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GALLIC ACID ESTER ANTIOXIDANTS FOR FISH OILS

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ABSTRACT

The report is a record of experiments conducted in an investigation at the College Park Laboratory as a wartime project to obtain information on the development of and the effectiveness of various gallic acid ester antioxidants in preventing the development of rancidity in fish oils and in inhibiting the decomposition of vitamin A in fish oils. A number of gallic acid ester antioxidants were prepared in the laboratory and tested with various fish oils. Several of the gallic acid esters served as good antioxidants. The alkyl gallates offered some protection to shark liver oils against the loss of vitamin A when the oils were blown with oxygen.

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INTRODUCTION

Considerable losses are suffered yearly in stored fishery products through deterioration of the oils contained therein. This degradation is manifested in the development of a rancid flavor, off odor, and discoloration of these products. Rancidity also is associated with the loss of vitamin A of fishery products, particularly in liver oils.

A large number and variety of compounds have been proposed as inhibitors of the development of rancidity in fats and oils. Some are particularly effective in vegetable oils, while others are more adaptable for use in animal fats. Most of these antioxidants have been developed for use in lard, shortenings, oleomargarine or other foods which usually contain a high percentage of fat. These generally contain little if any highly unsaturated fatty acids. Comparatively little work has been done on preventing or retarding rancidity in fish oils. These oils contain highly unsaturated fatty acids and are subject to serious losses because of rancidity. Therefore, an investigation was begun at the Service Laboratory in College Park, Maryland, as a wartime project to obtain information on the effectiveness of various antioxidants in preventing the development of rancidity in fish oils.

The objects of the experiments were: (1) to develop materials which would be effective antioxidants for highly unsaturated oils and (2) to develop materials which would inhibit decomposition of vitamin A in fish oils.

During the course of some earlier investigations conducted by the author and associates in another laboratory, it was found that protection was given to natural seed oils and to crude vegetable oils by substances of a phenolic nature which were related to the tannins. Various concentrates of tannins from tea and other sources were found to be quite effective in preventing rancidity in these vegetable oils. Later, gallic acid, which had been reported suitable by Golumbic and Mattill (1942), was found to be very effective as an

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NOTE: Chronic toxicity experiments with some of these antioxidants have been conducted at this Laboratory and are reported in the next article in this issue, "Feeding Tests With Gallic Acid Ester Antioxidants," pp. 19-20.

antioxidant but had the disadvantage of being rather insoluble in oil. It was found that 10 percent gallic acid in cottonseed oil, which had been partially hydrogenated as a mixture, had a strong antioxidant effect when added in small amounts to vegetable or animal oils. It is probable that under conditions of the hydrogenation, a triglyceride was formed with the gallic acid radical introduced in place of one or more of the fatty acid radicals. In the present investigation, the more promising antioxidants were incorporated into preparations of similar nature.

No conclusive evidence of the exact chemical nature of the autoxidation of fats and oils is available. It has been shown that rancidity of fats is associated with the formation of an ozone at the ethylenic double bond, or formation of monohydroperoxides, which in turn can and probably do act as catalysts to accelerate the production of additional unstable peroxides followed by the formation of aldehydes and ketones.

This is a chain reaction involving the activation of further molecules of the autoxidizable substance with the attendant liberation of energy in excess of that necessary to activate the same number of subsequent molecules. That a series of reactions induced at an ever-increasing rate takes place has been shown by the characteristic curves for rate of peroxide formation in natural fats. For fats containing no antioxidants, either natural or otherwise, the rate of peroxide formation immediately begins to increase logarithmically. The peroxide formation in fats containing antioxidants increases at a constant rate until the so-called end of the induction period is reached, at which time the antioxidant is largely destroyed. Thereafter, the rate of increase in peroxide formation is about the same as for a fat containing no antioxidant.

The mechanism of inhibition of rancidity is probably as follows: The esters of gallic acid and fatty acids combine to form triglycerides which are large molecules similar in size to the fatty glycerides themselves. These probably preferentially absorb the great amounts of the activation energy which are released and which normally cause the formation of peroxides. With efficient antioxidants there should be a constant slow rate of oxidation which is indicated by a straight-line relationship of peroxide formation with time.

Synthetic fatty triglycerides have been prepared successfully by direct esterification of fatty acids and glycerol. This method was used in these studies. Dry carbon dioxide was used as a catalyst for the reaction and to remove the water formed during esterification.

The glyceride gallate antioxidants are thick, viscous liquids. In making them, however, it is very possible that a small quantity of pyrogallol, or an ester of pyrogallol is formed during the synthesis. This was indicated by a brown tint which formed when they were treated with ferric chloride, as well as the blue-black color which developed with the gallates. The presence of the small quantity of substances containing pyrogallol was discovered late in the progress of this work. Pyrogallol probably has no toxic properties in the amounts present since no adverse effects were noted in rats fed the antioxidants in levels approximating five times the quantity used in oils as antioxidants. These chronic toxicity tests were then in progress for more than a year.

Chemically these gallic acid esters are similar to fatty acid modified alkyl resins. Substances of this nature are not crystallizable and do not distill without decomposition even under high vacuum. They are, therefore, practically impossible to separate in pure form. It was recognized that

there was a good possibility that migration of the hydroxyl groups might occur, and that the esterification reaction as indicated by formula would not be complete. There was also the possibility of cross polymerization and esterification of fatty acids with gallic acid hydroxyl groups. The characteristic blue-black color formed by the gallic acid radical with ferric chloride was depended upon as an analytical index to reveal the presence of this group. The insolubility of free gallic acid in oil and the values of the acid numbers found were used as an indication of the completeness of esterification.

The direct esterification procedure was used as it is a comparatively simple technique and could be carried out with equipment generally available, such as in a varnish kettle. These types of esters probably could be produced with fewer side reactions by esterifying triacetyl gallic acid or 4-methyl gallo-etheric acid with mono- and di-glycerides of fatty acids, followed by reestablishment of the gallic hydroxyl groups through hydrogenation or reduction with zinc and acid. The shorter and more direct method of production was chosen because any materials produced would have to be tested for chronic toxicity regardless of their composition. If no toxicity is indicated, it is the preferred method.

In the tests reported herein, all of those materials which contain gallic acid or its esters were added to the substrates in amounts equivalent to 0.1 percent of gallic acid radical. The other materials tested for comparative purposes were added at the 0.1-percent level unless otherwise stated.

During the course of oxidation of various deodorized fish oil substrates using a modified Swift test (King et al, 1933), it was found under the conditions of testing that the oils uniformly became rancid to taste at a peroxide value (p.v.) of about 20 millimoles per kilogram of oil. This peroxide value, therefore, has been taken as the value for comparison in calculating the protective factor for any particular antioxidant. The protective factor equals the time to reach p.v. 20 for the treated oil divided by the time to reach p.v. 20 for the untreated oil.

In evaluating antioxidants as inhibitors of vitamin A destruction, two protective factors were calculated. These were based on the ratio of the time necessary to destroy 10 and 20 percent of the vitamin content in a treated oil, to the time necessary to destroy 10 and 20 percent of the vitamin content in the untreated oil.

METHODS OF ANALYSES

PEROXIDE VALUES: Peroxide values were measured by a modification of the Wheeler method (1932). Samples of oil from 0.1 to 1 g. in weight, and which required a titration of not more than 15 ml. sodium thiosulfate solution, were weighed into small weighing vials. The vials plus samples were placed directly into dry, glass-stoppered 200 ml. Erlenmeyer flasks containing 50 ml. of a mixture of two parts of glacial acetic acid and one part of chloroform. After thorough mixing, one ml. of saturated potassium iodide was added, and the flask was held in the dark for three minutes. Fifty ml. of water were added together with a little starch solution, and the mixture was titrated with .002 N sodium thiosulfate. The data were expressed as millimoles of peroxide per kilogram of oil.

VITAMIN A: The vitamin A determinations were made by direct solution of the oil in isopropyl alcohol and a photometric estimation of absorption in a United Drug Company Vitamin A Meter. Some determinations were made using the unsaponifiable portion of the oil to rule out possible absorption by saponifiable materials added to the oils.

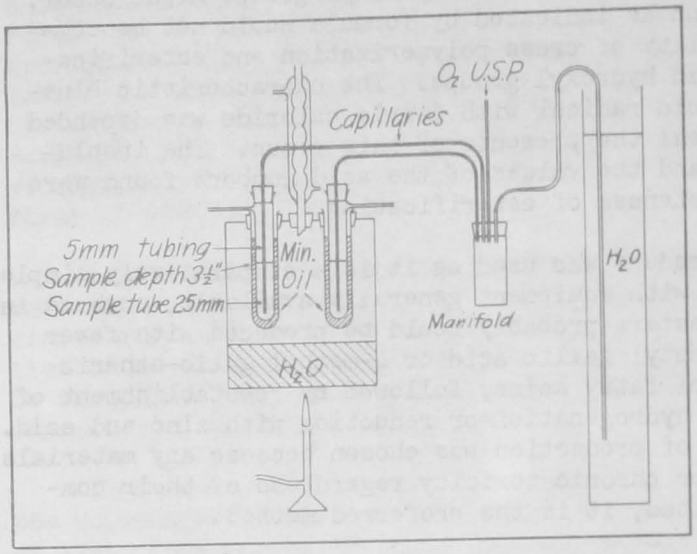


FIGURE 1 - RAPID TEST APPARATUS.

RAPID TEST FOR OXIDATION: The apparatus used embodies the principles of the well-known Swift stability test apparatus (King et al, 1933) except that in these experiments U.S.P. oxygen from a pressure cylinder was used (Fig. 1). The apparatus consists of a metal container fitted with a reflux condenser to return the moisture and to maintain a constant temperature of 100° C. within the boiling water bath. Large test tubes containing mineral oil are fitted into this metal container. These serve as oil baths in which the smaller test tubes containing the test sample are immersed. A manifold system is provided for introducing oxygen at a pressure of about 14 inches of water. The pressure is controlled by a water column manometer. Capillaries 0.1 mm. or less in diameter are placed in the bubbling-tube lines and are calibrated to allow a flow of 50 ml. of oxygen per minute at the before-mentioned manifold pressure. Pyrex test tubes, 25 mm. in diameter, are used as sample tubes. To these are sealed, near the top, horizontal short lengths of 7 mm. tubing which serve to limit the depth of immersion of the tubes in the oil bath and which are used as exit tubes for the oxygen. The gas flowing from these tubes is sniffed for rancid odors to determine how oxidation is proceeding.

All glass parts of the apparatus that came in contact with the samples were thoroughly cleaned with a wetting agent, acetone, and tap water. The parts were immersed in concentrated nitric acid at a temperature of about 90° C. for at least 3 hours, rinsed at least 10 times with tap water, and at least 6 times with distilled water, and oven-dried.

The various antioxidants tested were dissolved in the oil used as substrate by stirring with a clean glass tube through which passed a stream of hydrogen gas. Very gentle heat was used only with those samples which would not dissolve otherwise.

The oxidation apparatus was brought to operating temperature, which required about 10 minutes, before any samples were placed in it. The sample tubes were filled to a depth of 9 cm. and the bubbling tubes were inserted. The samples were allowed to remain in the apparatus without bubbling for 15 minutes to allow them to reach the required temperature. The oxygen system had been previously turned on without a connection with the sample tubes in order to flush out the air. At the end of the 15 minutes, the tubes were connected with the oxygen system.

Samples for the determination of peroxide value were taken periodically by momentarily interrupting the oxygen flow and using the bubbling tube as a sampler. Two samples were run simultaneously in this apparatus. The temperature of the oil in the sample tubes was determined periodically by using a similar tube which contained a thermometer. The temperature was found to remain constant at 99.5° C.

For the oxidation of oil samples to determine stability of vitamin A, the same apparatus was employed except that smaller sample tubes (15 x 150 mm.) were used. These were filled with oil to a depth of 6.5 cm.

STORAGE TESTS: Some samples were stored in glass bottles in the dark at 37° C. The glass bottles used were cleaned by the same methods as previously outlined for the rapid oxidation test. The bottles used were 5 cm. in diameter and 9.5 cm. deep and had mouths 3.75 cm. in diameter. Each bottle contained 50 g. of sample. The bottles were stoppered loosely with new cork stoppers.

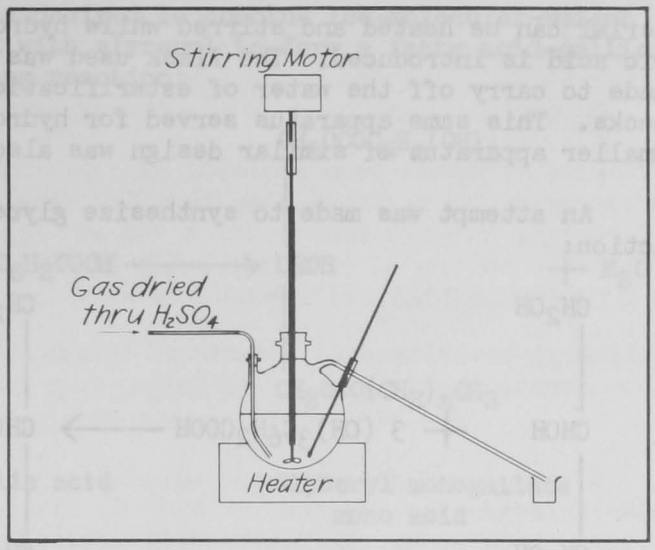


FIGURE 2 - ESTERIFICATION APPARATUS.

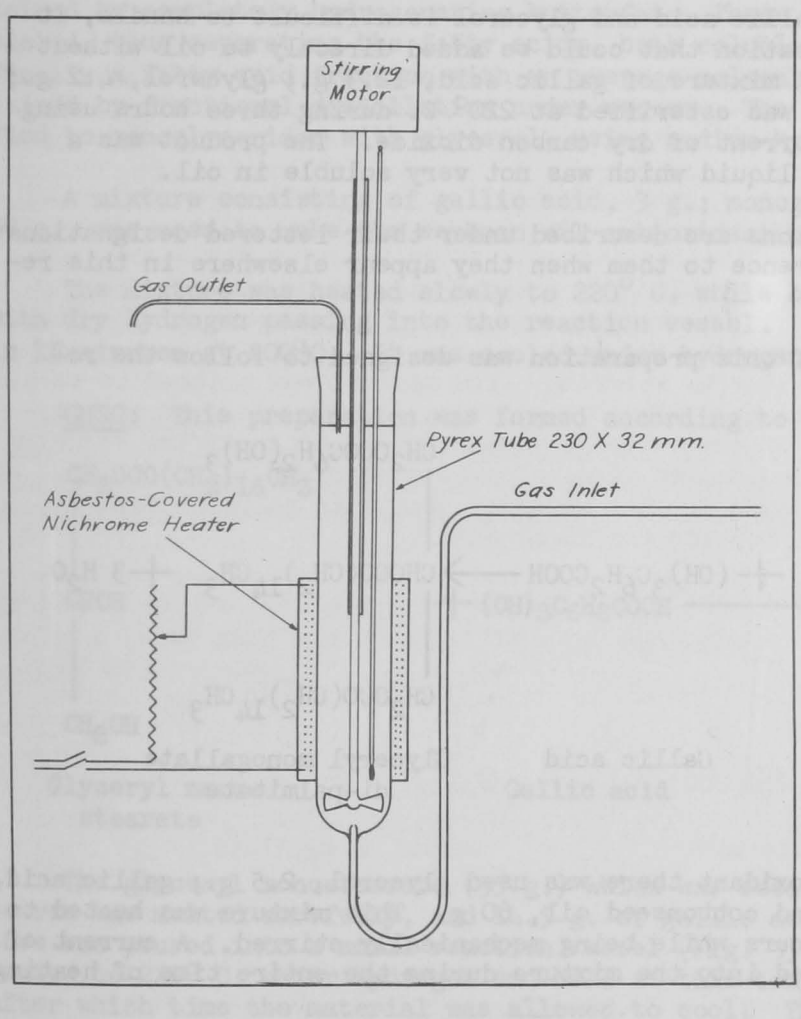


FIGURE 3 - ESTERIFICATION APPARATUS.

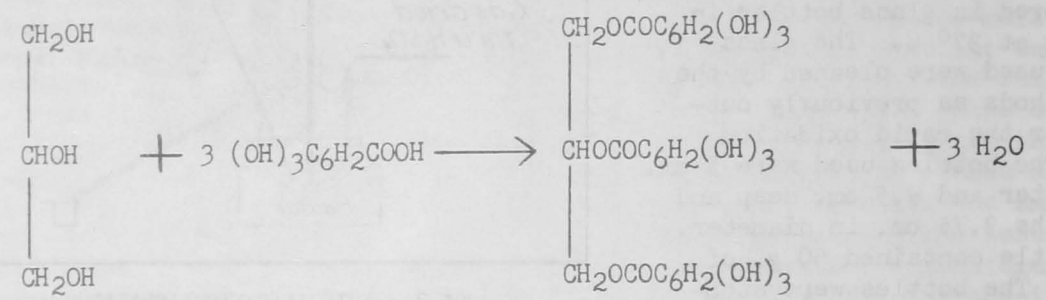
Samples for peroxide determination were taken from these bottles periodically by means of a clean glass tube. This sampling tube was used to thoroughly stir the oil, care being taken to avoid splashing oil on the sides of the bottles. This precaution was taken to avoid the formation of thin films of oil in the bottles which would subsequently oxidize rapidly, catalyzing the oxidation of the remainder of the sample.

PREPARATION OF ANTIOXIDANTS

The alkyl gallates were prepared by direct esterification using dry hydrochloric acid as a catalyst and crystallizing them from suitable solvents. The apparatus (Fig. 2) used to prepare most of the antioxidant preparations is a modified round-bottom, three-neck flask fitted with a thermometer well. The ma-

terial can be heated and stirred while hydrogen, carbon dioxide or dry hydrochloric acid is introduced. The flask used was of one liter capacity. Provision is made to carry off the water of esterification with a side tube from one of the necks. This same apparatus served for hydrogenation at atmospheric pressure. A smaller apparatus of similar design was also made from a pyrex tube (Fig. 3).

An attempt was made to synthesize glyceryl trigallate according to the reaction:

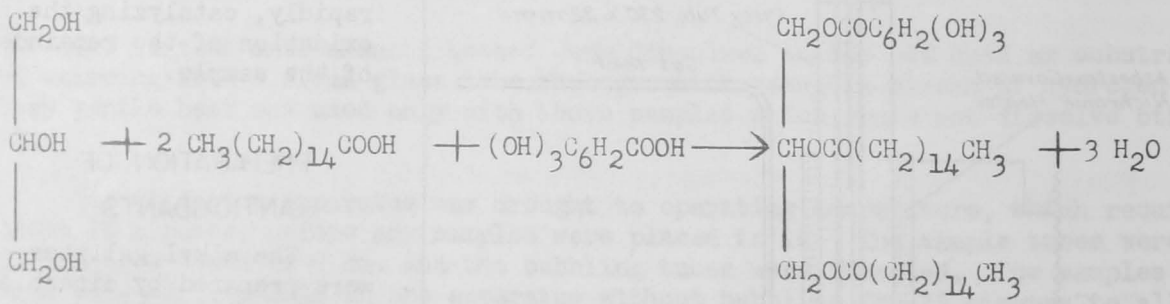


Glycerol Gallic acid Glyceryl trigallate

Because a mixture of gallic acid and glycerol is difficult to handle, it was desired to make a preparation that could be added directly to oil without first removing a solvent. A mixture of gallic acid, 12.3 g.; glycerol, 4.2 g.; and cottonseed oil, 73.0 g. was esterified at 220° C. during three hours using mechanical stirring and a current of dry carbon dioxide. The product was a dark reddish-brown, viscous liquid which was not very soluble in oil.

The following preparations are described under their lettered designations in order to facilitate reference to them when they appear elsewhere in this report.

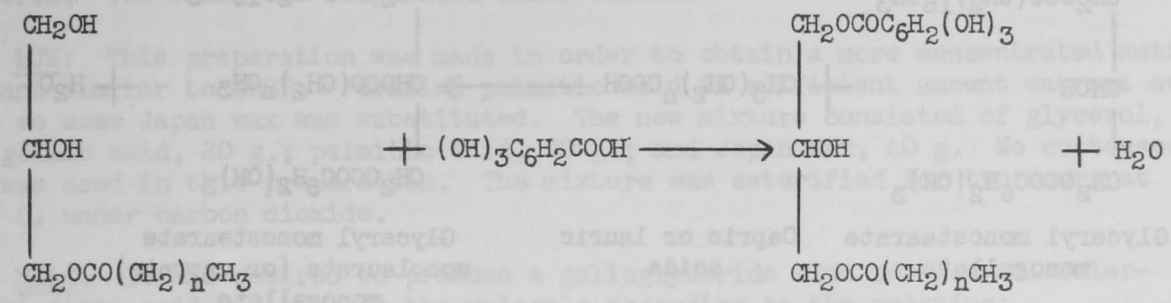
KMGP: The synthesis of this preparation was designed to follow the reaction:



Glycerol Palmitic acid Gallic acid Glyceryl monogallate di-palmitate

In preparing this antioxidant there was used glycerol, 2.5 g.; gallic acid, 2.5; palmitic acid, 6 g.; and cottonseed oil, 60 g. This mixture was heated to 200° to 210° C. for three hours while being mechanically stirred. A current of dry carbon dioxide was passed into the mixture during the entire time of heating and cooling.

KHMD: In this preparation it was desired to combine low-molecular-weight saturated fatty acids and gallic acid with glycerol to form a fatty acid-gallic di-glyceride according to the following reaction:



Monoglyceride of fatty acids having a length of 8 to 14 carbon atoms, with an average of 12 carbon atoms.

Gallic acid

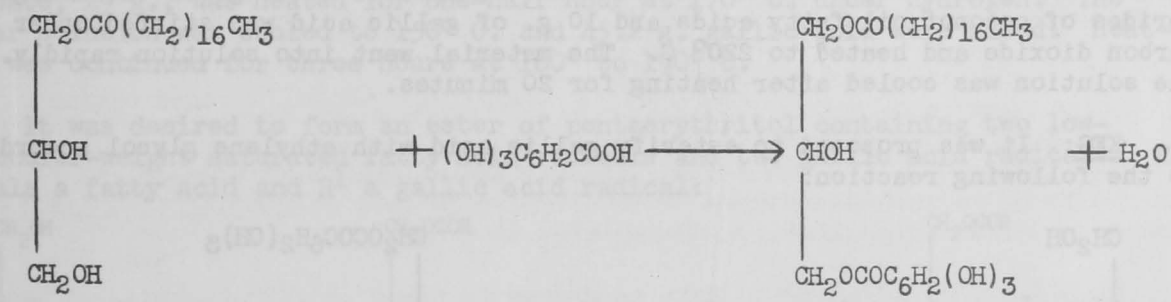
Glyceryl monogallate mono acid

The monoglycerides of low-molecular-weight saturated fatty acids were obtained by completely hydrogenating butterfat. These were saponified and acidulated, thus separating the fatty acids, both soluble and insoluble in water. Finally a fatty acid fraction with an average molecular weight of 218 was obtained by fractional distillation under vacuum. The fatty acids were esterified to monoglycerides with glycerol, using sodium hydroxide as a catalyst.

A mixture consisting of gallic acid, 3 g.; monoglycerides, 7 g.; and oil, 50 g., was used to make the soybean oil antioxidant preparation.

The mixture was heated slowly to 220° C. while being mechanically stirred with dry hydrogen passing into the reaction vessel. The solution became clear in 15 minutes at 200°C. It was cooled under hydrogen.

GMSG: This preparation was formed according to the following reaction:



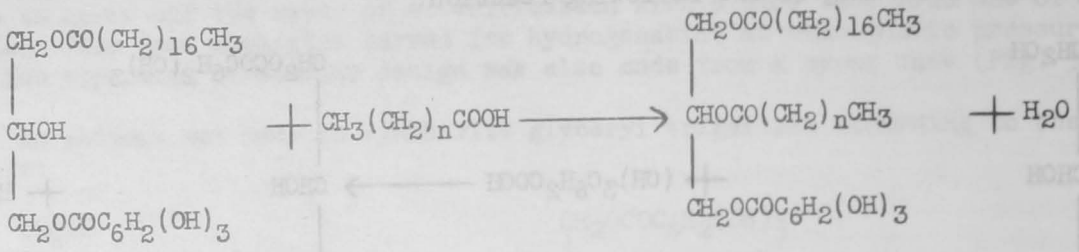
Glyceryl monostearate

Gallic acid

Glyceryl monostearate monogallate

The glyceryl monostearate (35 g.) which was obtained from a commercial source was melted carefully, and 14.5 g. of gallic acid were added. The mixture was poured into a small reaction vessel (Fig. 3). It was stirred and heated to 220° C. under hydrogen and held at this temperature for 39 minutes after which time the material was allowed to cool. The resultant product was a light-brown liquid which cooled to a brittle solid.

GSCG: This preparation was designed to introduce a low-molecular-weight fatty acid into the glyceride molecule GMSG.



Glyceryl monostearate monogallate | Capric or lauric acids | Glyceryl monostearate monolaurate (or caprate) monogallate

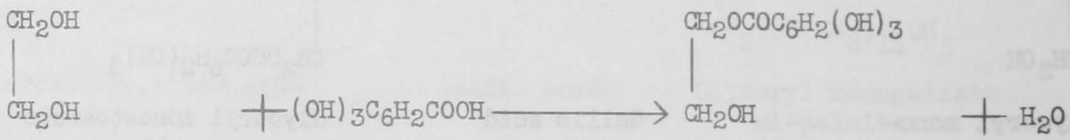
A mixture of glyceryl monostearate, 140 g., and gallic acid, 60 g., was held under hydrogen for one hour at 220° C. and cooled under hydrogen at 130° C.; 75 g. of low-molecular-weight fatty acids especially prepared from coconut oil were added. These had a mean molecular weight of 191. The temperature was brought to 230° to 240° C. and held for two hours. The product was cooled under hydrogen.

KMS40: This was similar to GMSG except that the reaction temperature was 225° C. and the time of heating was 40 minutes.

MCG: This preparation was similar to GMSG except that monoglycerides of low molecular weight fatty acids were used instead of glyceryl monostearate. The monoglycerides were made in the following manner:

Coconut oil was dried under vacuum. To 630 g. of oil were added 126 g. of a 20-percent solution by weight of U.S.P. glycerin containing 3.15 g. sodium hydroxide. The mixture was heated with continuous stirring under dry carbon dioxide. When the temperature reached 235° C., the mixture became clear. Heating was continued about 20 minutes. The mixture was then allowed to cool under carbon dioxide while being stirred. To the cool mixture was added 0.5 percent of 85 percent orthophosphoric acid with stirring. The mixture was allowed to settle overnight and the clear supernatant liquid was decanted. The yield of monoglycerides was about 80 percent of theoretical. A mixture of 40 g. of the monoglycerides of coconut oil fatty acids and 10 g. of gallic acid was stirred under carbon dioxide and heated to 220° C. The material went into solution rapidly. The solution was cooled after heating for 20 minutes.

KEG: It was proposed to esterify gallic acid with ethylene glycol according to the following reaction:

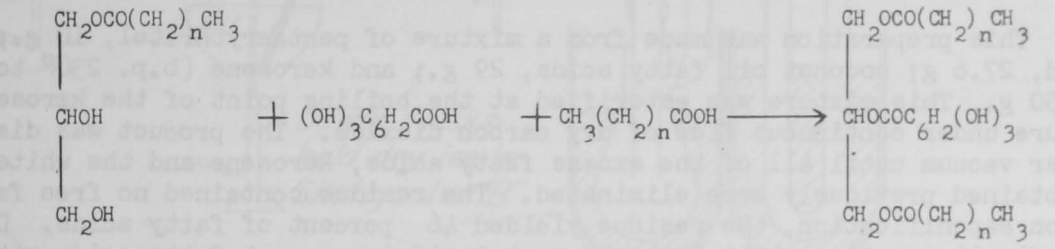


Glycol | Gallic acid | Glycol monogallate

Gallic acid with excess ethylene glycol was heated for eight hours at 190° C. under carbon dioxide. The excess glycol was removed by vacuum distillation. The residue was dissolved in ether, carbon black was added and the mixture was filtered. The ether was evaporated under vacuum.

KJW: This preparation was made in order to obtain a more concentrated antioxidant similar to KMGP. Purified palmitic acid in sufficient amount was not available so some Japan wax was substituted. The new mixture consisted of glycerol, 20 g.; gallic acid, 20 g.; palmitic acid, 20 g.; and Japan wax, 40 g. No cottonseed oil was used in this preparation. The mixture was esterified for two hours at 200° C. under carbon dioxide.

MCLG: It was desired to produce a galloglyceride with two low-molecular-weight fatty acid radicals in the molecule according to the reaction:



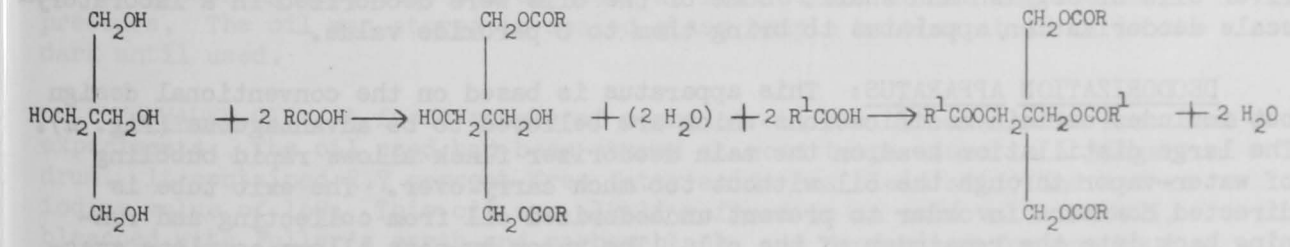
Glyceryl monolaurate or caprate — Gallic acid — Lauric or capric acid — Glyceryl dilaura- or capro-monogallate

It is possible, of course, that the gallic acid may attach itself to the terminal primary alcoholic group instead of the secondary position as shown. The mixture used consisted of monoglyceride of low-molecular-weight acid, 28 g.; low-molecular-weight fatty acid, 15 g.; and gallic acid, 7 g. This mixture was heated for two hours at 210° C. under carbon dioxide while being stirred mechanically.

PGC 190: It was planned to effect the formation of hydroxy-esters of pentaerythritol and coconut oil by alcoholysis (Burrell, 1944) and esterification of the free hydroxyl groups by gallic acid.

The mixture of coconut oil, 200 g.; pentaerythritol, 40 g.; and lithium lactate, 29 g.; was heated for one-half hour at 170° C. under hydrogen. The clear solution was cooled to 150° C. and 45.2 g. gallic acid were added. Heating was continued for three hours at 160° to 190° C.

It was desired to form an ester of pentaerythritol containing two low-molecular-weight saturated fatty acid radicals and two gallic acid radicals. R equals a fatty acid and R^1 a gallic acid radical:



Pentaerythritol — Fatty acid — Gallic acid — Ester of pentaerythritol

Gallic acid and pentaerythritol both being solids require a fluid medium when being esterified. Kerosene, the medium chosen, was shaken thoroughly with mercury to remove sulphur compounds, washed repeatedly with sulfuric acid to re-

move aromatic compounds, and distilled. A fraction boiling at 230° to 245° C. was selected. The mixture used consisted of gallic acid, 27.6 g.; pentaerythritol, 10 g.; coconut oil fatty acid, 29 g.; and kerosene, 50 g.

The coconut oil fatty acids were specially prepared by hydrogenation, saponification, acidulation and vacuum fractional distillation to obtain a fatty acid fraction that was completely saturated and had an average molecular weight of 197.

Esterification was carried out at 220° to 230° C. under a flow of carbon dioxide until the esters were in solution. This required about two hours. Upon distillation under vacuum, white crystals were deposited in the condenser along with the first fraction of kerosene to distill over. The crystals were collected and washed free of kerosene with petroleum ether. The crystals proved to be pyrogallol.

PGCR: This preparation was made from a mixture of pentaerythritol, 10 g.; gallic acid, 27.6 g; coconut oil fatty acids, 29 g.; and kerosene (b.p. 230° to 260° C.), 50 g. This mixture was esterified at the boiling point of the kerosene for 1.5 hours under continuous flow of dry carbon dioxide. The product was distilled under vacuum until all of the excess fatty acids, kerosene and the white crystals obtained previously were eliminated. The residue contained no free fatty acids. Upon saponification, the residue yielded 46 percent of fatty acids. Difatty acid digallo-pentaerythritol should contain 49.4 percent fatty acids with a molecular weight of 197. Some loss of low-molecular-weight fatty acids was expected through solution and volatilization, consequently the amount found was considered satisfactory.

CMGP: This product was made similar to KMGP but coconut oil rather than cottonseed oil was used because of the more saturated fatty acids. The mixture consisted of coconut oil, 400 g.; gallic acid, 25 g.; glycerol, 25 g.; and palmitic acid, 70 g. This mixture was heated for one-half hour at 175° C. under a flow of dry carbon dioxide with stirring. One g. of lithium lactate was added and the temperature was raised to 280° C. for 15 minutes. The preparation was allowed to cool slowly under the carbon dioxide with constant stirring.

A considerable number of other preparations were made during this study which proved unsatisfactory for one reason or another. The data are not reported.

PREPARATION OF SUBSTRATE OILS

The oils used as substrates in the testing of the various antioxidants were soybean; the body oils of menhaden, pilchard and salmon; seal blubber oil; and the liver oils of dogfish and shark. Some of the oils were deodorized in a laboratory-scale deodorization apparatus to bring them to 0 peroxide value.

DEODORIZATION APPARATUS: This apparatus is based on the conventional design but includes certain modifications which are believed to be advantageous (Fig. 4). The large distillation head on the main deodorizer flask allows rapid bubbling of water-vapor through the oil without too much carry-over. The exit tube is directed downward in order to prevent undeodorized oil from collecting and running back into the remainder of the oil. The vapor by-pass allows complete evacuation of air at the start and prevents bubbling of air through the oil at the end of a run when the vacuum is broken. Efficient traps provide maintenance of a high degree of vacuum.

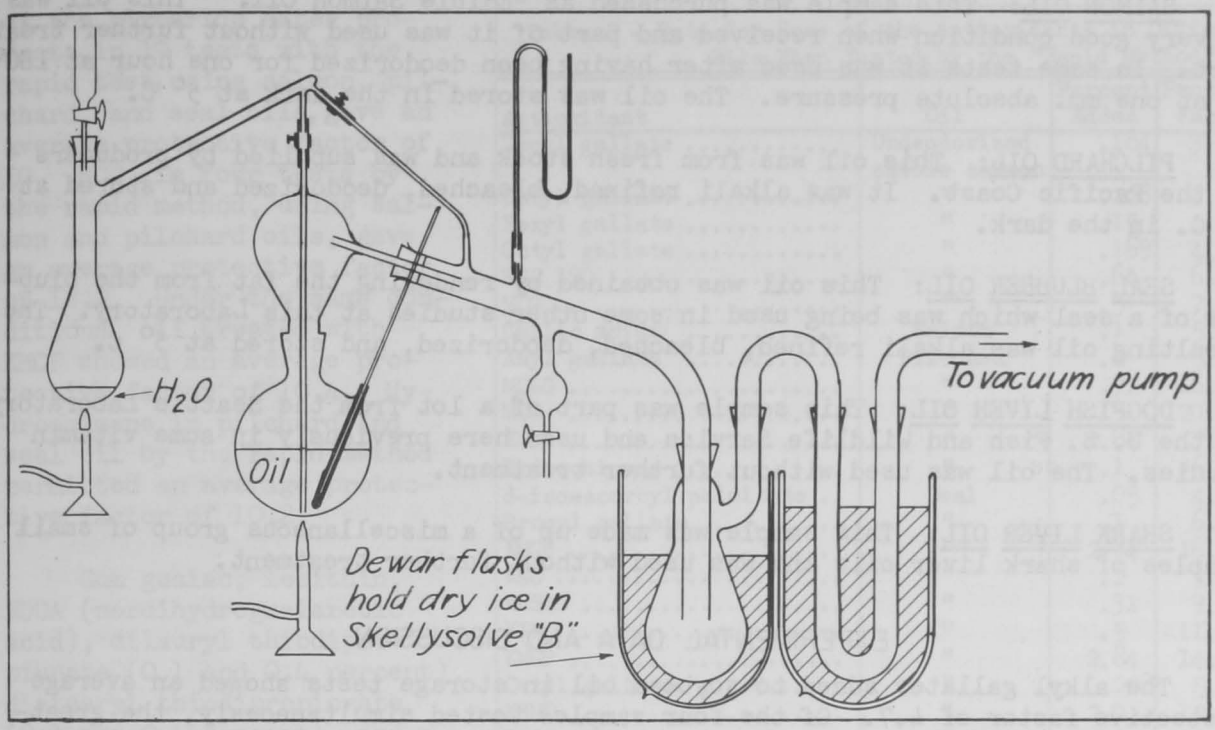


FIGURE 4 - OIL DEODORIZATION APPARATUS

A standard taper joint is used to connect the head with the main flask, which facilitates filling and cleaning. The flow of water-vapor is controlled by a Hoffman clamp which permits throttling of the tube connection at the top of the head. Direct heat instead of the usual oil bath could be used since a thermometer well was built into the apparatus. Controlled amounts of water-vapor were used to agitate the contents of the flask to prevent local heating. The modified apparatus also has a small flask with a stopcock sealed into the system to catch any carry-over of liquid. This prevents the accumulation and solidification of material in the two traps.

Information on the source of oils, description of oils and treatment given follows:

SOYBEAN OIL: This was a sample of crude solvent-extracted soybean oil. The oil was alkali refined and bleached according to the standard methods of the National Cottonseed Products Association (1945). The oil was deodorized in the deodoring apparatus for one hour at 180° C. and at one mm. absolute pressure. The oil was stored in sealed glass sample bottles at 5° C. in the dark until used.

MENHADEN OIL: A fresh sample of menhaden oil was not available for these experiments. The oil used had been stored at room temperature in a 55-gallon drum. It contained 2.7 percent free fatty acids (as oleic acid) and had an iodine value of 169. This oil was alkali refined to an acid value of .05, bleached with Fuller's earth and carbon black, and deodorized for one hour at 180° C. at one mm. absolute pressure. It was stored in sealed glass bottles in the dark at 5° C. until used.

SALMON OIL: This sample was purchased as "Edible Salmon Oil." This oil was in very good condition when received and part of it was used without further treatment. In some tests it was used after having been deodorized for one hour at 180° C. at one mm. absolute pressure. The oil was stored in the dark at 5° C.

PILCHARD OIL: This oil was from fresh stock and was supplied by producers on the Pacific Coast. It was alkali refined, bleached, deodorized and stored at 5° C. in the dark.

SEAL BLUBBER OIL: This oil was obtained by rendering the fat from the blubber of a seal which was being used in some other studies at this Laboratory. The resulting oil was alkali refined, bleached, deodorized, and stored at 5° C.

DOGFISH LIVER OIL: This sample was part of a lot from the Seattle Laboratory of the U. S. Fish and Wildlife Service and used here previously in some vitamin studies. The oil was used without further treatment.

SHARK LIVER OIL: This sample was made up of a miscellaneous group of small samples of shark liver oils and was used without further treatment.

EXPERIMENTAL DATA AND DISCUSSION

The alkyl gallates added to soybean oil in storage tests showed an average protective factor of 4.7. Of the four samples tested simultaneously, the greatest deviation from this average was a protective factor of 0.5 (Table 1). With menhaden oil, these same alkyl gallates showed in storage tests a protective factor of 2.7 with the greatest deviation within a series being a protective factor of 0.8. Six alkyl gallates used with salmon oil in the rapid test showed an average protective factor of 4.0 with the greatest deviation amounting to a protective factor of 0.7.

Table 1 - Data for Various Antioxidants in Soybean and Menhaden Oils Stored in Glass in the Dark at 37° C.

Antioxidant	Oil	Percent Added	Protective Factor[1]
Ethyl gallate	Soybean	.124	4.25
Methyl gallate	"	.117	5.12
KMGP	"	2.84	10.64
Decyl gallate	"	.184	4.2
Cetyl gallate	"	.228	5.2
Petroleum ether extract of gallic acid	"	Extract from 50 g. in 50 g. oil	1.62
Cetyl gallate	Menhaden	.228	2.5
Ethyl gallate	"	.124	2.5
Methyl gallate	"	.117	2.5
Glycol monogallate	"	.133	3.25
Decyl gallate	"	.184	3.34
KMGP	"	2.84	12.00
Gallic acid[2]	"	.1	3.0

1/ Time to reach peroxide value of 20 for treated oil. Time to reach peroxide value of 20 for untreated oil.
2/ In deodorization apparatus for 10 minutes at 200° C.

The average protective factor for the alkyl gallates according to the rapid test (Table 2) and using salmon, pilchard, and seal oils, was 4.7. The low protective factors obtained with menhaden oil are probably due to the bad condition of the original oil together with a probable high iron content due to long storage in a steel drum while having a high free fatty-acid content.

Under the storage conditions used, antioxidant KMGP, the one glycerol ester antioxidant available at the time the storage tests were started, showed protective factors of 10.6 and 12.0 with soybean and menhaden oils, respectively. Ten

of the glyceride ester products, in 19 tests with the rapid test, using salmon, pilchard, and seal oils, gave an average protective factor of 10. MCG, in four tests by the rapid method, using salmon and pilchard oils, gave an average protective factor of 12.2. Under the same conditions, oil treated with KMGP showed an average protective factor of 10.4. Hydroquinone in pilchard and seal oil by the rapid method permitted an average protective factor of 10.2.

Gum guaiac, lecithin, NDGA (nordihydroguaiaretic acid), dilauryl thiodipropionate (0.1 and 0.4 percent), distearyl thiodipropionate (0.1 and 0.4 percent), and a mixture of 0.02 percent thiodipropionic acid and 0.08 percent dilauryl thiodipropionate, according to the rapid test, induced an average protective factor of 1.3. Another sample of beta,beta thiodipropionic acid in salmon oil, however, gave a protective factor of 5.9 by the rapid test.

The sample of NDGA received arrived late in the progress of this work and just one test was conducted using this material. In this one test, a protective factor of only 1.9 was obtained by the rapid method.

Thiodipropionic acid gave a protective factor of 5.9 in salmon oil when introduced before deodorization but this same acid and its esters gave little or no protection to salmon oil when introduced in the manner described by the producers of these materials. Resin guaiac, mixed tocopherols and lecithin lengthen the keeping quality of salmon oil very little (Table 2).

Table 2 - Data for Some of the Antioxidants in Different Oils When Tested by the "Rapid Method"

Antioxidant	Oil	Percent Added	Protective Factor[1]
Decyl gallate	Undeodorized edible salmon	.184	3.29
Cetyl gallate	"	.228	3.72
Hexyl gallate	"	.15	4.29
Octyl gallate	"	.169	4.29
PGC 190	"	.64	6.71
MCG	"	.5	7.85
Gallic acid[2]	Salmon	.1	5.24
Amyl gallate	Pilchard	.2	5.2
MCLG	"	.72	16.5[3]
MCG	"	.5	20.0
KMGP	"	2.84	7.0[3]
Hydroquinone	"	.1	6.16
d-isoascorbyl palmitate	Seal	.05	4.56
Propyl gallate	"	.1	6.28
KMGP	"	2.84	16.3
KEG	"	.2	3.8
KMS40	"	.31	9.7
KJW	"	.5	11.0
KMGP	"	2.84	14.4
Cetyl gallate	"	.228	5.8
KHMD	"	2.0	12.0
Hydroquinone	"	.1	14.2
MCG	Salmon	.5	11.5
CMGP	"	2.1	10.1
MCG	Pilchard	.5	9.66
Resin guaiac[2]	Salmon	.1	1.31
Mixed tocopherols[2]	"	.02	1.46
Lecithin[2]	"	.1	1.66
Beta, beta thiodipropionic acid[2]	"	.1	5.85
Propyl gallate	"	.1	4.0
GSCG	"	.46	5.38
CMGP	"	2.1	9.0
KHMD	Undeodorized edible salmon	2.0	4.07
KMGP	"	2.84	4.07
MCLG	"	.72	8.0
NDGA	Salmon	.1	1.86
Dilauryl thiodipropionate	"	.1	1.00
" "	"	.4	1.67
Distearyl "	"	.1	1.00
" "	"	.4	1.54
Thiodipropionic acid	"	.02	-
Dilauryl thiodipropionate	"	.08	1.00[4]

1/ Time to reach peroxide value of 20 for treated oil. / Time to reach peroxide value of 20 for untreated oil.

2/ In deodorization apparatus for 10 minutes at 170° C.

3/ Protective factor on this sample was calculated as follows: Time to reach peroxide value of 10 for treated oil. / Time to reach peroxide value of 10 for untreated oil.

4/ The oil substrate in this case had been deodorized two weeks before use and had an initial peroxide value of eight.

Table 3 - Data on Stability of Vitamin A in Liver Oils When Tested by the "Rapid Method"

Antioxidant	Oil	Percent Added	Protective Factor 1/ At 10% Depletion	Protective Factor 1/ At 20% Depletion
Cetyl gallate	Dogfish, 20,000 U.S.P. units Vitamin A per g.	.228	3.57	4.33
Octyl gallate	"	.189	6.00	6.45
Decyl gallate	"	.184	5.71	5.9
Amyl gallate	"	.147	5.71	6.33
KMGP	"	2.84	12.7	12.27
KHMD	"	2.00	15.15	16.00
PGCR	"	.24	2.79	3.14
Crystals from PGCR	"	.24	8.82	13.7
Hydroquinone	"	.1	14.7	14.1
None	Dogfish, deodorized 30 min. at 150° C.	-	1.6	1.6
Amyl gallate	"	.147	11.38	10.0
Crystals from PGCR	Unsaponifiable matter from shark oil in cocomut oil 80,000 U.S.P. units Vitamin A per g.	.24	1.74	1.84
Crystals from PGCR	Shark		10.4	8.1

1/Protective Factor = Time to reach 10 (or 20) percent depletion of vitamin A for treated oil. / Time to reach 10 (or 20) percent depletion of vitamin A for untreated oil.

In seal oil, d-isoascorbyl palmitate with propyl gallate, 0.05 percent each, gave a protective factor of 4.5, while propyl gallate alone at 0.1 percent gave a protective factor of 6.0. The d-isoascorbyl palmitate was not tested alone.

Data on the effect of the use of the antioxidants in various oils are shown in Figures 5 to 20. (See pages 15-17)

In respect to stability of vitamin A, the alkyl gallates tested produced an average protective factor (at 10 percent depletion) of 5.3 (Table 3). One sample of dogfish liver oil was deodorized with amyl gallate in solution and was subsequently oxidized. This process gave a protective factor of 11.4 or just about double that found when amyl gallate had been simply dissolved in the oil. KMGP, KHMD and hydroquinone gave protective factors of 12.7, 15.2 and 14.7, respectively. Figures 21 to 25 present data on shark liver oils showing the effect of added antioxidants on the vitamin A content of the oils when blown with oxygen. (For Figures 21 to 25 see page 18.)

CONCLUSIONS

These data show that the gallic acid esters are good antioxidants for fish oils. Of the special compounds containing gallic acid, KMGP, MCG, KHMD and CMGP are the best antioxidants of those tested.

Alkyl gallates offered some protection to shark liver oils against the loss of vitamin A when the oils were blown with oxygen. KMGP and KHMD gave high protective factors comparable to those obtained with hydroquinone.

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Figures 5 to 20 present data on various oils showing peroxide value versus time of storage or time of blowing.

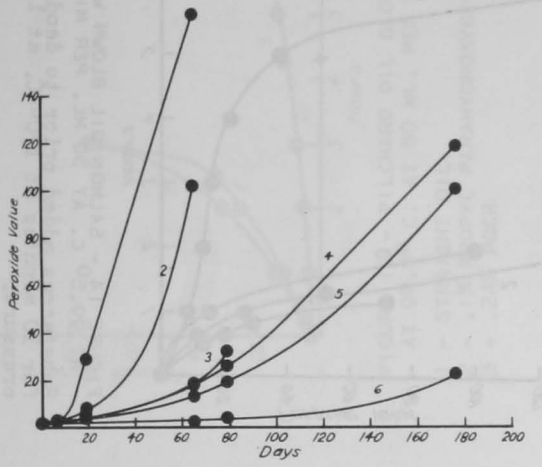

FIGURE 5 - SOYBEAN OIL STORED IN GLASS IN DARK AT 37° C.

1 - STRAIGHT OIL
2 - 3.33% KMCC
3 - .184% DECYL GALLATE
4 - .1245% ETHYL GALLATE
5 - .117% METHYL GALLATE
6 - 2.48% KMGP

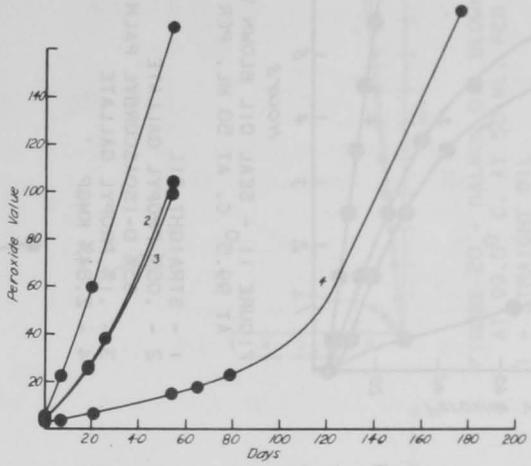

FIGURE 6 - MENHADEN OIL STORED IN GLASS IN DARK AT 37° C.

1 - STRAIGHT OIL
2 - .1245% ETHYL GALLATE
3 - .117% METHYL GALLATE
4 - 2.84% KMGP

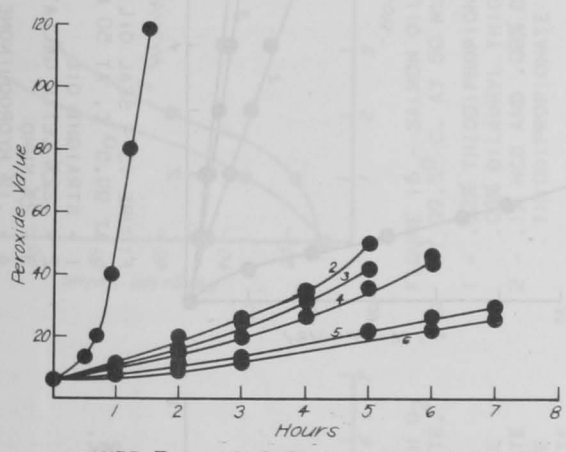

FIGURE 7 - UNDEODORIZED EDIBLE SALMON OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .184% DECYL GALLATE
3 - .2285% CETYL GALLATE
4 - .15% HEXYL GALLATE OR .169% OCTYL GALLATE
5 - .64% PGC190
6 - .5% MCG

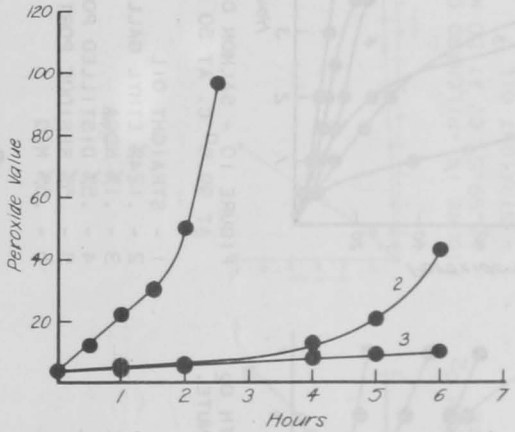

FIGURE 8 - PILCHARD OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .2% AMYL GALLATE
3 - .72% MCIG

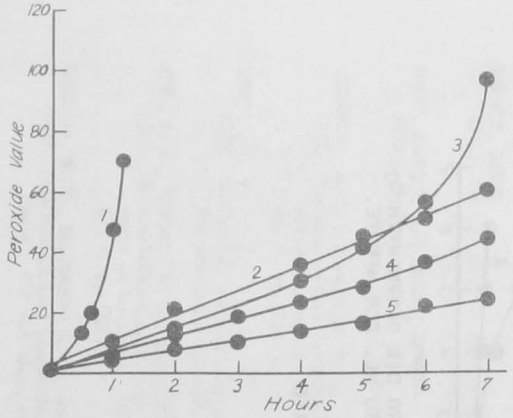

FIGURE 9 - SALMON OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .347% PECG
3 - .1% PROPYL GALLATE
4 - .46% GSCG
5 - 2.1% CMGP

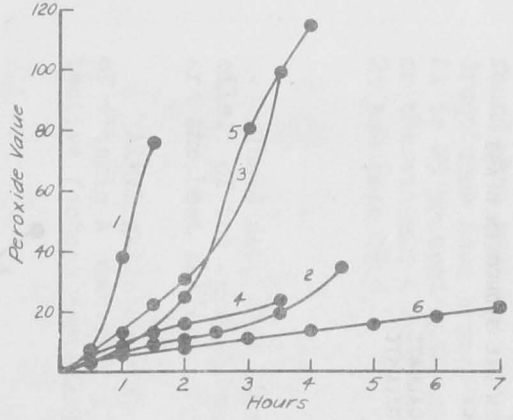

FIGURE 10 - SALMON OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .124% ETHYL GALLATE
3 - .1% NDGA
4 - .5% DISTILLED PORTION MCG
5 - .5% RESIDUE PORTION MCG
6 - .5% MCG

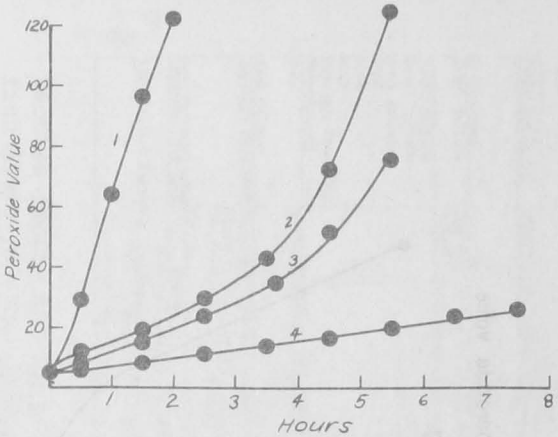

FIGURE 11 - SEAL OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .05% PROPYL GALLATE
.05% D-ISOASCORBYL PALMITATE
3 - .1% PROPYL GALLATE
4 - 2.84% KMGP

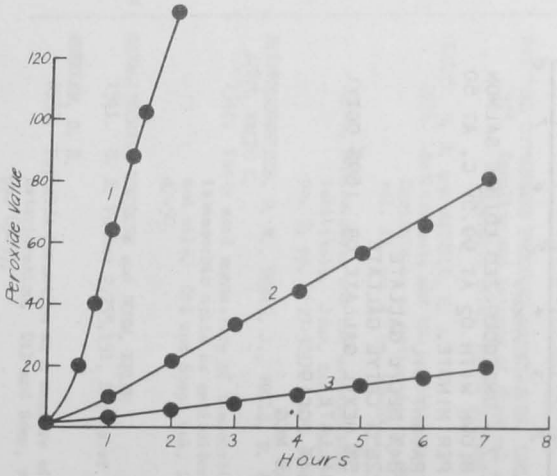

FIGURE 12 - SEAL OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .2% KEG
3 - 2.84% KMGP

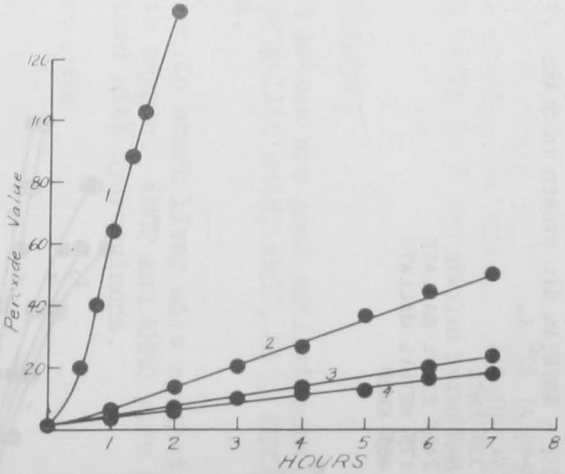

FIGURE 13 - SEAL OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .22% CETYL GALLATE
3 - 2% KHMD
4 - .1% HYDROQUINONE

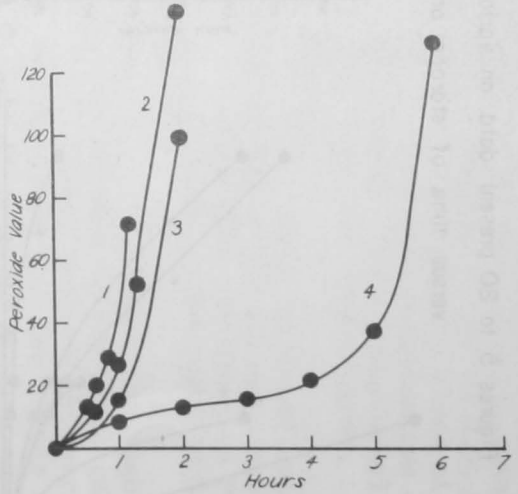

FIGURE 14 - SALMON OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

Substances added prior to deodorization for 10 minutes at 170° C. at 1 mm. abs. pressure.

1 - STRAIGHT OIL
2 - .1% RESIN GUAIAC
3 - .1% LECITHIN
4 - .1% BB' THIODIPROPIONIC ACID

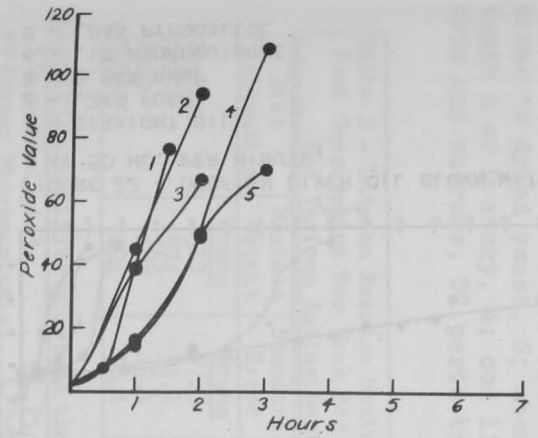

FIGURE 15 - SALMON OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .1% DILAURYL THIODIPROPIONATE
3 - .1% DISTEARYL THIODIPROPIONATE
4 - .4% DILAURYL THIODIPROPIONATE
5 - .4% DISTEARYL THIODIPROPIONATE

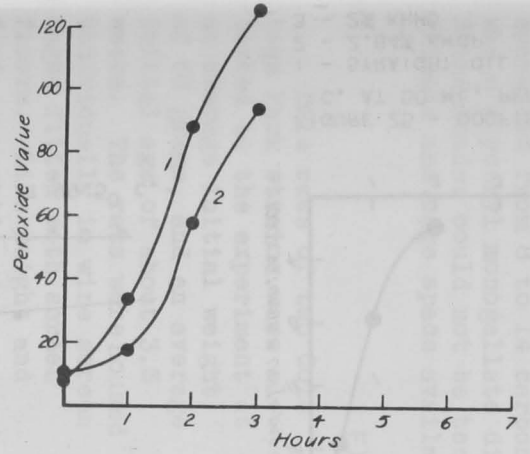

FIGURE 16 - SALMON OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - .02% THIODIPROPIONIC ACID AND .08% DILAURYL THIODIPROPIONATE
2 - .1% MCG AND .08% DILAURYL THIODIPROPIONATE

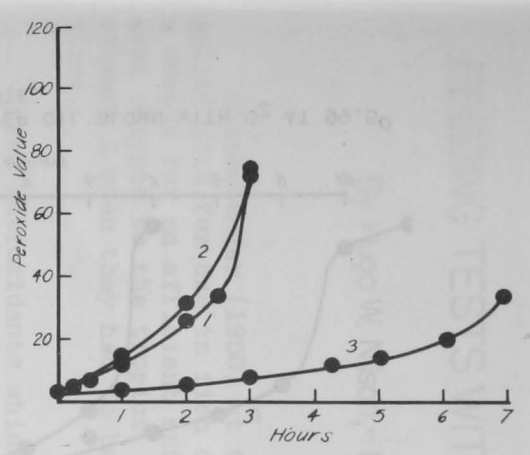

FIGURE 17 - PILCHARD OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .1% PROPYL PARAHYDROXYBENZOATE
3 - .24% PGCR

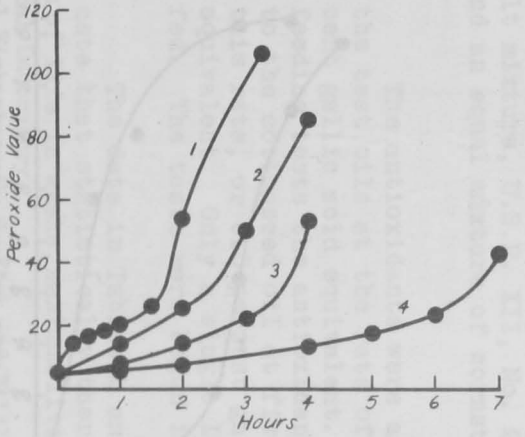

FIGURE 18 - PILCHARD OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .5% COCONUT OIL (HYDROGENATED WITH 10% GALLIC ACID)
3 - 1% SAME
4 - 2% SAME

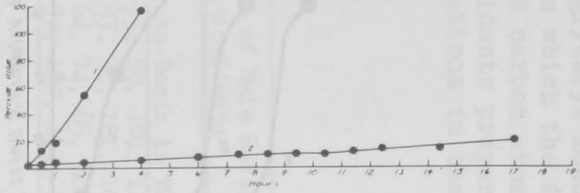

FIGURE 19 - PILCHARD OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - 5% MCG

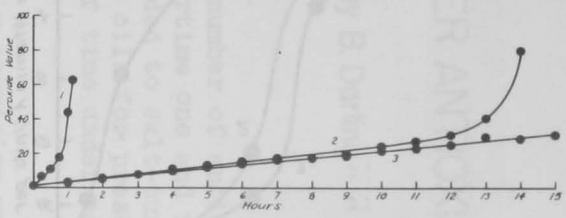

FIGURE 20 - SALMON OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - 2.1% CMGP
3 - .5% MCG

Figures 21 to 25 present data on shark liver oils,

showing the effect of added antioxidants on the vitamin A content of the oils when blown with oxygen.

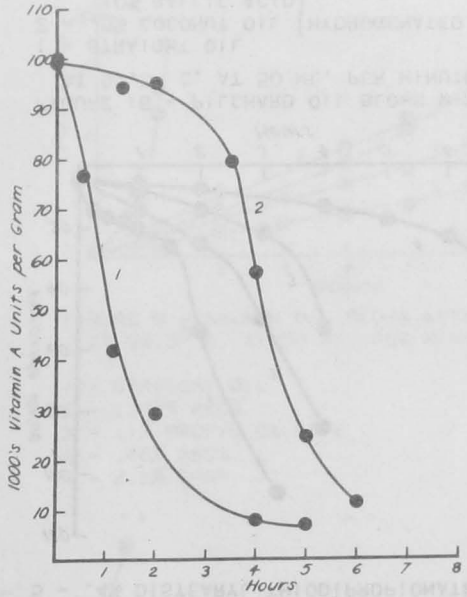

FIGURE 21 - SHARK LIVER OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .24% PGCR

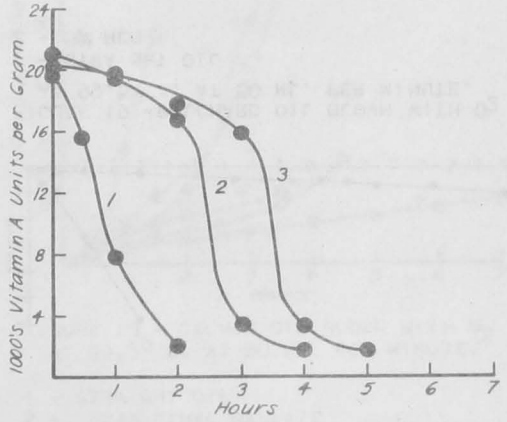

FIGURE 23 - DOGFISH LIVER OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .2285% CETYL GALLATE
3 - .169% OCTYL GALLATE

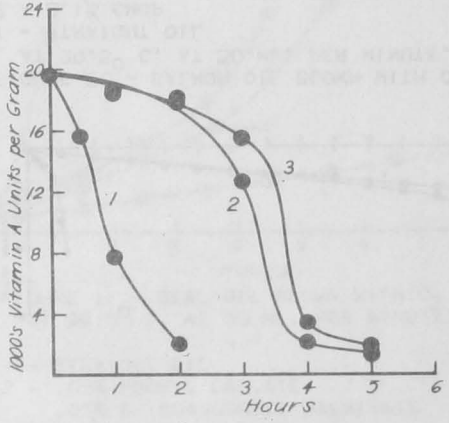

FIGURE 24 - DOGFISH LIVER OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .184% DECYL GALLATE
3 - .147% AMYL GALLATE

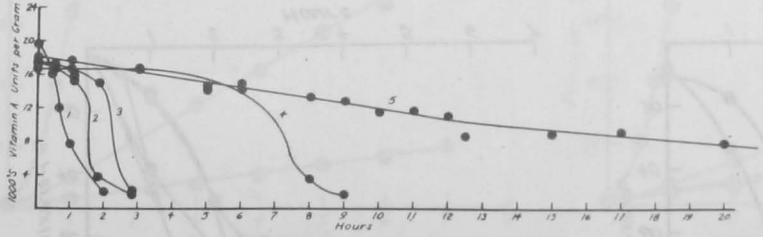

FIGURE 22 - DOGFISH LIVER OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - .24% PGCR
3 - 2.84% KMGP
4 - .1% HYDROQUINONE
5 - .24% PYROGALLOL

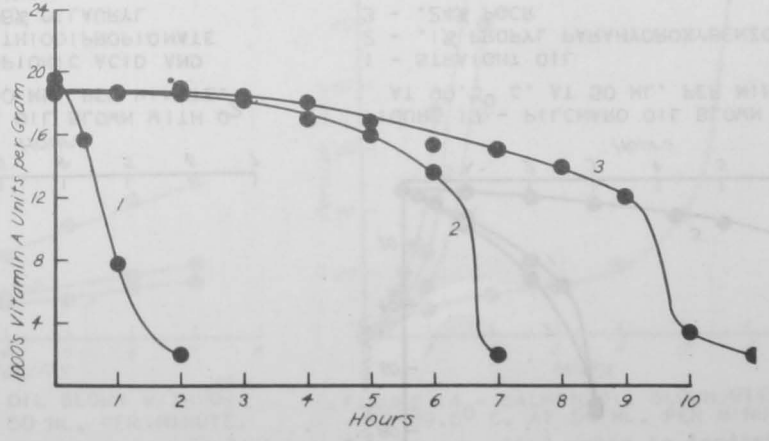

FIGURE 25 - DOGFISH LIVER OIL BLOWN WITH O_2 AT 99.5° C. AT 50 ML. PER MINUTE.

1 - STRAIGHT OIL
2 - 2.84% KMGP
3 - 2% KHMD