. The effect of this reduction in percent ferric oxide was dramatically illustrated in Figure 1 where the TBA number for Celite 319 at the end of 10 days storage was approximately 20 times greater than that of the Celite Analytical Filter-Aid. That the rate of autoxidation can be decreased even more was shown by plotting the TBA numbers resulting from chemical analysis of the acid treated Celite Analytical Filter-Aid and comparing them with the untreated surfactant.

It is believed that this procedure could be used to study the catalytic oxidation action of metals, the pro-oxidant effect of

FIGURE 1. THE EFFECT OF PURIFICATION OF SURFACTANT ON THE ACCELERATED AUTOXIDATION OF FROZEN ROCKFISH.



hemoglobin, and the function of metal deactivators or synergists in frozen seafoods.

B. Influence of Temperature on the Development of Oxidative Rancidity.

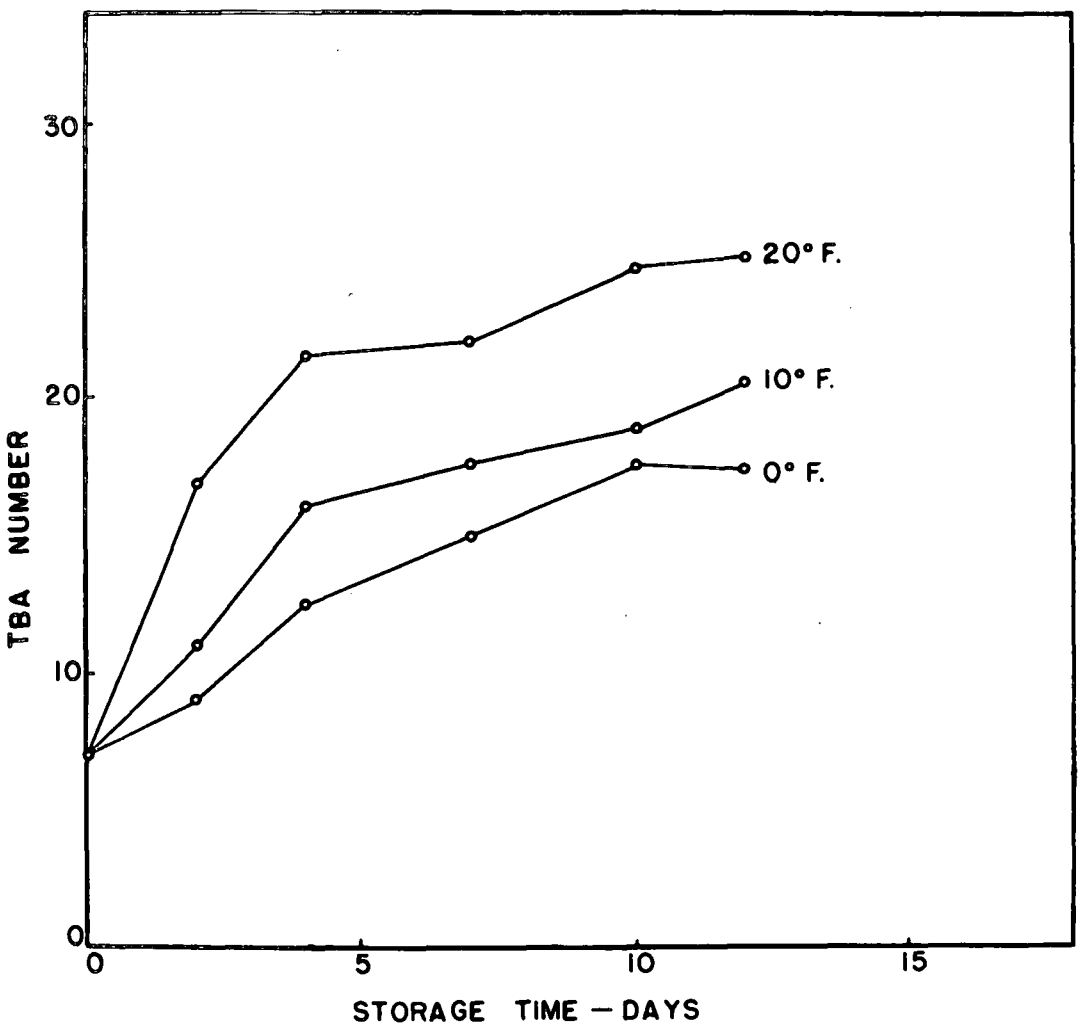
The use of low temperature storage has been recommended by technologists for the maintenance of good quality in frozen seafoods. They emphasized the importance of storing fishery products at 0°F. or below and demonstrated that the necessity for low temperature storage increases as the length of the storage period was increased (67, p. 94). The effect of storage temperature on the rate of autoxidation of a fish-surfactant mixture as shown in Figure 2 indicates that the rate of autoxidation is approximately doubled for what would be an 18°F. rise in temperature. This was also noted by Lea (36, p. 138) who further states that the effect of temperature on the oxidative reaction is of the order usually found for chemical processes.

It would seem apparent, that by this accelerated procedure, it may be possible to evaluate the various storage variables in a matter of days. Previous experience has shown that weeks or even months would be necessary to achieve a similar state of oxidation with whole or even ground fillets. The advantages of evaluating antioxidants or other additives by this method are equally apparent.

C. Effect of Fluctuating Temperatures on the Autoxidation of Seafoods.

Pottinger (46, p. 19-27) emphasized the need for keeping frozen seafoods at a constant temperature. He found appreciable differences,

FIGURE 2. THE EFFECT OF STORAGE TEMPERATURE ON ACCELERATED AUTOXIDATION OF FROZEN FISH.



due to storage temperatures, in the storage life of the samples. The samples held at 0°F. or below had two to three times the storage life of the samples held at 15°F. He also pointed out that rancidity and discoloration occurred much sooner and progressed more rapidly with samples that were loosely wrapped and where care had not been exercised in eliminating the air pockets. In referring to the samples held under fluctuating storage temperature conditions, Pottinger (46, p. 19) stated that based on palatability scores, volatile acid numbers, "drip" determinations and visual examination, there was no adverse effect on the quality of frozen fish in the temperature range covered (0° to - 10°F. and 0° to 15°F). He concluded that the average temperature encountered during the fluctuation periods would seem to be the deciding factor on the quality of the frozen fillets. The effect of fluctuations in storage temperature on the rate of autoxidation of frozen seafood was shown in Figure 3. The degree of oxidative rancidity increases rapidly with an increase in the number of fluctuation periods. This rapid increase in the rate of autoxidation is in direct contrast with results shown in Table 1 where very little change is noted in TBA numbers of commercial packaged rockfish fillets from the same lots evaluated over the same period of time. The samples described (Figure 3) were ground and mixed with purified surfactant prior to storage at 0°F. whereas the samples referred to in Table 1 were stored as commercially packaged fillets.

FIGURE 3. THE INFLUENCE OF TEMPERATURE FLUCTUATIONS ON THE ACCELERATED AUTOXIDATION OF FROZEN ROCKFISH.

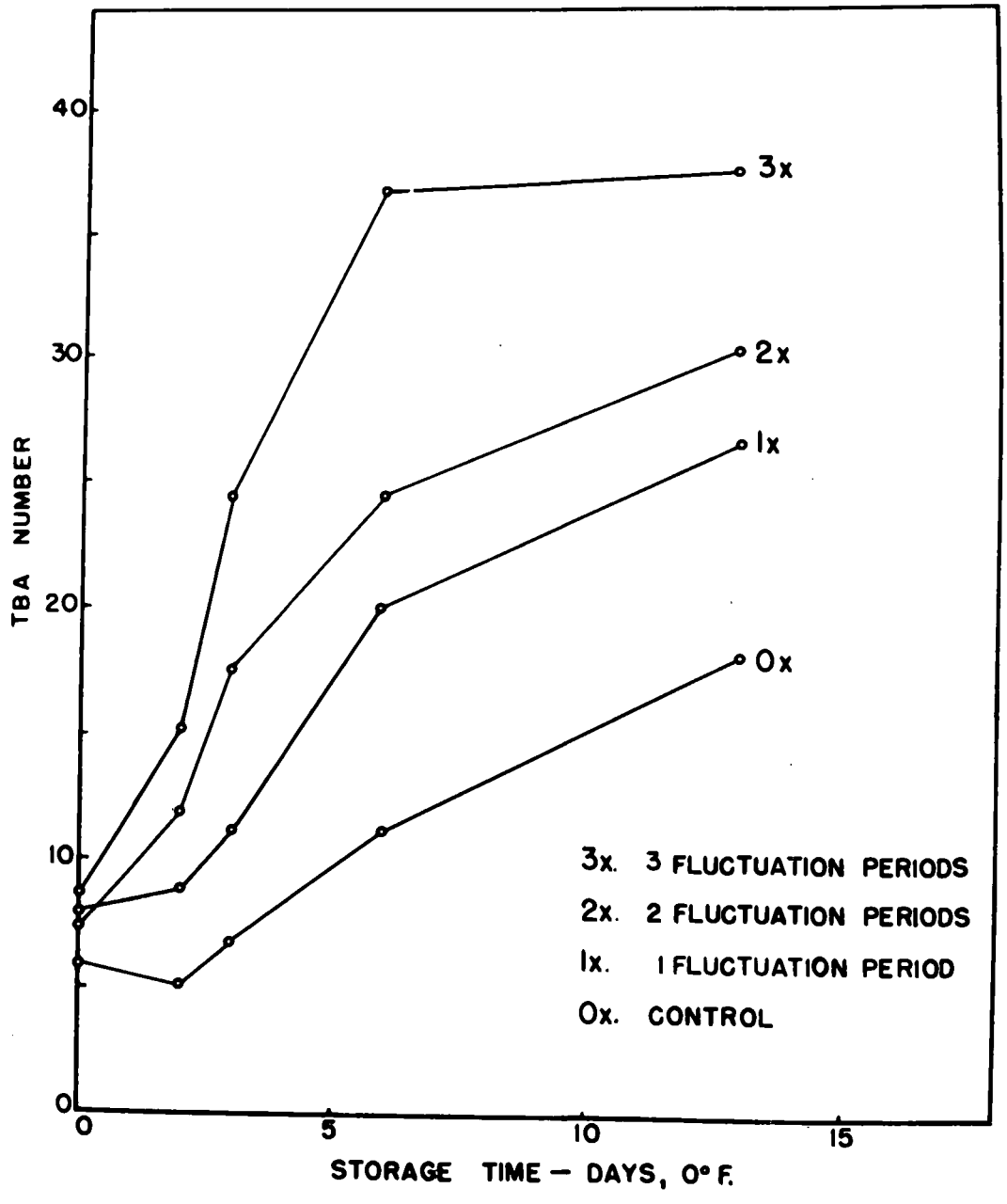


Table 1

EFFECT OF PRE-STORAGE TEMPERATURE FLUCTUATIONS ON THE RATE OF AUTOXIDATION OF COMMERCIALLY PACKAGED ROCKFISH FILLETS

Number of Temperature Fluctuations	Days Storage at 0°F.		
	0	5	20
0*	1.14	1.24	1.21
1		1.15	1.20
2		1.20	1.43
3		1.38	1.56

*(Identifiable to lots 0x, 1x, 2x, 3x).

The data shown in Figure 3 and Table 1 indicate that the accelerated autoxidation method could be used for rapid evaluation of the various processing and storage variables associated with frozen seafoods. As noted in Figure 3, very definite increases in the rates of autoxidation were shown for the samples mixed with surfactant, whereas little or no change was shown for the untreated samples even after 20 days of storage. Application of the accelerated method to frozen seafoods would therefore permit evaluation of the product before shipment to the retail market. This experiment also suggests the possible application of this accelerated autoxidation procedure to the evaluation of processing variables in frozen vegetables and precooked frozen foods as well.

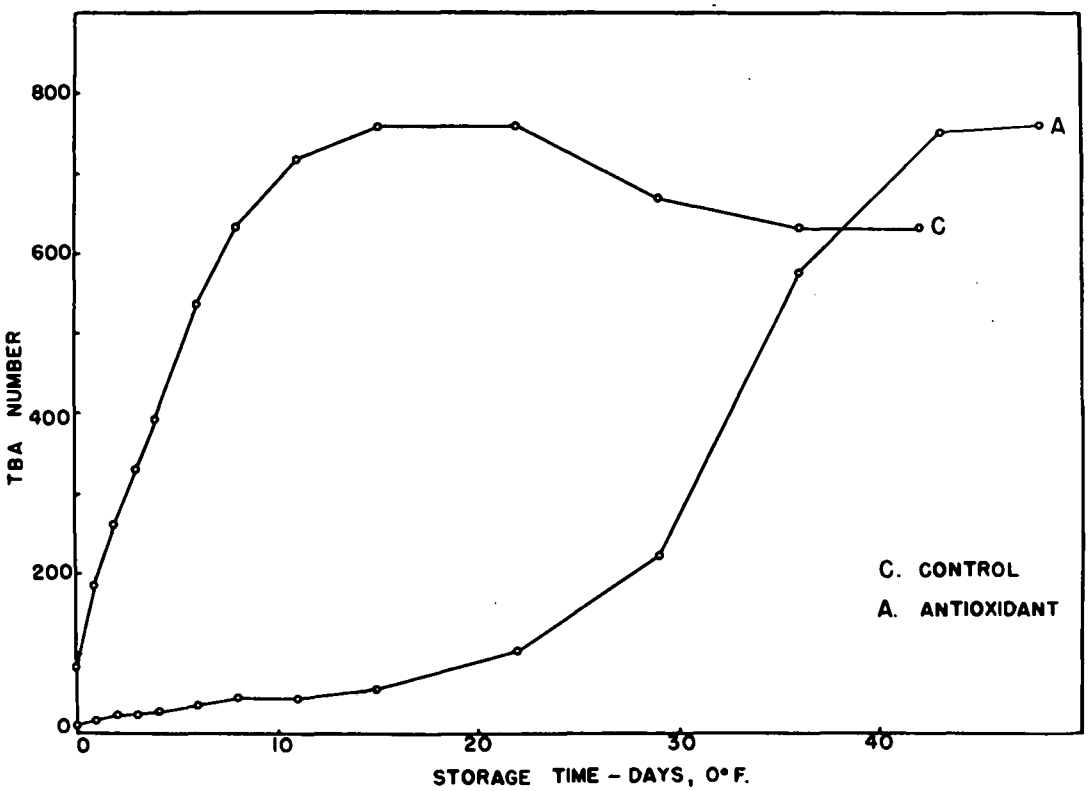
D. Influence of Antioxidants on the Accelerated Autoxidation of Frozen Salmon.

The need for antioxidants to protect frozen seafoods and thereby increase their storage stability has been the subject of considerable research and development (4, p. 250-256; 5, p. 254-260; 51, p. 1-6; 61, p. 137-154; 62, p. 237-247). In spite of the advantages shown by the application of antioxidants to both fish and other foods, they have not received a very enthusiastic reception by the fisheries industry. This lack of acceptance, in spite of the great need, may be due in part to the difficulty of application and the costs involved. Sample variations and lengthy storage requirements often make a clear-cut evaluation of an antioxidant treatment a formidable task.

Figure 4 dramatically illustrates the effect of a synergistic mixture of phenolic antioxidants added to an accelerated oxidation reaction involving ground silver salmon steaks.

The effectiveness of the NDGA solution in prolonging the induction period is clearly seen. It should also be noted that less than 30 days is needed to evaluate the stabilizing effect of the antioxidant instead of the many months usually required. The extremely high TBA number obtained for the rancid salmon tissue is apparently due to the high fat content (8.7 percent) and the highly unsaturated fats which are present.

FIGURE 4. THE EFFECT OF AN ANTIOXIDANT ON THE ACCELERATED AUTOXIDATION OF FROZEN SALMON.



II. Chemical and Sensory Evaluation of the Packaging Treatment

Analysis of variance (40, p. 309-324) calculations were made on the sensory evaluations of "Tenderness", "Rancidity" and "Overall Desirability" and significance determined at the five percent level. Since this investigation was initiated in August, no sensory evaluations were made until after classes began and the panel was trained. The first scheduled taste panel session was set up to evaluate samples that had been stored at 0°F. for four months. Thereafter, taste panel sessions were held on the first Tuesday and Thursday of each month, with the exception of January, until the tests were completed with the evaluation of the nine month storage sample.

Results of analysis of variance calculations for the evaluation of "Rancidity" and "Overall Desirability" indicated a significant difference, at the five percent level, between the treatment means. The method of least significant difference (40, p. 233-238) was used to determine which treatments were significant. It was shown that the carton was significantly different from the pouch and can during every storage period evaluated except the first one. This was not the case with "Tenderness" evaluations where no significant difference was noted between treatments throughout the duration of the storage study.

To elucidate the above statistical analyses, the means of the observations for each package treatment pertaining to a particular flavor evaluation (Tables 2 - 4) were plotted against storage time. The erratic results (Figure 5) shown for tenderness evaluations, as

Table 2

TENDERNESS SCORES OF FROZEN ROCKFISH FILLETS AS AFFECTED BY PACKAGING TREATMENT AND STORAGE
(Mean Flavor Score)

Months Storage at 0°F.	Treatment		
	Commercial Carton	Plastic Pouch	Tin Can
4	5.19	4.96	5.35
5	4.57	5.20	5.42
6	5.16	4.87	4.84
7	4.58	5.37	5.41
8	5.28	5.46	5.23
9	4.93	5.03	4.93

Table 3

RANCIDITY SCORES OF FROZEN ROCKFISH FILLETS AS AFFECTED BY PACKAGING TREATMENT AND STORAGE
(Mean Flavor Score)

Months Storage at 0°F.	Treatment		
	Commercial Carton	Plastic Pouch	Tin Can
4	4.85	5.26	5.53
5	4.36	5.18	5.34
6	4.62	5.16	5.67
7	4.98	5.96	5.48
8	4.57	5.62	5.66
9	4.48	5.32	5.03

Table 4

DESIRABILITY SCORES OF FROZEN ROCKFISH FILLETS AS AFFECTED BY PACKAGING TREATMENT AND STORAGE
(Mean Flavor Scores)

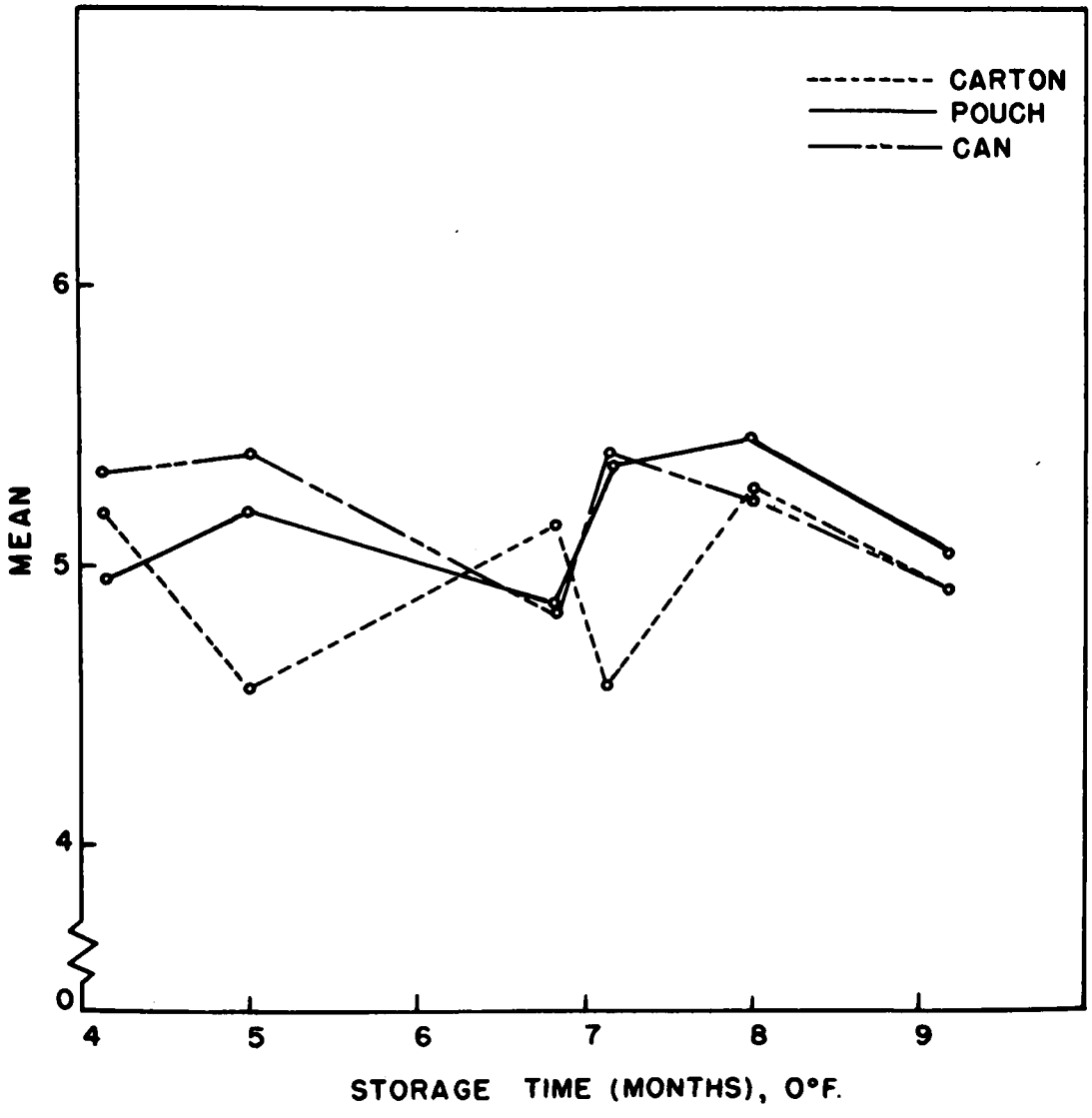
Months Storage at 0°F.	Treatment		
	Commercial Carton	Plastic Pouch	Tin Can
4	4.58	4.74	5.14
5	4.30	4.91	5.16
6	4.33	4.98	5.49
7	4.51	5.66	5.30
8	4.66	5.46	5.50
9	4.32	5.05	3.68

Table 5

CHEMICAL DETERMINATION OF OXIDATIVE RANCIDITY IN FROZEN ROCKFISH FILLETS AS AFFECTED BY PACKAGING TREATMENT
(TBA Number)

Months Storage at 0 F.	Treatment		
	Commercial Carton	Plastic Pouch	Tin Can
4	2.21	1.33	1.24
5	3.08	2.42	1.77
6	3.86	2.29	1.98
7	4.76	2.70	1.58
8	3.26	2.12	1.85
9	4.00	2.07	1.78

FIGURE 5. EFFECT OF PACKAGING CONTAINERS ON TENDERNESS OF FROZEN ROCKFISH FILLETS AS INDICATED BY SENSORY EVALUATION.



affected by packaging containers, clearly indicates that the tasters could not consistently differentiate between the treatments. This substantiates the results indicated by analysis of variance calculations. Figure 6 shows the effect of packaging containers on the development of rancidity as evaluated by a trained taste panel. That the tasters consistently noted a difference between the treatments is quite apparent. It should also be noted that the fillets packaged in commercial carton were rated much lower than those packaged in plastic pouches and tin cans. This would tend to indicate that seafoods packaged in commercial cartons do not have the same protection from atmospheric oxygen as those packaged in the pouch and can. This result was also shown by the method of least significant difference. This same preference for fillets packaged in plastic pouches and tin cans was shown in Figure 7 where the tasters were asked to evaluate the samples as to "Overall Desirability". It was interesting to note, in reviewing Figures 5, 6, and 7, that overall desirability is primarily dependent upon the evaluation of rancidity. It should also be noted that although changes in flavor scores were detected during the overall storage period, little or no difference was shown between the score for the fourth and ninth month evaluation. This cannot be adequately explained except to note that sensory evaluations are influenced by outside factors that cannot be controlled.

To ascertain if a possible relationship existed between sensory and chemical evaluations, TBA determinations were made on random samples of each treatment served to the panel. The results (Table 5)

FIGURE 6. EFFECT OF PACKAGING CONTAINERS ON DEGREE OF OXIDATIVE RANCIDITY IN FROZEN ROCKFISH FILLETS AS INDICATED BY SENSORY EVALUATION.

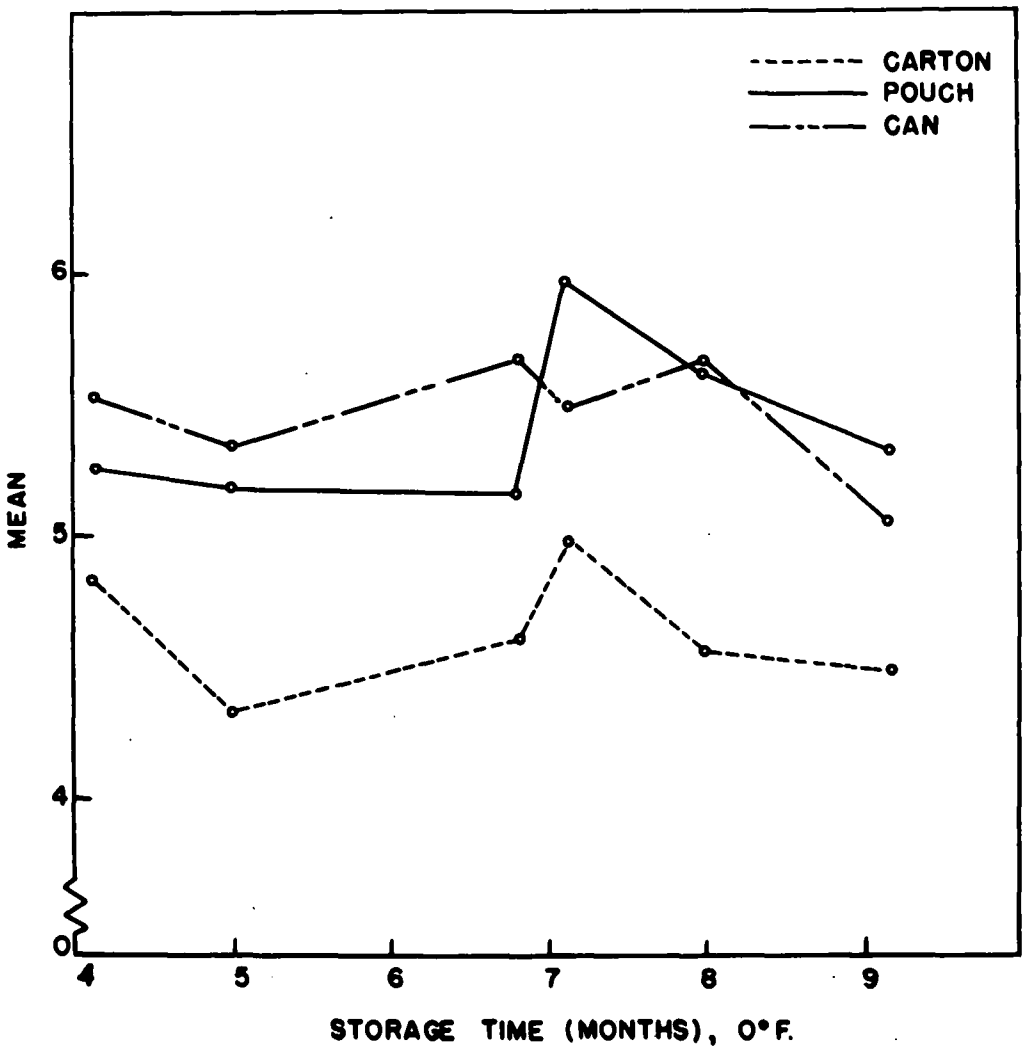
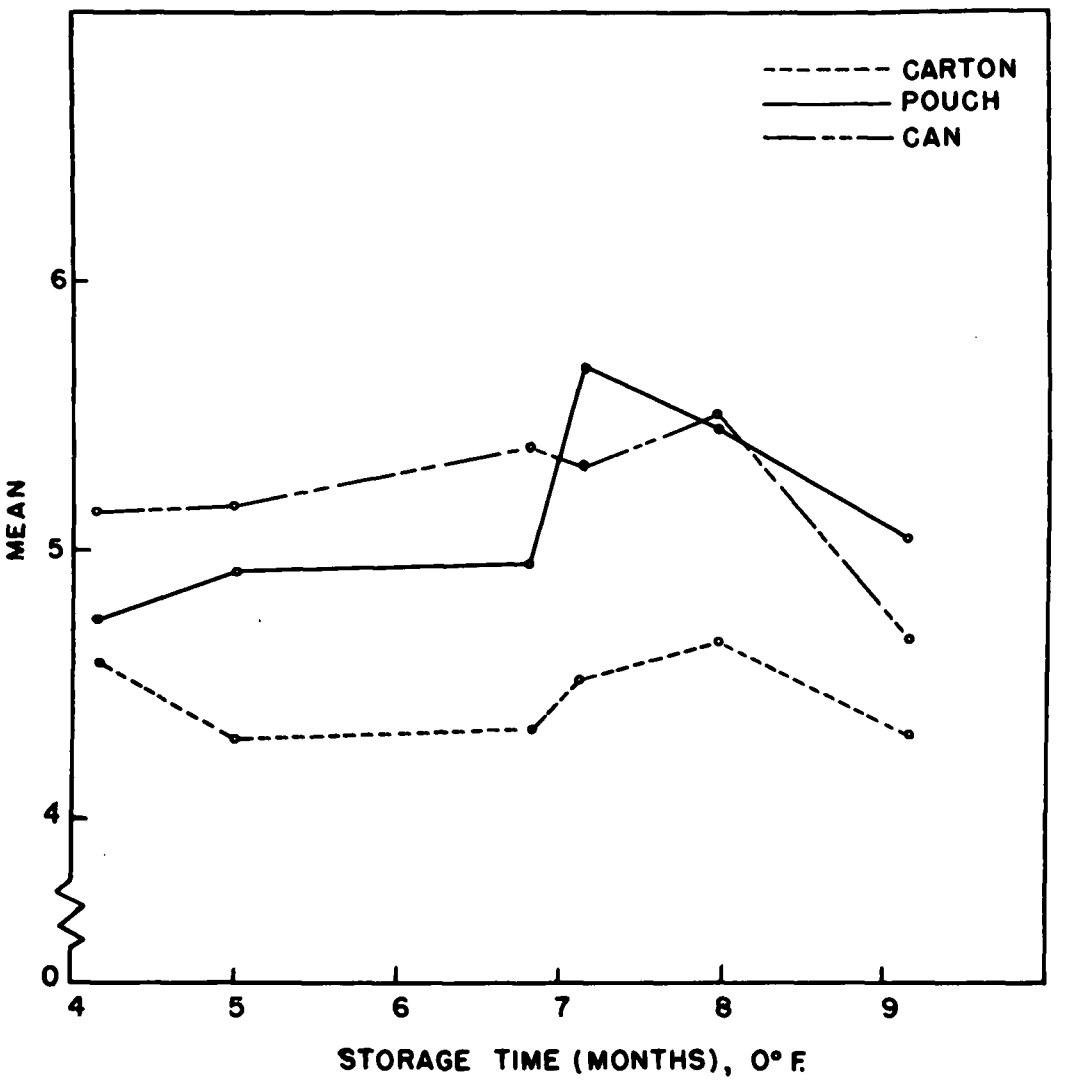


FIGURE 7. EFFECT OF PACKAGING CONTAINERS ON OVERALL DESIRABILITY OF FROZEN ROCKFISH FILLETS AS INDICATED BY SENSORY EVALUATION.



were plotted against storage time (Figure 8), and clearly substantiate the results indicated by the sensory evaluations for rancidity. However, the chemical analysis indicated that the degree of rancidity increased with storage time and that this increase was greatest in those fillets packaged in commercial cartons.

Although both methods of evaluation (Figure 9) point out that the commercial carton offers the least protection against autoxidation of frozen rockfish fillets, a positive relationship could not be shown due to insufficient data.

FIGURE 8. EFFECT OF PACKAGING CONTAINERS ON DEGREE OF OXIDATIVE RANCIDITY IN FROZEN ROCKFISH FILLETS AS DETERMINED BY CHEMICAL EVALUATION.

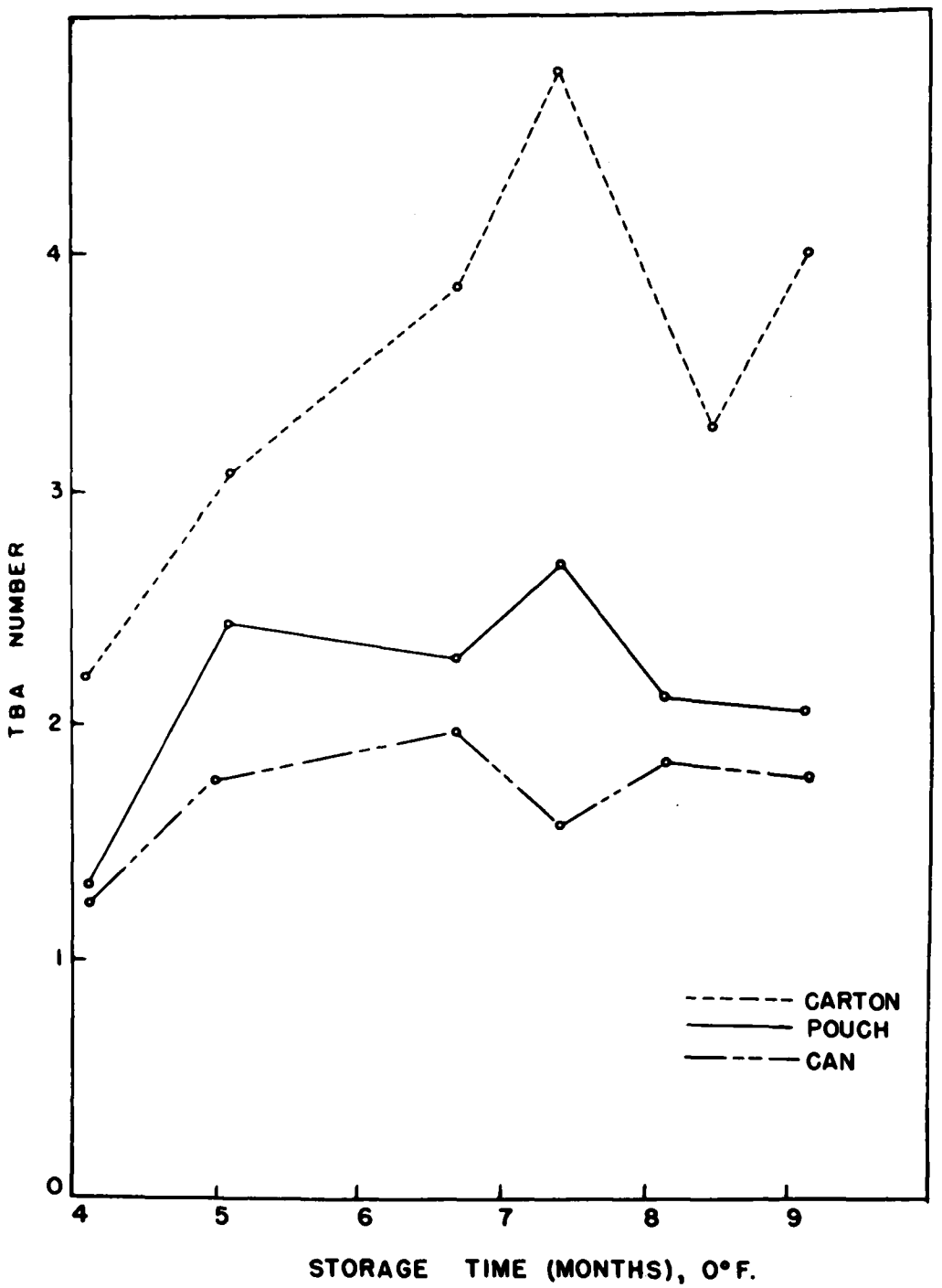
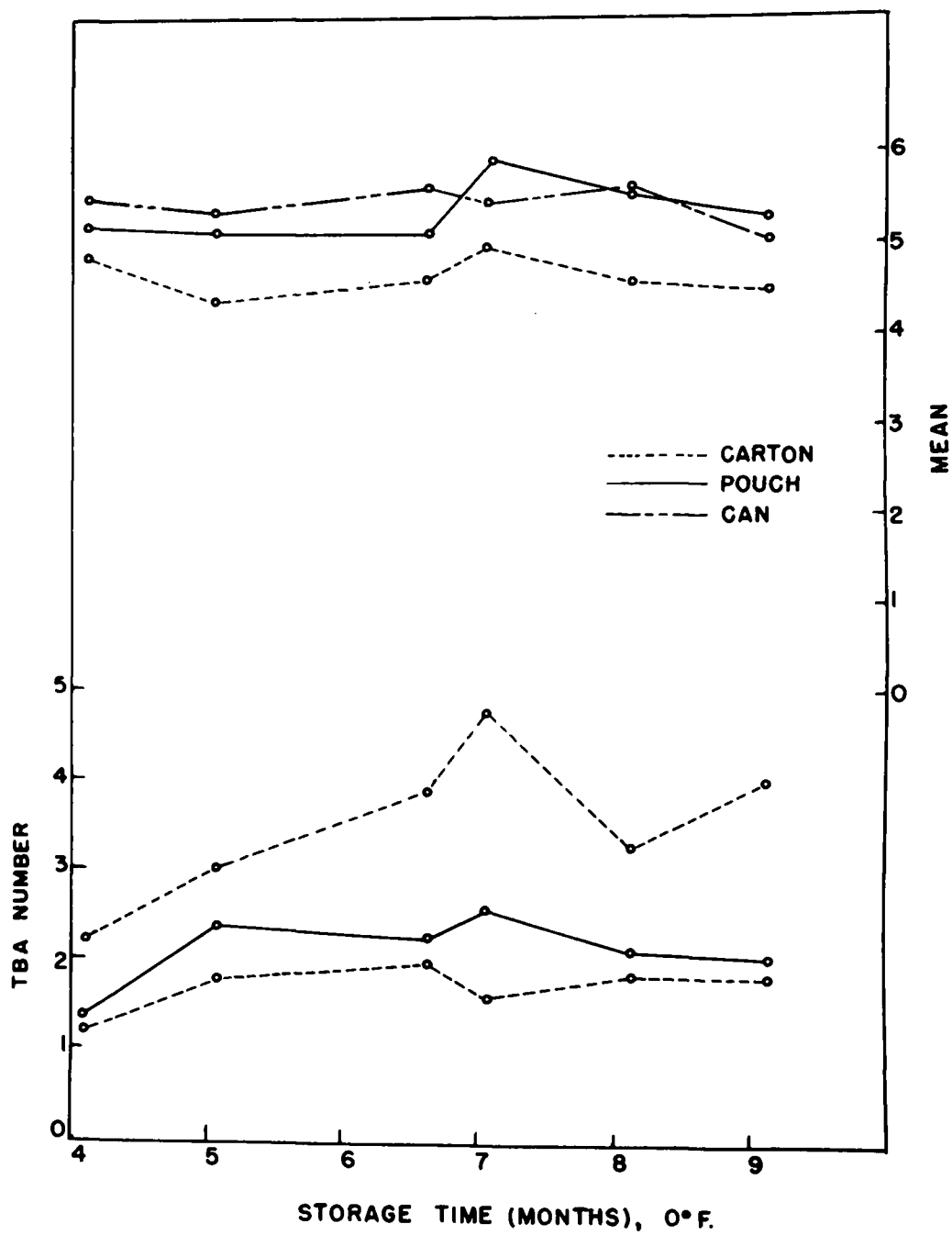


FIGURE 9. A COMPARISON OF SENSORY AND CHEMICAL EVALUATIONS FOR OXIDATIVE RANCIDITY RESULTING FROM VARIOUS PACKAGING TREATMENTS OF FROZEN ROCKFISH FILLETS.



SUMMARY AND CONCLUSION

An accelerated autoxidation procedure for frozen seafoods and fats and oils was presented. This method depends on the use of a purified diatomaceous silica, Celite Analytical Filter-Aid, as a support for the material to be tested. The increase in surface area that results, permits the autoxidation reaction to proceed at an accelerated rate, even at sub-freezing temperatures.

The effect of temperature on the rate of autoxidation was determined as well as the effect of fluctuating temperatures on the storage of frozen seafoods. The application of this procedure to evaluate antioxidants was demonstrated.

Representative types of packaging containers were used to demonstrate the effect of packaging on the rate of oxidative rancidity in frozen seafoods. A possible relationship between chemical and sensory evaluations was also shown.

The use of this accelerated autoxidation procedure for the evaluation of processing variables pertaining to certain frozen vegetables and precooked frozen foods was suggested.

Conclusions.

1. The necessity of an inert matrix as a support media for the proposed accelerated autoxidation procedure was shown by the catalytic effect of iron (expressed as ferric oxide) on the frozen seafood samples.

2. An increase in storage temperature, as well as fluctuating

temperatures, is shown to greatly increase the rate of oxidative rancidity in frozen seafoods. The need for storing seafoods at a constant temperature of 0°F. or below was demonstrated.

3. The storage life of frozen silver salmon, as evaluated by the TBA method, was noticeably extended by the incorporation of an NDGA antioxidant solution with the ground fish flesh.

4. The proposed accelerated autoxidation procedure permitted the very rapid evaluation of quality in frozen seafoods as affected by temperature, catalysts and antioxidants.

5. Analysis of variance calculations of taste panel evaluations for tenderness, rancidity, and overall desirability indicated that a significant difference existed between the treatment means for rancidity and overall desirability but not for tenderness.

6. The method of least significant difference indicated that both the plastic pouch and the tin can were significantly different from the commercial carton as concerns rancidity and overall desirability. There was no significant difference between the pouch and the can.

7. It was shown that sensory evaluation of frozen rockfish fillets as to overall desirability is primarily dependent on the degree of rancidity present in the sample.

8. The greater susceptability to autoxidation of frozen fish packaged in commercial cartons as compared to Mylar/Saran/Poly laminated pouchs and "C" enameled tin cans was shown by both sensory and chemical evaluations. This would tend to indicate that a possible relationship existed between sensory and chemical evaluations for oxidative rancidity in packaged, frozen seafoods.

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