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IN VEGETABLE OILS

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STUDIES WITH SELECTED ANTIOXIDANTS IN VEGETABLE OILS

By

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ABSTRACT

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The effect of the antioxidant, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox C) was evaluated and compared with currently used antioxidants in coconut, peanut and corn oils. Oxidative changes were determined by peroxide value, conjugated diene absorption and measurement of weight increase. Good correlations were obtained among these methods, particularly between peroxide values and conjugated diene absorption.

Trolox C when used alone has been found to be the most effective among the antioxidants tested in both natural aging and accelerated ($63^{o}C$) tests. The order of effectiveness in decreasing order was Trolox C, TBHQ, BHA or BHT and citric acid. Trolox C when used in combination with BHA or BHT displayed a negative synergism. However, a huge increase in stability of oils was obtained when treated by Trolox C in combination with BHA or BHT and citric acid.

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INTRODUCTION

Coconut, peanut and corn oils are vegetable oils currently used for preparing food in Indonesia. Coconut oil is the oil extracted from the coconut palm (Cocos nucifera, Linn.). With an oil yield up to 65 percent, copra, the main product of the palm, is perhaps the richest material for vegetable oil extraction. Copra and coconut oil are traditional commodities in the world markets for oilseeds, oils and fats. Total world exports of copra and coconut oil are estimated at about 1.45 million tons of oil equivalent which represent a share of approximately 10 to 11 percent of the world's total export trade in oils and fats (Thampan, 1975).

About two-thirds of the world's peanut crop (Arachis hypogaea) is crushed for oil. Peanuts supply about a fifth of the world's edible oil production and they comprise a third of the world's trade in edible oils and oil bearing materials (Woodroof, 1973). Now legumes including peanuts are the crops being recommended by the Indonesian government, therefore peanuts will have a more important role in Indonesia in the near future.

Corn, known botanically as Zea mays Linnaeus is one of the world's most versatile seed crops. The production of

corn only to obtain oil is uneconomical. Corn oil is a by-product of both wet and dry milling (Reiners et al., 1970) so that the amount of it available depends on the demand for the other corn products.

Coconut oil has both food and non-food uses. It is used as cooking oil, margarine and confectionery. It is also employed in the manufacture of soaps and detergents. Corn and peanut oils are mainly used for edible purposes such as cooking oil, salad oil and margarine. From a nutritional point of view, peanut and corn oil are important because these oils are rich in essential fatty acids. In recent years, there have been a dramatic increase in the use of corn and peanut oil due largely to the increasing awareness of the importance of polyunsaturated fatty acids in the diet. Although coconut oil has a low unsaturated fatty acid content, it has a greater digestibility coefficient and economically is cheaper than those other oils.

Coconut, peanut and corn oils are susceptible to oxidative deterioration. The development of synthetic antioxidants has played a vital role in the marketing of vegetable oils by retarding oxidation. Several chemical compounds having antioxidant efficacy in fats and oils have been cleared for use by governmental regulatory agencies. Some new antioxidants have been developed recently, although these have not been released for human consumption.

The recent trend toward increased use of vegetable oils in the human diet has emphasized the need for better

antioxidant systems than those currently available. The present study was designed to gain knowledge in the effect of new and currently available antioxidants on the stability of coconut, peanut and corn oils. This work was based on the Schaal oven test at temperature of 63^{o}C and storage stability tests at room temperature. Peroxide value, conjugated diene absorption and weight gain were measured to follow the oxidative changes in the samples. The antioxidants used in this work were Trolox C (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), CA (citric acid) and TBHQ (tertiary butylhydroquinone).

LITERATURE REVIEW

Mechanism of Oxidative Rancidity.

Lipid oxidation is very important and of much interest to the food chemist because it results in the formation of off flavors and odors, destruction of essential fatty acids, formation of brown pigments, and alteration of pigments and flavors. The common feature of oxidative rancidity is the reactivity of the unsaturated fatty acid moieties in lipids. In a refined fat or oil the oxidation usually proceeds through autocatalytic processes. The term "auto catalysis" refers to a reaction which increases in rate with time due to the formation of products which themselves catalyze the reaction (Dugan, 1961; Lundberg, 1962).

Oxidation of fatty substances is believed to take place in three stages (Dugan, 1962; Sherwin, 1974):

(a) Initiation: This probably corresponds to the oxidation induction period of a fat or oil, and during this stage fat or oil molecules convert to unstable free radicals which can catalyze further free radical formation in the substrate. This may be depicted as:

$$\underset{\text{(fat molecule)}}{RH} \xrightarrow{\text{Initiator(s)}} \underset{\text{(free radical)}}{R^{\cdot}} + H^{\cdot}$$

Various agents such as light (especially in the UV region) and heavy metals (particularly copper and iron) are principal initiators of autoxidation.

(b) Propagation: Free radicals which have formed can combine with molecular (atmospheric) oxygen to form more radicals and hydroperoxides. This reaction may be depicted as:

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$$

(peroxy free radical)

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$$

(hydroperoxide)

$$ROOH \longrightarrow RO^{\cdot} + {}^{\cdot}OH$$

$${}^{\cdot}OH + RH \longrightarrow R^{\cdot} + H_2O$$

During propagation, especially in the presence of catalytic agents, decomposition of the hydroperoxides leads to formation of a wide variety of aldehydes, ketones, acids, etc., responsible for the obnoxious odor and flavor characteristics of rancid food fats and oils.

c) Termination: Termination of the oxidation chain reaction occurs when the free radicals (autocatalysts) are deactivated or destroyed. This may occur in various ways such as:

$$R^{\cdot} + R^{\cdot} \longrightarrow RR$$

$$R^{\cdot} + ROO^{\cdot} \longrightarrow ROOR$$

Various extraneous influences may be present that affect the rate of oxidation. These factors are temperature,

light radiation, various metals, metal salts, and organic compound of metals, oxidative enzymes such as lipoxidases and photochemical pigments which act as accelerators in the presence of light (Lundberg, 1962).

The reaction of RH + O_2 ------> ROOH requires a change in total spin, RH and ROOH being in singlet states while O_2 is in triplet state, moreover the reaction is endothermic by about 64 kcal/mole (Waters, 1946). Both the energy and spin barriers could be overcome however if instead of ordinary triplet state O_2, singlet state O_2 was the active species. Since the plant pigments could serve as sensitizer, the photosensitized production of singlet O_2 is believed to be the mechanism for the production of fatty acid hydroperoxides. The two most likely mechanisms of photooxidation involve either a biradical moloxide (Schonberg, 1935):

$$^1S \xrightarrow{hv} {}^1S^* \longrightarrow {}^3S^*$$

$$^3S^* + {}^3O_2 \longrightarrow .S\text{-}O\text{-}O.$$

$$.S\text{-}O\text{-}O. + RH \longrightarrow ROOH + {}^1S$$

or singlet O_2 as the reactive intermediate (Rawls and van Santen, 1970):

$$^1S + h\nu \longrightarrow {}^1S^* \longrightarrow {}^3S^*$$

$$^3S^* + {}^3O_2 \longrightarrow {}^1O_2{}^* + {}^1S$$

$$^1O_2{}^* + RH \longrightarrow ROOH$$

$$ROOH \longrightarrow \text{free radical product.}$$

S is the sensitizer, the superscript refers to the spin multiplicity and the asterisk indicates electronic excitation.

In the structure of chlorophyll, a five membered ring condensed to the porphyrin system contains a carbonyl group. Rawls and van Santen (1970) reported that the chlorophyll reaction involved the non singlet O_2 oxidation mechanism since electronically-excited carbonyl groups are known to be very effective proton abstractors and since the visible light is sufficient to excite the carbonyl n ------> Π^* state. The following mechanism was proposed:

chl + hv ------> chl*

* = n ------> Π^* excited state of the carbonyl group.

chl* + RH ------> R· + (chlorophyll product)

R· + O_2 ------> RO_2·

RO_2· + RH ------> ROOH + R·

Metal Catalysts.

Heavy metals, particularly those possessing two or more valency states with a suitable oxidation - reduction potential between them (e.g. Cu, Co, Fe, Mn, Ni, etc.), generally increase the rate of oxidation deterioration of fats and oils.

Swern (1964) stated that copper in particular, is a very strong prooxidant, being effective in a concentration

of much less than one p.p.m. Tappel (1955) listed copper as a well known catalyst for the oxidation of unsaturated fats. Cooney et al. (1958) stated that both copper and iron were potent oxidative catalysts in cottonseed oil. Vioque et al. (1965) demetalized olive and soybean oils by passing them through columns packed with cation exchange resins. This lowered the trace metal content and increased the stability of the oils.

Mertens et al. (1971) studied trace metals and the flavor stability of margarine. Levels of 0.1 p.p.m. copper led to rapid flavor deterioration. In order to expect good flavor stability, the maximum copper amounts which can be tolerated are about 0.02 p.p.m. Berger (1975) stated that variation in metal content accounts for stability and anti-oxidant activity variation in many fats and oils.

Measurement of Lipid Oxidation.

The acceptability of a food product depends on the extent to which the oxidative rancidity has occured. Thus some criterion for assessing the extent of oxidation is required. This can be followed by determining the total consumption of oxygen, by determining the amount of a product of lipid oxidation or by measuring the decrease in the concentration of unsaturated lipids. Many methods have been developed and among these are peroxide value, conjugated diene absorption and weight gain methods.

Peroxide Value.

The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Therefore it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. The iodometric methods of Lea (1931) and Wheeler (1932) are widely used, and these are based on the measurement of the iodine produced from potassium iodide by the peroxides present in the oil. The iodine, liberated in a stochiometric ratio of two atoms of iodine for each atom of active oxygen, can be quantitated by titration with sodium thiosulfate.

According to Mehlenbacher (1960), the two principal sources of error in these methods are (a) the absorption of iodine at unsaturated bonds of the fatty material, and (b) the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated. Lea (1931) attempted to eliminate this error by filling the sample tube with nitrogen at the beginning of the test and assuming that the vaporization of chloroform thereafter would prevent the reentry of oxygen into the tube. Wheeler (1932) used a homogenous solution in an attempt to eliminate the need for shaking thereby minimizing the effect of oxygen.

Conjugated Diene Absorption Method.

Oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption due to the formation of conjugated diene and triene hydroperoxides. Fatty

acids with conjugated unsaturation absorb strongly in the region 230 to 375 nm, diene unsaturation at 233 nm and triene unsaturation at 268 nm. The magnitude of change is not readily related to the degree of oxidation because the effects upon the various unsaturated fatty acids vary in quality and magnitude. However, the changes in the ultraviolet spectrum of a given substance can be used as a relative measurement of oxidation (Gray, 1978).

Oils containing linoleate or more highly unsaturated fatty acids are oxidized to conjugated diene systems that can be measured by ultraviolet absorption at 233 nm. Farmer and Sutton (1943) indicated that absorption increased proportionately to the uptake of oxygen and to the formation of peroxides in the early stages of oxidation.

Angelo et al. (1975) studied the autoxidation of peanut butter by measuring the peroxide value and the increase in absorption at 234 nm due to diene conjugation. They concluded that the conjugated diene hydroperoxide (CDHP) method can be used as an index of progressive staling in place of, or in addition to, the peroxide value. The CDHP method is faster than the peroxide value method, is much simpler, requires no chemical reagents, does not depend upon chemical reaction, and can be conducted on smaller samples. This method is applicable for the analysis of peroxides in vegetable oils produced containing polyunsaturated fatty acids.

Weighing Method.

The technique of following the rate of oxidation of oils by weighing small samples at intervals during storage has been used from time to time for at least 75 years (Olcott et al., 1958). They found the weighing procedure to be a convenient method for estimating the relative effectiveness of antioxidants in marine and other oils and purified fatty esters. The procedure involved weighing the oil into beakers and the additives are then put in as aliquots of solutions in volatile solvents. In constant draft oven at 50 or 60^{o}C the solvents are removed quantitatively as judged by constant weight in subsequent weighing. Fukuzumi and Ikeda (1969) used a vacuum desiccator at constant temperature (30 ± 1^{o}C) for 1 hour, at 1 mm Hg pressure to remove the solvents.

Fukuzumi et al. (1976) studied the effect of new antioxidants, such as phenothiazine derivatives on the autoxidation of methyl linoleate and used the weighing method to estimate the induction period. They found that the induction period evaluated from the weighing method gives almost the same value as that from the peroxide value.

Antioxidants.

Antioxidants are substances capable of slowing the rate of oxidation in autoxidizable material. The choice of an antioxidant for a given purpose is governed by the requirement of the system and the characteristics of the antioxidants

available. Desirable features of an antioxidant include the following: it must be effective at low concentrations, non-toxic, conveniently and safely handled, and low in cost, and it must not impart undesirable characteristics to the system in which it is used (Dugan, 1963).

All antioxidants are structurally similar in that they contain unsaturated benzene rings plus either hydroxy or amino groups. Stuckey (1962) divided antioxidants into phenols, amines and aminophenol groups. Most natural and synthetic food grade antioxidants belong to the phenolic class of compounds.

Although the presence of hydroxy or amino groups on the aromatic ring is necessary for antioxidant activity, the potency of a given compound can be greatly enhanced by the introduction of certain substituents into the proper position on the aromatic nucleus. Morawetz (1949) and Thompson and Symon (1956) showed, after evaluating many phenolic compounds, that alkyl substitution in the ortho and para positions greatly enhance the potency of a given compound. The addition of the tertiary butyl group in the ortho position seems to be particularly effective in this respect.

Mechanism of Antioxidant Action.

Observations from a number of studies have indicated that more than one type of action may occur depending upon the conditions of the reaction and the type of system being studied. Dugan (1963) noted that antioxidants might be

considered to function in two ways, either as inhibitors of free radical formation or as peroxide decomposers.

Antioxidants that function as free radical inhibitors react with free radicals to form inert products as in a termination step in the chain reaction mechanism. Studies by Bolland and Ten Have (1947) led to a proposal of a simple mechanism in which the antioxidants acted as hydrogen donors or free radical acceptors. From the kinetics of the reaction, it appeared that the free radical acceptors (AH_2) react primarily with $RO_2^{\cdot}$ and not with $R^{\cdot}$, as follows:

$$RO_2^{\cdot} + AH_2 \dashrightarrow ROOH + AH^{\cdot}$$

$$AH^{\cdot} + AH^{\cdot} \dashrightarrow A + AH_2$$

Boozer et al. (1955) proposed a different mechanism, involving complex formation, as follows:

$$RO_2^{\cdot} + AH_2 \dashrightarrow (RO_2AH_2)^{\cdot}$$

$$(RO_2AH_2)^{\cdot} + RO_2^{\cdot} \dashrightarrow \text{stable product.}$$

The peroxide decomposers act as catalysts to decompose peroxides initially present as well as those that are formed during further oxidation. An important feature of this decomposition process is that the primary stable products are not free radicals. This naturally rules out the decomposition of peroxides by metals such as copper, cobalt and iron (Dugan, 1963).

Synergists and Synergism.

The term synergism refers to the cooperative action of two or more agents in such a way that the total effect is greater than the sum of the individual effects taken independently (Dugan, 1963). The original description of antioxidants and synergists (Olcott and Matill, 1936) differentiated between substances which were effective alone in relatively low concentration (tocopherols, phenols) and those which had little activity by themselves, but were effective in combination with the phenolic inhibitors. They were called synergists (citric and ascorbic acids, phospholipids, etc.).

It has been well demonstrated that the chelation of metals is one of the principal mechanisms involved, where one of the antioxidants is a metal chelating agent. Kraybill et al. (1949) showed that BHA exhibits synergism with certain acids, including citric, as well as with hydroquinone, methionine, lecithin, and thiodipropionic acid. Citric acid was more effective against iron and nickel than against copper, and ascorbic acid was effective against copper but ineffective against iron (Morris et al., 1950). Cowan et al. (1962) with soybean oil and lard, showed that both sorbitol and citric acid were acting as a metal inactivator and did not have a true synergistic effect. Citric acid readily forms stable complex salts with many metallic ions (Lockwood and Irwin, 1963), thus it is able to retard

the increasing rate of free radical formation. Zeldes and Livingston (1971) identified three radicals while studying paramagnetic resonance spectra of radicals present during photolysis of aqueous solutions of citric acid.

Smith and Dunkley (1962) showed ferrous ion was more effective than ferric ion in the peroxidation of linoleate. It was proposed that a perhydroxyl radical was produced by the reduced metal ion. Strouse et al. (1977) reported that triionized citrate formed a tridentate chelate with Fe (II) in which the protonated hydroxyl group, the central carboxyl group, and one terminal carboxyl group are coordinated to a single Fe (II) ion. Both oxygen atoms of the other terminal carboxyl group were coordinated to two other symetry-related Fe (II) ions. Hexaquoiron (II) was the counter ion.

Cort et al. (1975) stated that it was not the metals per se, but their oxidation state which were important. Ascorbate converted Fe^{3+} to Fe^{2+} and Cu^{2+} to the lower oxidation states. It was the higher oxidation which reacted with tocopherol, Trolox C and ascorbic acid. Berger (1975) reported that ascorbic acid had some antioxidant activity and possibly it acted as a somewhat inefficient free radical scavenger. On the other hand, citric acid was particularly valuable as a chelating agent.

Synergism has been produced also in fat by a combination of two antioxidants (Mahon and Chapman, 1953; Dugan et al., 1954). There are some theories regarding to synergism. These may be as metal scavenger, peroxide decomposer,

and sparing agents, as in the interaction of phenolic antioxidants or the interaction of other agents with phenolic antioxidants (Dugan, 1963). Ikeda and Fukuzumi (1977) with methyl linoleate showed that nucleic acid acted as a synergist with tocopherol through H bonding which protected tocopherol from direct air oxidation.

The Use of Synthetic Antioxidants in Coconut, Peanut and Corn Oils.

The effect of various antioxidants on the stability of coconut, peanut and corn oils has been reported by many researchers. Dugan et al. (1950) reported that BHA when used alone and in combination with propyl gallate and citric acid had been found useful in preventing rancidity in frying oils such as corn oil and peanut oil and in the food prepared in these oils.

The addition of citric acid during the cooling stage of deodorization or of monoisopropyl citrate (Gooding et al., 1950) or monoglyceride citrate (Brown and Gooding, 1955) following deodorization negates the prooxidant effect of trace metal to a large degree, principally that of traces of iron and copper, which occur in all vegetable oils. The effect is to reestablish the inherent stability of vegetable oils owing to the naturally occuring antioxidants contained in vegetable oils.

Tollenar and Vos (1958) in an extended study found a small protective factor with octyl gallate in various

vegetable oils tested at 100°C and 34°C. Their room temperature storage test with BHT and gallates in various combinations in corn oil showed that these antioxidants under these conditions had no effect. They also mentioned the reason why animal fats yield considerably better results with antioxidants than vegetable oils as follows:

a) Vegetable oils generally have a higher iodine value than animal fats. Antioxidants however exercise a stronger influence on the oxidation of oleic esters than on linoleic and linolenic esters. Although oxidative rancidity does not constitute a major problem for coconut oil, the positive action of phenolic antioxidants on this vegetable oil with relatively low unsaturation has been demonstrated in storage tests.

b) Vegetable oils contain many more natural antioxidants than animal fats. The supplementary addition of antioxidants has less effect in the vegetable oil group.

c) Fats as a rule are packed entirely differently from oils, packed fat frequently has unlimited quantities of oxygen available.

d) The natural flavor constituents in vegetable oils and fats are entirely different from those present in animal fats and react differently to the addition of antioxidants.

Fritsch et al. (1971) demonstrated that the addition of BHA, BHT and citrate to coconut oil increased the AOM

stability to about 350 hr even though the initial stability was 30 or 250 hr. These results suggested that the high resistence to oxidation of coconut oil might not be entirely due to its low level of unsaturation but due to the presence of natural antioxidants in varying amounts. Semiquantitative GLC showed no difference in the amount of tocopherols in coconut oils with low and high stabilities. Thewalt et al. (1969) reported detecting phenols other than tocopherols in amounts up to 100 p.p.m. in coconut oil samples.

MATERIAL AND METHODS

Oils.

Coconut, peanut and corn oil were used for this study. Coconut oil was obtained from PVO International Inc. Boonton N.J. 07005, corn oil from CPC International Inc. Argo Ill. 60501 while Shedd's pure peanut oil was purchased from a local store. All of the oils were refined and had no added antioxidants.

Antioxidants and Chemicals.

Five antioxidants were used for this study. These were Trolox C, a trivial name for 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Roche Inc); BHA for butylated hydroxyanisole: a mixture of 2- and 3-tert-butyl-4-hydroxy-1-methoxy benzene (Eastman Kodak Company); BHT for butylated hydroxytoluene: 3,5-ditert-butyl-4-hydroxy toluene (Ashland Chemical Company); Citric acid monohydrate (Fisher Scientific Company); and TBHQ for 2-tert-butyl hydroquinone (Eastman Kodak Company).

All the reagents used during analysis were analytical grade.

Procedure.

In each series, 50 g of the oils were weighed into beakers. Some served as control and accurately measured

amounts of the antioxidant solution in absolute alcohol were added to others. The oil was stirred by magnetic stirrer for ten minutes at room temperature to assure the homogenous distribution of the antioxidants, then filled into weighed petticups. Solvent was carefully removed in a vacuum oven at a constant temperature of 30°C and pressure of 1 mm Hg, as judged by constant weight in subsequent weighing. After they were weighed accurately, the samples were placed in a constant temperature oven at 63°C. Other samples were stored at room temperature for long term storage.

Peroxide value, conjugated diene absorption and weight gain determinations were made to follow oxidative changes in the samples.

Determination of Peroxides.

Peroxides in the oils were measured by the iodometric method of Wheeler. The petticups containing oil were removed from the oven, cooled in a desiccator, weighed and transferred into a 200-300 ml Erlenmeyer flask where the samples were dissolved in 30 ml of glacial acetic acid - chloroform solution (3/2, v/v). Then 0.5 ml of saturated potassium iodide solution was added and swirled to provide mixing. After two minutes, 30 ml of distilled water was added and the sample was titrated with standardized sodium thiosulfate solution. Starch indicator (1 percent, w/v) was added when near the end point. The solution was titrated to disappearance of the blue color.

The peroxide content was determined as meq/kg of sample.

Conjugated Diene Absorption.

About ten mg of oil were weighed accurately into small petticups and placed into test tubes. Ten ml of pure iso-octane (2,2,4-trimethyl pentane) was added to the sample and the oil was shaken in a Fisher mini shaker to assure complete dilution. Conjugated diene absorption was measured at 233 nm by a Beckman DU spectrophotometer using pure iso-octane as a blank. If the absorption was too large, dilution was accomplished by taking 1 ml of sample solution and mixing with 9 ml of pure iso-octane in order to bring the absorbance within the proper limits. Conjugated diene absorption was calculated at a dilution of 1:1000 (w/v).

Iso-octane Purification.

The iso-octane used in the spectral determination of conjugated diene absorption should have an absorbance at 233 nm of not more than 0.07 when compared with distilled water. In order to conform to this requirement, the iso-octane was purified by passage through a 50 cm column of silica gel.

Weight Gain Technique.

This simple method is based on the concept that oxygen absorption is accompanied by a finite gain in weight of the oil undergoing the oxidative process. In this

technique, the petticups containing oil were removed from the oven, allowed to cool in a desiccator for 10 minutes, weighed and then placed in the oven.

Preparation of Methyl Esters.

Methyl esters were prepared by a rapid procedure described by Metcalfe et al. (1966). A total of 4 ml of 0.5 N methanolic NaOH was added to approximately 150 mg of oil in a 50 ml volumetric flask. This mixture was heated in a steam bath for about 5 minutes until the oil went into solution. A total of 5 ml BF_3-methanol was added to the flask and the mixture was boiled for 2 minutes. Enough saturated NaCl solution was added to the flask to float the methyl esters up, then the entire mixture was transferred into a separatory funnel. About 20 ml of petroleum ether (b.p 30-60^{o}C) was added to the separatory funnel and the funnel was shaken vigorously for 1 minute and the layers were allowed to separate. The lower aqueous layer was drained off and discarded. The petroleum ether layer was drained through filter paper into a 50 ml beaker. The solvent was then evaporated on a 60^{o}C water bath or removed by a gentle stream of air at room temperature. The esters were then ready for GLC analysis.

Fatty Acid Composition of Oils.

Chromatographic analysis of methyl esters was performed using a Beckman GC-5 equipped with a hydrogen flame detector.

A coiled stainless steel column 1/8 in x 6 ft packed with 10% DEGS-PS on 80/100 Supelcoport (Supelco Inc.) was used for methyl ester separation. The column oven temperature was 160°C, the injection temperature was maintained at 185°C and the detector at 205°C. The nitrogen carrier was adjusted to 27 ml/minute. The flow rate of hydrogen and oxygen were 26 ml/minute and 250 ml/minute respectively. The emerging peaks were identified by comparing retention time to those of standard mixtures of known fatty acid methyl esters. These standard were Supelco F&OR mix #1 for corn oil, F&OR mix #3 for peanut oil and F&OR mix #5 for coconut oil. Peak areas were calculated by multiplying peak height time peak width at half height and the percentage of total fatty acids were determined.

Free Fatty Acid (FFA) Determination.

The Official AOCS method (1974) was used for free fatty acid determination. In this method, 50 g of sample was weighed into an Erlenmeyer flask. Fifty ml of hot neutralized alcohol and 2 ml of phenolphthalein (1% in 95% alcohol) were added to the solution. The solution was titrated with 0.1 N standard NaOH to the appearance of the first permanent color which must persist for 30 seconds.

The percentages of free fatty acids in corn and peanut oils were calculated as oleic acid while in coconut oil it was expressed as lauric acid.

Iodine Value.

The Hanus method was used for the iodine value determination. The iodine value is a measure of unsaturation and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample. The Hanus solution was prepared by dissolving 13.2 g of iodine in 1 liter of glacial acetic acid (99.5%) and enough bromine was then added to almost double the halogen content.

In this method 0.1 - 0.2 gram corn or peanut oil or 0.5 gram coconut oil was placed in a dry 500 ml glass stoppered flask containing 10 ml of chloroform. Twenty five ml of Hanus solution was added and the solution was allowed to stand for 30 minutes in the dark with occasional shaking. Ten ml of 15% potassium iodide was added, shaken and followed by the addition of 100 ml of freshly boiled and cooled water washing down any free iodine that might be on the stopper. The solution was titrated with 0.1 N $Na_2S_2O_3$ until the yellow color had almost disappeared. The starch indicator was then added and the titration continued until the blue color had disappeared entirely. A blank determination was conducted simultaneously with that for the sample.

RESULTS AND DISCUSSION

The oils used in this study were refined and had no added antioxidants. The majority of oils are usually marketed in refined condition thus it seemed appropriate to study the effect of antioxidants on refined oils. The results presented in this study were performed at least duplicate determinations.

The Properties of Original Oils.

The properties of original oils in terms of free fatty acids content, iodine value, peroxide value and conjugated diene absorption are shown in Table 1. All samples exhibited low free fatty acids content.

Among these samples, corn oil exhibited the highest iodine value while peanut and coconut oils had the medium and the lowest iodine values respectively. The data agree with the literature values reported by Swern (1964). The susceptibility of fats and fatty acids to oxidation is associated with the presence of unsaturated bonds. This reaction leads to the formation of primary, secondary and tertiary oxidation products which may make the oils unsuitable for consumption. Thus corn oil was more susceptible to oxidative deterioration than peanut oil which in turn was more susceptible than coconut oil.

Table 1. Properties of original oils

Oils	Free fatty acids (%)	Iodine value (cg/g)	Peroxide value (meq/kg)	Conjugated Diene absorption at 233 nm*
Corn	0.082	116.81	12.2	0.358
Peanut	0.024	92.49	14.9	0.212
Coconut	0.044	10.30	8.3	0.124

*Calculated at a dilution of 1:1000 (w/v).

The degree of oxidation that has taken place in fats and oils can be expressed in terms of peroxide value and conjugated diene absorption. As shown in Table 1, all samples exhibited substantial amounts of peroxides. These indicated that the oxidative process had been started. It was noted that although corn oil had a lower peroxide value than peanut oil it exhibited higher conjugated diene absorption.

Gas Liquid Chromatography (GLC) Analysis.

The original oils were also analyzed for fatty acid composition by gas liquid chromatography as shown in Table 2. The data show that the coconut oil contained appreciable quantities of saturated $C_{8:0}$ to $C_{14:0}$ fatty acids, whereas the corn and peanut oils contained low levels of saturated fatty acids but high levels of C_{18} unsaturated fatty acids.

In the coconut oil, lauric and myristic made up over 75% of the total acids. It contained also caproic, capric, caprylic, palmitic, oleic and linoleic. The fatty acids detected were in agreement with the data reported by Sreenivasan (1968). The small differences in the percentages of each acid may be due to the difference in growth conditions or varieties of coconut trees.

Worthington and Hammons (1977) studied variability in fatty acid composition among peanut genotypes. They reported that the two major fatty acids, oleic and linoleic

Table 2. Fatty acid composition of original oils

Fatty acid*	Fatty acid (%)		
	Coconut oil	Peanut oil	Corn oil
8:0	2.9	-	-
10:0	3.3	-	-
12:0	53.6	-	-
14:0	21.9	-	-
16:0	8.1	10.9	12.1
18:0	2.3	1.5	1.8
18:1	6.3	55.1	28.8
18:2	1.6	27.7	56.2
18:3	-	0.8	0.8
20:0	-	0.8	0.3
22:0	-	1.9	-
24:0	-	1.4	-

*The notation used to describe fatty acids is number of carbon atom:number of double bonds.

ranged between 36-69% and 14-40%, respectively, and together made up 75-85% of the total fatty acids. The very long chain (C_{20}-C_{24}) fatty acids made up 4-9%, palmitic acid 7-13%, and stearic acid 2-5% of the total fatty acids. The fatty acid composition of peanut oil as shown in Table 2, agrees with the values reported by Worthington and Hammons (1977).

As shown in Table 2, the corn oil contained a high percentage of linoleic acid. It contained also palmitic, stearic, oleic, linolenic and arachidic acids. Linoleic and oleic acid made up 85% of total acids. These data were in agreement with the range of values tentatively adopted by the Food and Agriculture Organization/World Health Organization Codex Alimentarius Committee on Fats and Oils (Spencer and Herb, 1976).

Correlations Among Peroxide Value (PV), Weight Gain and Conjugated Diene Absorption (CDA) Tests.

Peroxide value (PV) determination is the most widely used chemical method to measure oxidative rancidity of fats and oils. Oxidation of polyunsaturated fatty acids produces peroxides and the position of the double bonds shifts to a conjugated form. Conjugated linkages absorb light in the ultraviolet region of the spectrum and have absorption maxima at the wave length of 233 nm. Although the conjugated diene absorption (CDA) test has not been adopted for day to day quality control in the food industry, its usefulness for

oil products has been indicated. Some researchers use the weight gain procedure as a convenient method for estimating the extent of oxidative rancidity.

Fukuzumi and Ikeda (1969) and Ikeda and Fukuzumi (1977) used the weighing method, UV and IR spectra to follow the extent of autoxidation. They found that the induction period evaluated from the weighing procedure gave almost the same value as that from the peroxide method. Ke et al. (1977) used weight gain, PV, TBA and free fatty acid tests to follow the oxidation rate while studying the potency of antioxidants on mackerel skin lipid as the tested model system.

Angelo et al. (1975) plotted PV against the corresponding conjugated diene hydroperoxide (CDHP) values to test for possible correlation between the two methods in determinations of shelf life stability of peanut butters. They found a linear relationship between CDHP and PV values with a high correlation coefficient which indicated that the results from the two methods were highly correlative. The linear regression equation had a negative intercept and positive slope.

To test for possible correlation among PV, weight gain and CDA methods in this study, the results from one method were plotted against the corresponding values of the other methods measured on the same sample of oil stored at $63^{o}C$ (Figure 1, Figure 2 and Figure 3). The linear regression

equations for the three methods are shown in Table 3. It was noted that the regression equation between PV and CDA had a negative intercept and positive slope thus it was consistent with the finding reported by Angelo et al. (1975).

Table 4 shows the correlation coefficients among PV, weight gain and CDA measured on coconut, peanut and corn oils stored at a temperature of 63°C. High correlations were demonstrated between PV and CDA of all samples observed. The correlation between PV and weight gain as well as CDA and weight gain were high enough but weaker than the correlation between PV and CDA. These weaker correlations may be due to the vaporization of volatile oxidation products which affected the measurements made by the weight gain method.

As mentioned, the present study has demonstrated that the CDA test is more sensitive and correlates better with peroxide development than that of the weight gain method in vegetable oils. The high correlations observed between PV and CDA may be explained, in part, by the suitability and accuracy of the CDA method for monitoring the oxidative changes in place of or in addition to PV. The advantages of CDA over PV are that it is faster and simpler, it does not depend on chemical reaction or color development and can be carried out on much smaller samples.

It was interesting to note that the correlation coefficient between PV and CDA of corn oil was higher than that of peanut oil which in turn was greater than that of coconut

Table 3. Regression equations among PV, weight gain and CDA of oils during storage at 63°C

Oil	PV vs CDA	PV vs weight gain	CDA vs weight gain
Coconut	Y = -10.98 + 171.41 X	Y = 7.76 + 410.69 X	Y = 0.12 + 2.23 X
Peanut	Y = -10.41 + 77.84 X	Y = 19.91 + 244.17 X	Y = 0.44 + 2.95 X
Corn	Y = -15.24 + 59.56 X	Y = 45.52 + 205.09 X	Y = 1.03 + 3.41 X

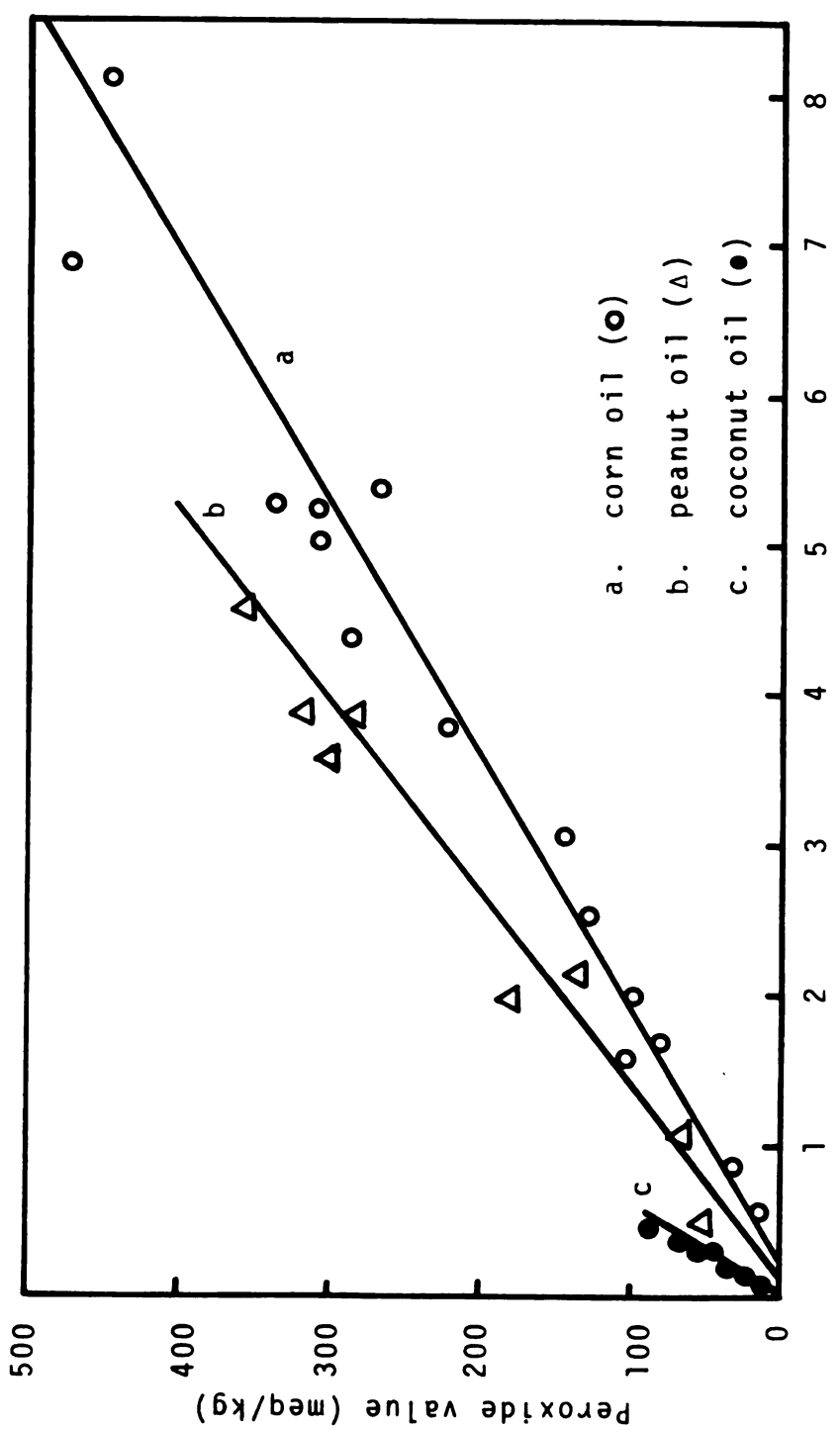

Figure 1. Peroxide value vs conjugated diene absorption of oils stored at 63°C.

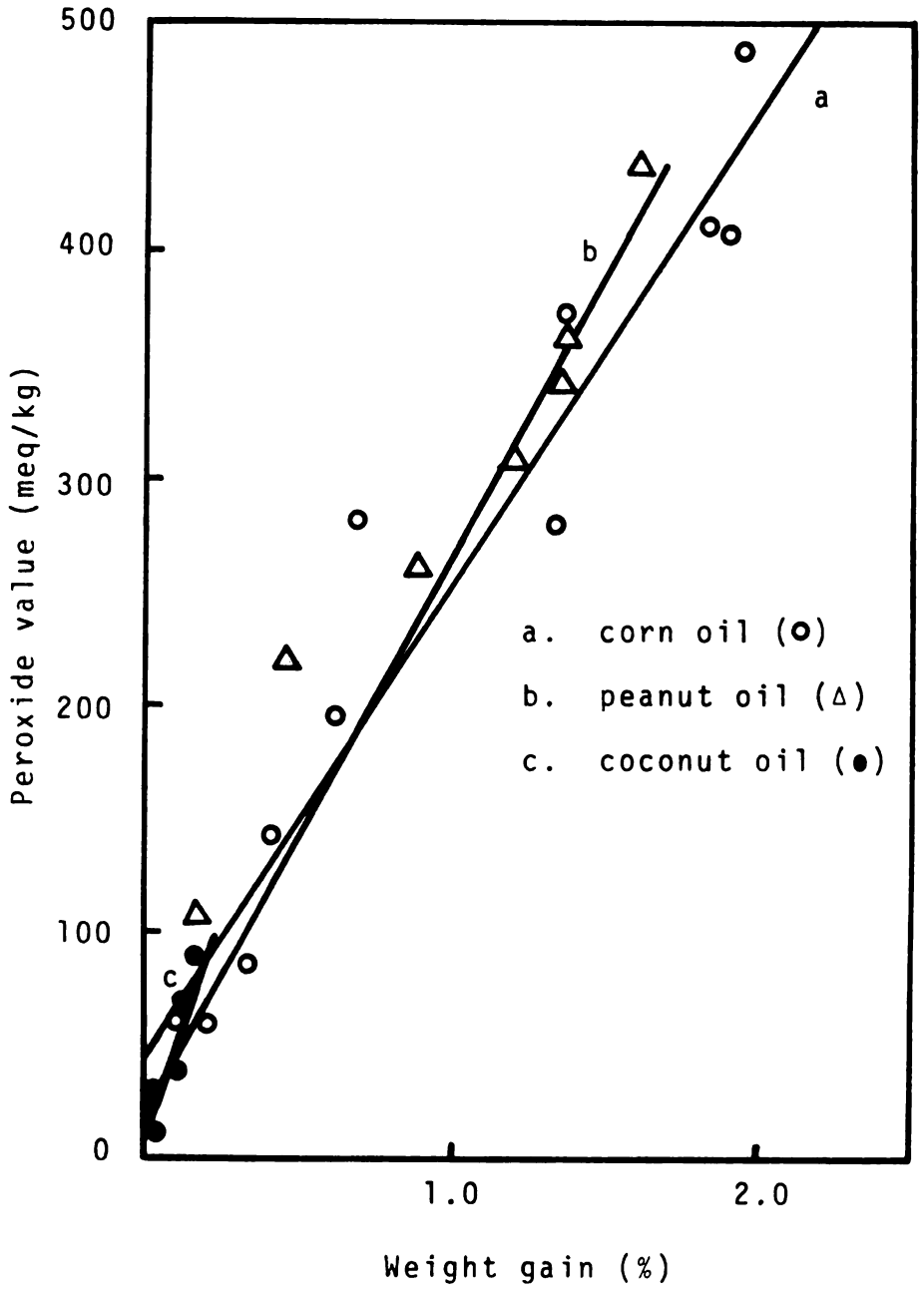

Figure 2. Peroxide value vs weight gain of oils stored at 63°C.

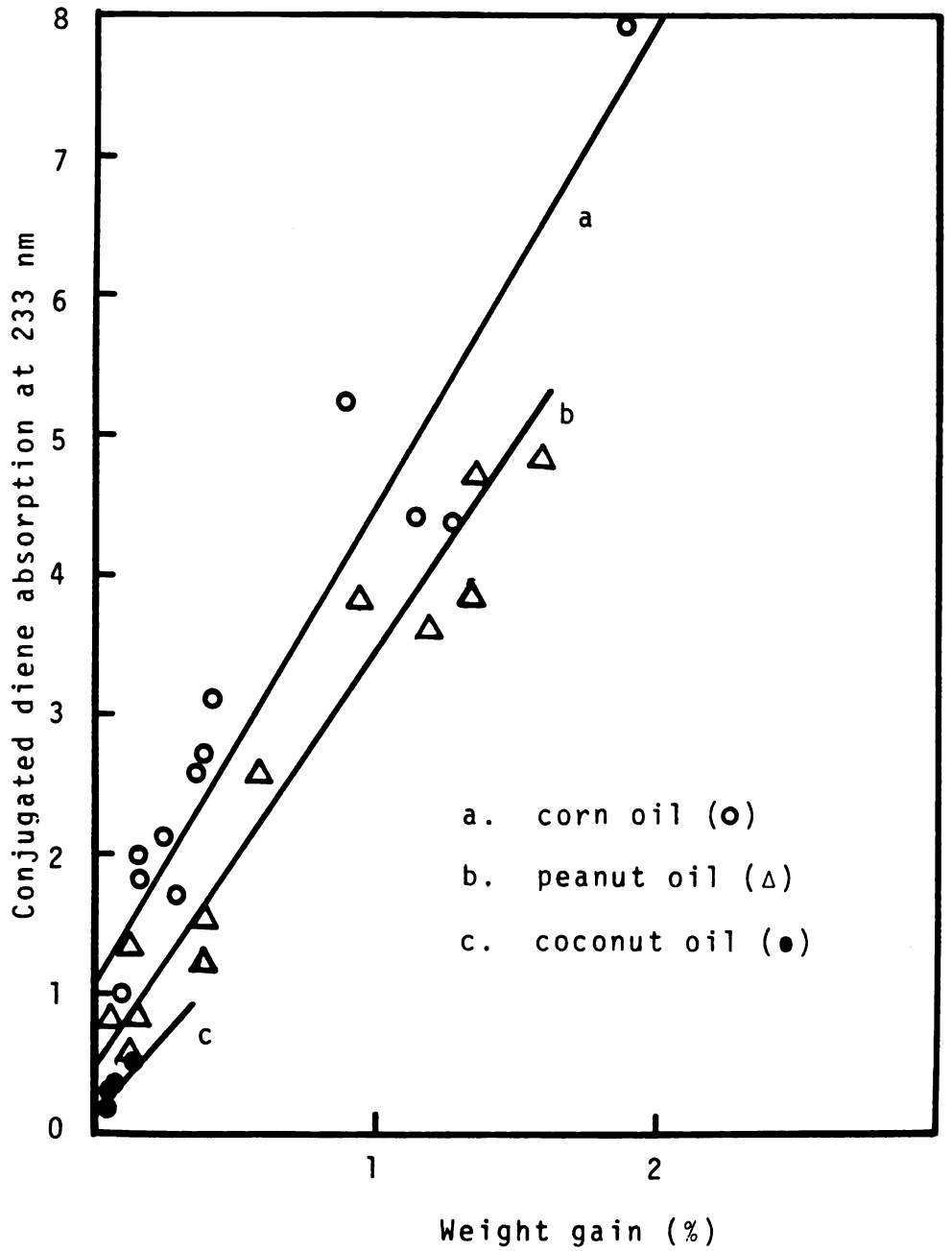

Figure 3. Conjugated diene absorption vs weight gain of oils stored at 63°C.

Table 4. Correlation coefficients among PV, CDA and weight gain of oils during storage at 63°C[a]

Oil	Correlation coefficient*		
	PV vs CDA	PV vs weight gain	CDA vs weight gain
Coconut	0.954	0.887	0.864
Peanut	0.975	0.972	0.935
Corn	0.981	0.882	0.890

[a]Results of both control and antioxidant treated oils were pooled for computing correlation coefficients.

*Indicates significant ($P < 0.01$).

oil. Thus the CDA method was more reliable when conducted in vegetable oils containing high level than those with low levels of polyunsaturated fatty acids.

As can be seen in Table 4, the correlations between PV and CDA, PV and weight gain, and CDA and weight gain of peanut oil were 97.5%, 97.2% and 93.5% respectively. It seemed that PV, CDA and weight gain were excellent methods to measure the oxidative changes in peanut oil. On the other hand, the correlations between PV and weight gain, and between CDA and weight gain of corn and coconut oils were less than 90%. Thus the weight gain test is not so accurate as to replace PV in following the oxidative deterioration of coconut and corn oils.

The Effect of Type and Concentration of Antioxidants Upon Oil Stability.

The type and concentration of antioxidants play an important role in applying antioxidants to vegetable oils to achieve optimum results. There are significant differences between antioxidants with regard to their performance characteristics in different types of oils.

Sherwin's review (1972) noted that propyl gallate was an extremely effective antioxidant from the stand point of its ability to improve the shelf life of vegetable oils whereas BHA and BHT were not particularly effective in this type of application. Sherwin and Thompson (1967) used cottonseed, soybean and safflower oils to show that the

higher the concentration of BHA, BHT, TBHQ or propyl gallate, the greater the AOM stability.

Skinner et al. (1970) and Cort et al. (1975) reported that a tocopherol derivative 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid was effective in both animal fats and vegetable oils in contrast to tocopherol which had poor activity in the prevention of peroxidation of vegetable oils. Dugan (1956) showed that tocopherols at a concentration of 0.10% had lower effectiveness as measured by AOM stability than a concentration of 0.05% in lard.

In this study, Trolox C, BHA and BHT each with two different concentrations were compared. The results are shown in Table 5, Figure 4 and Table 6. It is evident from Table 5 that peroxide value increased gradually in both treated and untreated samples of peanut oil during the storage time over a period of four weeks. Analysis of variance indicated highly significant treatment effects in the development of peroxide values ($P < 0.01$). Duncan's New Multiple Range Test was used to test the differences among the means. As indicated in Table 5, a significant difference between the peroxide value achieved and the initial peroxide value of control sample was observed during a one week storage period. Samples containing Trolox C 0.02% and Trolox C 0.01% required 4 weeks and 3 weeks respectively to reach this point. Samples containing BHA or BHT required 3 weeks regardless of the concentration.

Table 5. Mean peroxide values (meq/kg) of peanut oil during storage at 63°C.

Antioxidant	Time (weeks)				
	0	1	2	3	4
Control	14.9^{a}	49.9^{def}	121.9^{m}	268.4^{n}	426.5^{o}
Trolox C 0.01%	15.0^{a}	16.6^{a}	23.2^{a}	67.1^{ghi}	222.7^{p}
Trolox C 0.02%	14.3^{a}	15.5^{a}	20.4^{a}	22.2^{a}	37.7^{bcd}
BHA 0.01%	15.3^{a}	25.3^{abc}	71.6^{ghij}	85.0^{jkl}	325.1^{q}
BHA 0.02%	14.2^{a}	21.4^{a}	63.3^{fgh}	89.7^{l}	196.4^{r}
BHT 0.01%	15.0^{a}	24.2^{ab}	62.7^{fgh}	80.2^{ijk}	305.4^{s}
BHT 0.02%	14.2^{a}	21.5^{a}	56.8^{efg}	77.3^{ijk}	122.5^{t}

Standard Error of Means = 3.561

^{ab} = values sharing common letters were not significantly different (by Duncan's New Multiple Range Test) $P < 0.01$.

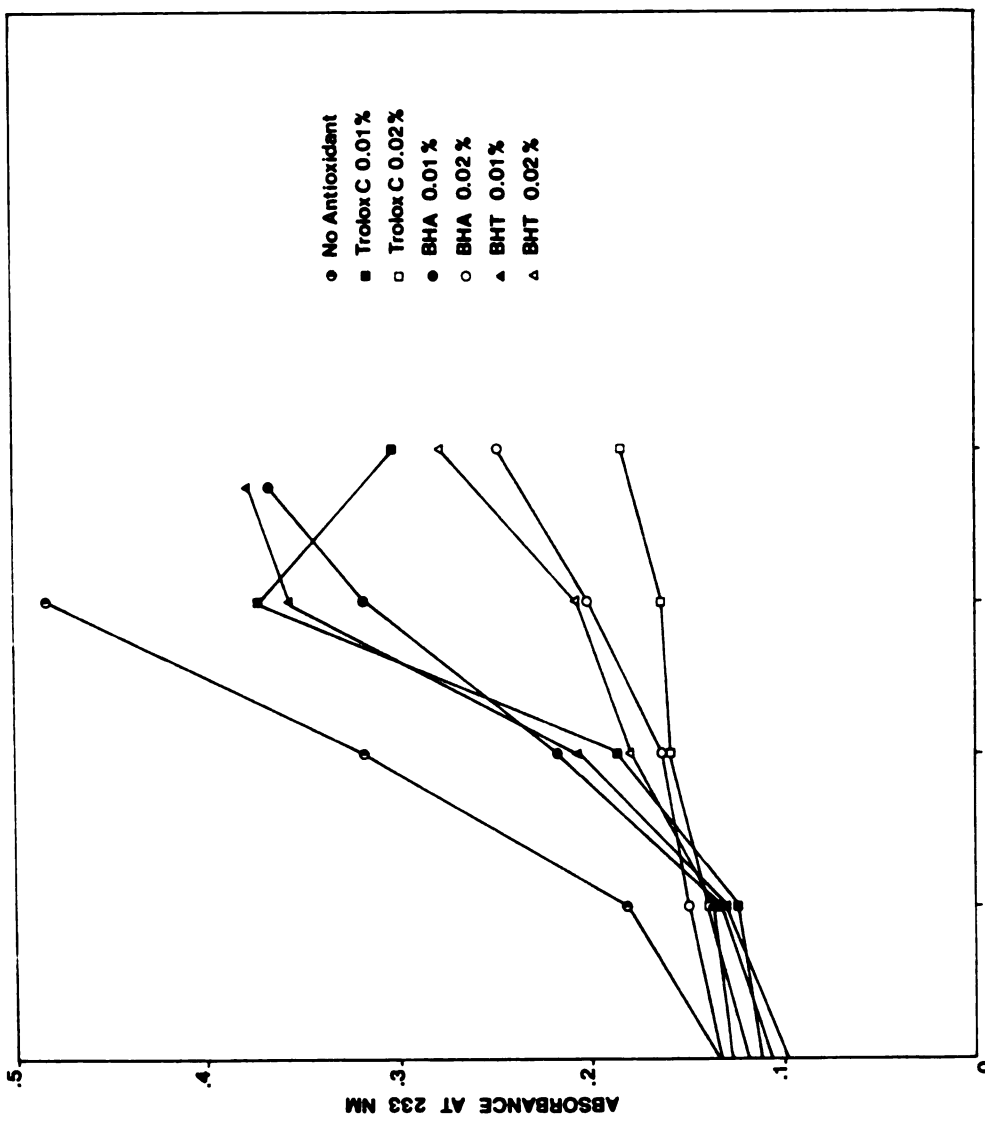

Figure 4. Conjugated diene absorption vs time for coconut oil during storage at 63°C.

Table 6. Mean weight gain (%) of corn oil during storage at 63°C.

Antioxidant		Time (weeks)			
		1	2	3	4
No antioxidant		0.189^{bc}	0.402^{ef}	1.392^{i}	2.496^{j}
Trolox C	0.01%	0.080^{ab}	0.190^{bc}	0.490^{f}	1.336^{i}
Trolox C	0.02%	0.020^{a}	0.053^{a}	0.415^{ef}	0.475^{k}
BHA	0.01%	0.138^{ab}	0.337^{e}	0.778^{g}	1.988^{l}
BHA	0.02%	0.041^{a}	0.215^{cd}	0.487^{f}	1.080^{h}
BHT	0.01%	0.125^{abc}	0.314^{de}	0.724^{g}	1.917^{m}
BHT	0.02%	0.030^{a}	0.205^{cd}	0.378^{ef}	1.041^{h}

Standard Error of Means = 0.0292.

^{abc} = values sharing common letters were not significantly different (by Duncan's New Multiple Range Test) $P < 0.01$.

The difference between samples containing Trolox C at 0.01% and 0.02% was not significant for the first three weeks. There was also no significant difference in inhibitory effects between BHA 0.01% and 0.02% or BHT 0.01% and 0.02% for the first three weeks. It was noted that the concentration of 0.01% for these antioxidants seemed to be optimum in retarding the peroxidation of peanut oil. As measured by peroxide method, Trolox C had greater efficacy than either BHA or BHT, which confirmed the results obtained by Cort et al. (1975).

Figure 4 displays the development of conjugated diene absorption measured on coconut oil during storage at 63°C over a period of 8 weeks. As can be seen, the control sample had the highest rate of conjugated diene absorption development. The sample containing Trolox C had higher stability than that containing either BHA or BHT. The effects of antioxidant concentration were clearly noted for all antioxidants used after a six weeks storage period.

Table 6 shows the mean weight gain of corn oil during storage at 63°C. for four weeks. Analysis of variance indicated a highly significant difference between treatments. As can be seen, the antioxidants used had inhibitory effects to various degree upon oxygen uptake as indicated by lower weight gains than that of the control sample. As in peanut oil, Trolox C had better efficacy than either BHA or BHT in corn oil. As the concentration increased, the inhibitory effect also increased. It was noted that the

effect of antioxidant concentration was more significant in corn oil than in peanut oil.

As mentioned, all antioxidants used exhibited higher effectiveness as the concentration increased. The use of antioxidants in concentration as small as possible is an important consideration in applying the antioxidants to vegetable oils. BHA and BHT are very soluble in oils. The solubility of Trolox C in peanut and corn oils is 0.18 g/100 g while its toxicity as measured by LD_{50} is greater than 1,000 mg/kg of body weight in mice, rats and rabbits (Cort et al., 1975). The maximum concentration of antioxidants permitted in vegetable oils are defined by regulations. BHA and BHT are food approved antioxidants, while Trolox C has not been released for human consumption. This study established that the concentration of 0.01% for Trolox C in vegetable oils gives good results.

Effects of Combinations of Antioxidants Upon Oil Stability

Trolox C, BHA, BHT and citric acid were used for this study and evaluated at a temperature of 63°C. Peroxide value, conjugated diene absorption and weight gain were measured on samples during the storage period.

Table 7 shows the stabilization of peanut oil at 63°C. as measured by the weight gain method. Figure 5 exhibits the development of peroxide value in peanut oil over a period of four weeks. Figure 6 displays the change of conjugated diene absorption observed in the same samples. As can be seen, a similar pattern was obtained.

Table 7. Stabilization of peanut oil at 63°C.

Antioxidant	Oven days*	Protective factor	Synergism (days)
1. No antioxidant	13	1.0	
2. Trolox C 0.01%	22	1.7	
3. BHA 0.01%	17	1.3	
4. BHT 0.01%	18	1.4	
5. CA 0.005%	14	1.1	
6. Trolox C 0.01% + BHA 0.01%	23	1.8	-3
7. Trolox C 0.01% + BHT 0.01%	23	1.8	-4
8. Trolox C 0.01% + BHA 0.01% + CA 0.005%	41	3.2	+14
9. Trolox C 0101% + BHT 0.01% + CA 0.005%	44	3.4	+16

*days to reach a weight gain of 0.2%

$$\text{Protective factor} = \frac{\text{Stability of the sample containing antioxidant}}{\text{Stability of the control sample}}$$

Rancid odor was noted in peanut oil when the weight gain reached 0.2%. This point was equivalent to a peroxide value of about 70 meq/kg and conjugated diene absorption of about 1.03. As indicated in Table 7, all antioxidants and their combinations had inhibitory effects to various degrees upon oxidative deterioration in peanut oil. The protective factor was calculated by the ratio of the stability of the sample containing antioxidant and the stability of the control sample, thus the higher the protective factor the greater the effectiveness of the antioxidant.

Cort et al. (1975) revealed that 0.02% Trolox C in thin layer test at temperature of 45^{o}C had two to four times the antioxidant activity of BHA and BHT in vegetable oils and animal fats. As can be seen from Table 7, the peanut oil treated with 0.01% Trolox C displayed good stability and required 22 days to reach a weight gain of 0.2%. The protective factor of Trolox C was 1.7. The peanut oil treated with BHA and BHT exhibited lower stability and required 17 and 18 days respectively to reach the same point. The protective factor of BHA was 1.3 while that of BHT was 1.4. Thus Trolox C, in the petticup test at 63^{o}C in peanut oil, had 1.2 to 1.3 times the antioxidant activity of BHA and BHT. These were not as high as the values reported by Cort et al. (1975) and may be due to the higher temperature used as well as lower concentrations of antioxidants. It was postulated by Cort et al. (1975) that high temperature destroyed part of the efficacy of

Trolox C.

Cort et al. (1975) showed synergism of Trolox C in lard and soybean oil with ascorbic acid and ascorbyl palmitate as measured by the active oxygen method. Ascorbic acid is not soluble in oil but very active. The lack of solubility actually enhances the stability of ascorbic acid in oil. Ascorbates convert Fe^{3+} to Fe^{2+} and Cu^{2+} to the lower oxidation states, thus the conversion of Trolox C to its quinone form which is catalyzed by these trace metals, can be inhibited.

As shown in Table 7, 0.01% Trolox C showed a negative synergism in combination with either 0.01% BHA or 0.01% BHT in peanut oil. The addition of 0.005% citric acid provided a positive synergism. The citric acid probably had a role similar to that of ascorbic acid.

As displayed in Figure 5, the control sample exhibited the highest rate of peroxide development with time, which in turn was followed by the samples containing citric acid, BHA, BHT and Trolox C respectively. Trolox C in combination with BHA had a slightly higher rate than when in combination with BHT. Trolox C in combination with either BHT or BHA and citric acid had the lowest rate. A similar trend was noted, when it was evaluated by conjugated diene absorption (Figure 6). The order of antioxidant efficacy in decreasing order was Trolox C, BHT, BHA and citric acid.

Table 8 exhibits stabilization of corn oil at $63^{o}C$ based on peroxide measurement. The stability was calculated

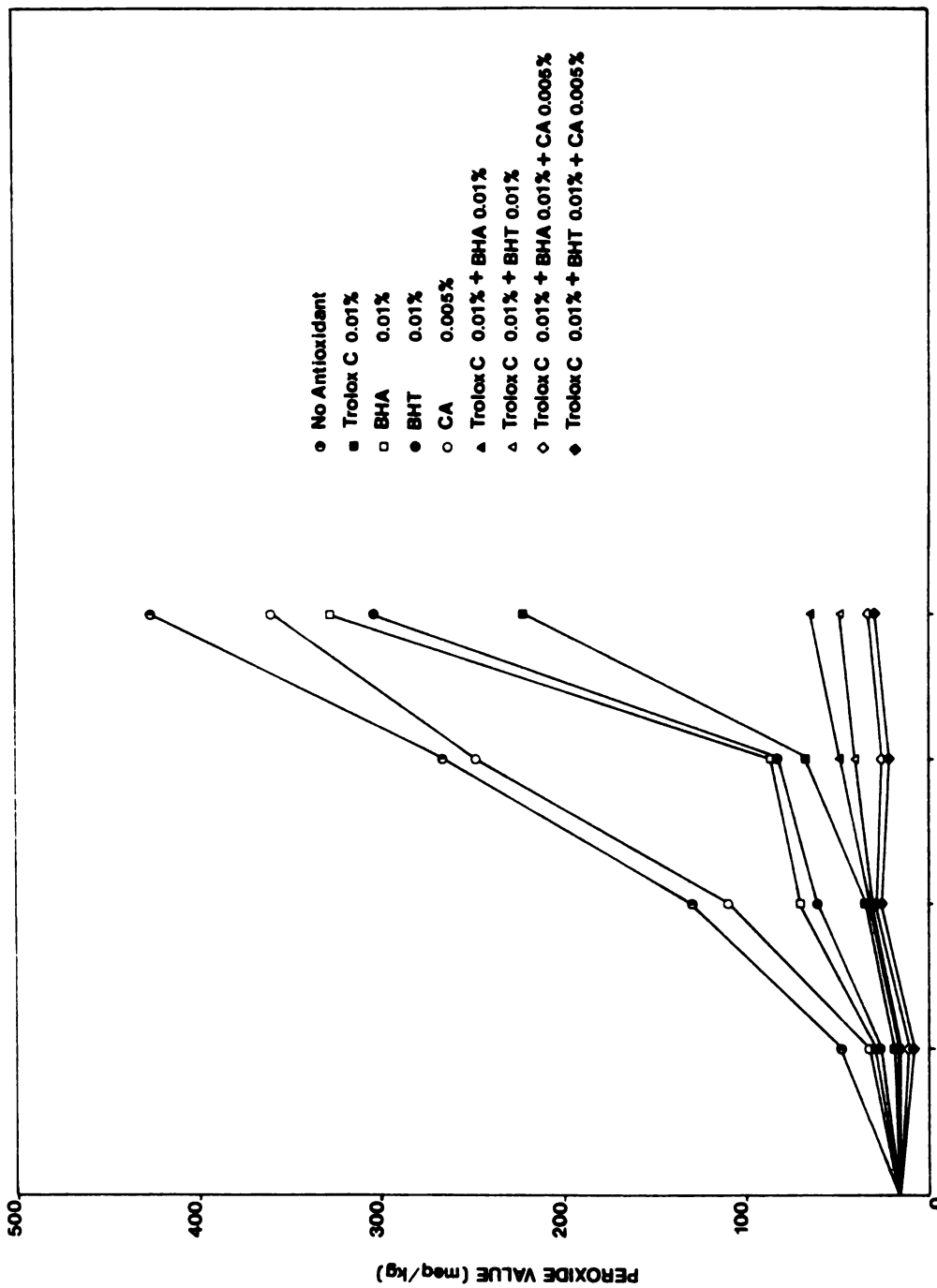

Figure 5. Peroxide value of peanut oil during storage at 63°C.

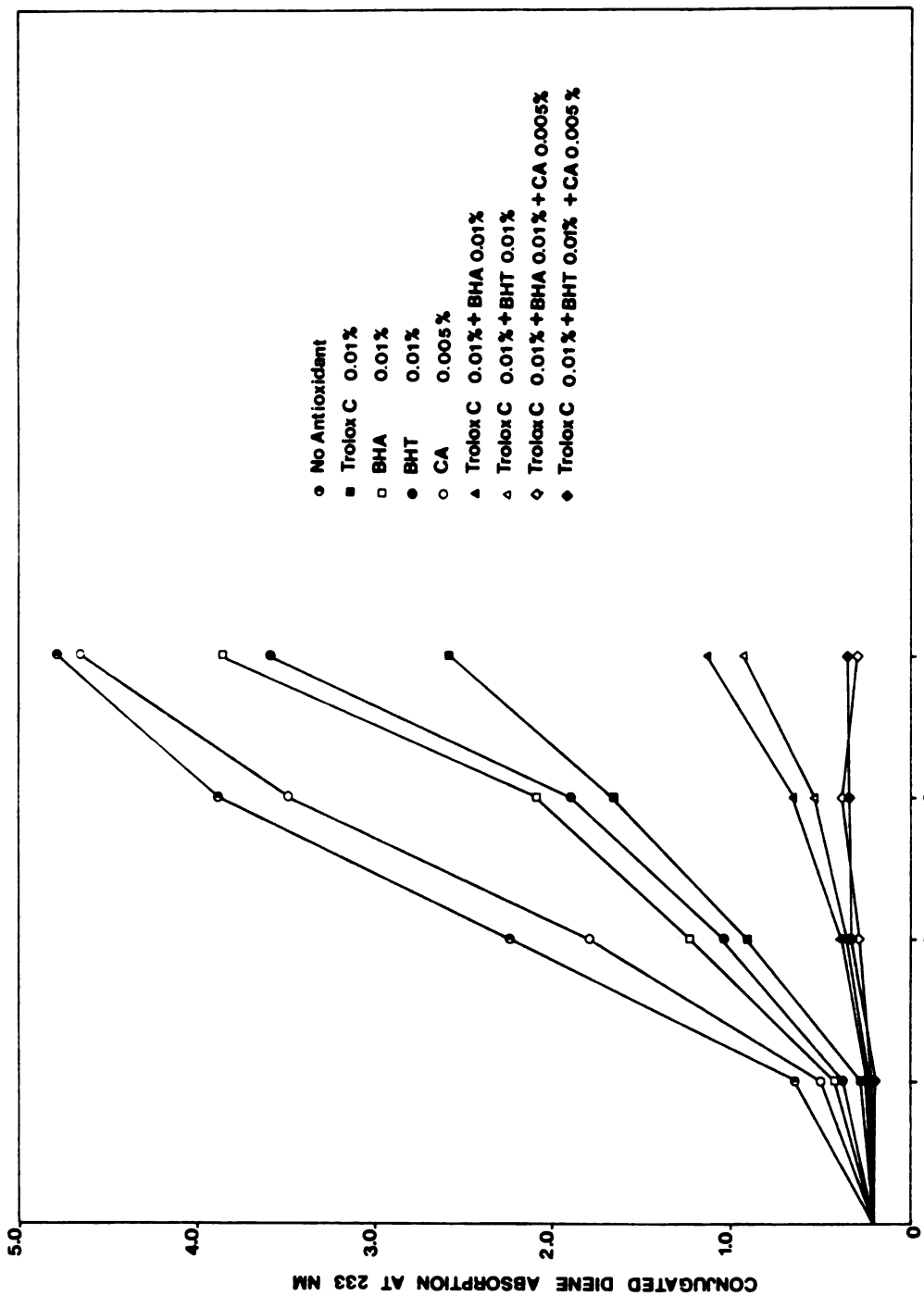

Figure 6. Conjugated diene absorption of peanut oil during storage at 63°C.

Table 8. Stabilization of corn oil at 63°C.

Antioxidant	Oven days*	Protective factor	Synergism (days)
1. No antioxidant	7	1.0	
2. Trolox C 0.01%	15	2.1	
3. BHA 0.01%	12	1.7	
4. BHT 0.01%	10	1.4	
5. CA 0.005%	8	1.1	
6. Trolox C 0.01% + BHA 0.01%	16	2.3	-4
7. Trolox C 0.01% + BHT 0.01%	16	2.3	-2
8. Trolox C 0.01% + BHA 0.01% + CA 0.005%	18	2.6	-3
9. Trolox C 0.01% + BHT 0.01% + CA 0.005%	18	2.6	-1

*days to reach a peroxide value of 70 meq/kg

$$\text{Protective factor} = \frac{\text{Stability of the sample containing antioxidant}}{\text{Stability of the control sample}}$$

as the number of days required to reach the peroxide value of 70 meq/kg. This end point corresponded with a weight gain of 1.2% and conjugated diene absorption of 1.431. Corn oil treated with 0.01% Trolox C had a higher stability than the samples with BHA, BHT and citric acid. The order of effectiveness in decreasing order was Trolox C, BHA, BHT and citric acid. Trolox C had about 1.2 to 1.5 times the antioxidant activity of BHA and BHT. This value was lower than that reported by Cort et al. (1975) probably by the greater temperature employed. It was interesting to note that BHT had greater effectiveness than that of BHA in peanut oil. On the other hand BHA showed higher activity in corn oil than did BHT. In corn oil, 0.01% Trolox C in combination with either 0.01% BHA or BHT displayed a negative synergism. The addition of 0.05% citric acid to these samples reduced the negative value of the synergism.

Figure 7 shows the development of oxygen uptake as measured by weight gain in corn oil. Here it is seen that the amount of oxygen absorbed varied with antioxidants throughout the storage period. Figure 8 displays the absorbance at 233 nm due to diene conjugation. It seemed that a similar trend existed.

Results of tests performed with coconut oil appear in Table 9, Figure 9 and Figure 10. Since Trolox C in combination with another antioxidant never reached an end point over a period greater than two months, synergism in coconut oil could not be calculated. Table 9 shows mean conjugated

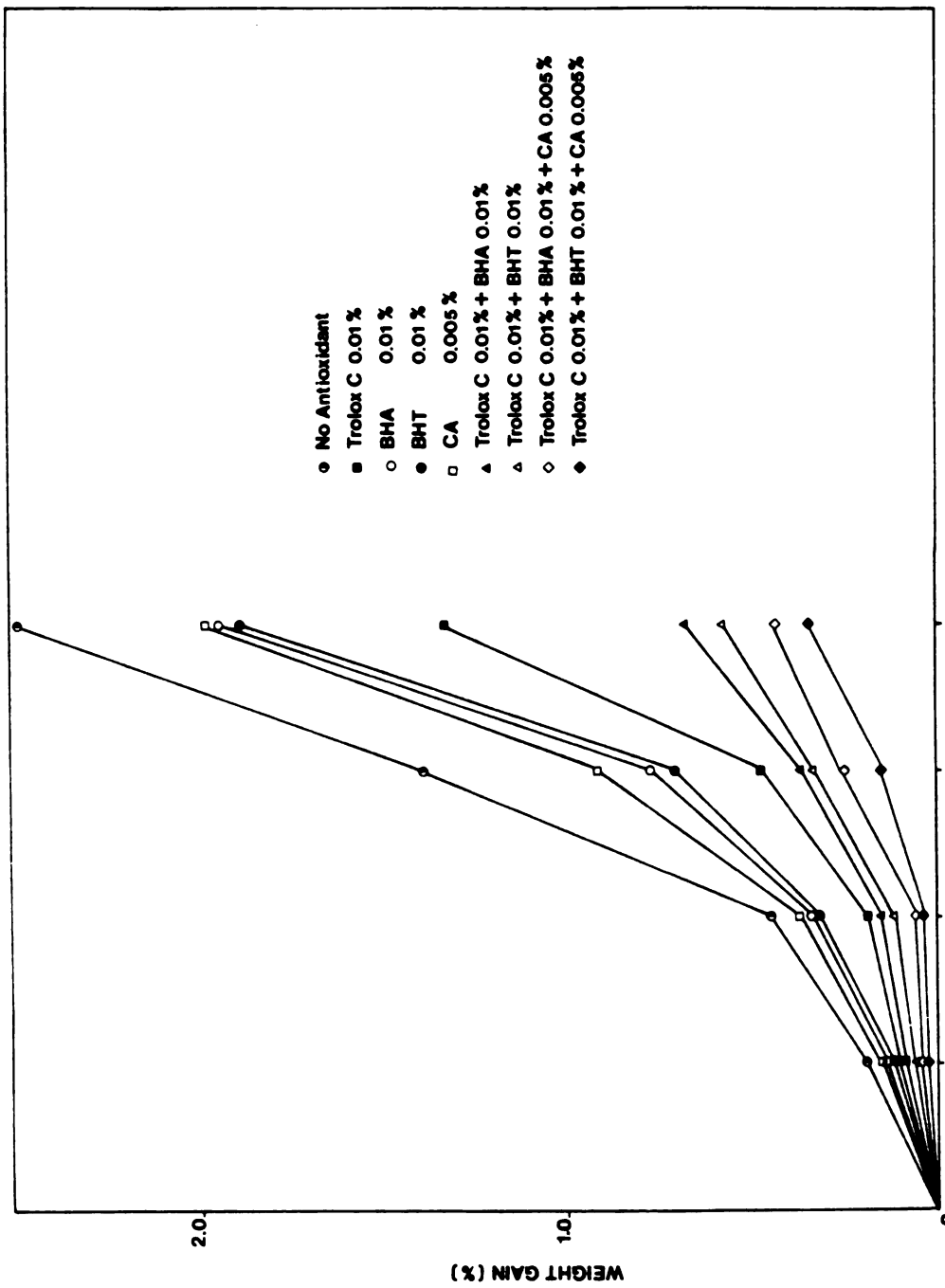

Figure 7. Weight gain of corn oil during storage at 63°C.

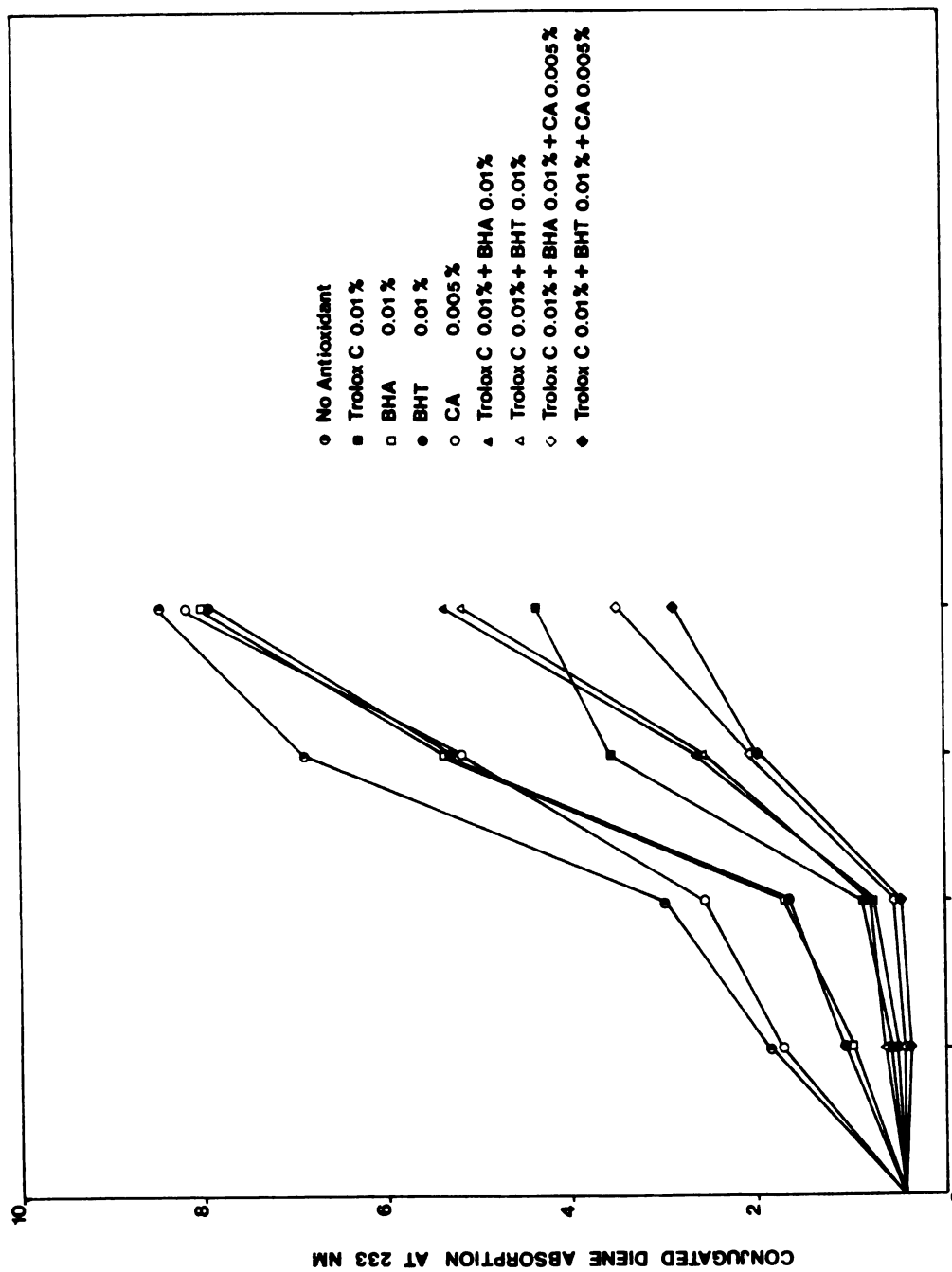

Figure 8. Conjugated diene absorption of corn oil during storage at 63°C.

Table 9. Mean conjugated diene absorption of coconut oil during storate at 63°C for 8 weeks*

Antioxidant**	Time (weeks)				
	0	2	4	6	8
No antioxidant	0.124^{bcdef}	0.181^{jk}	0.318^{o}	0.487^{v}	0.341^{pq}
Trolox C	0.110^{ab}	0.127^{cdef}	0.187^{k}	0.378^{rs}	0.305^{no}
BHA	0.105^{ab}	0.139^{efg}	0.218^{l}	0.321^{op}	0.372^{rs}
BHT	0.098^{a}	0.136^{efg}	0.213^{l}	0.357^{qr}	0.380^{st}
CA	0.121^{bcdef}	0.167^{ijk}	0.293^{n}	0.413^{u}	0.394^{tu}
Trolox C + BHA	0.110^{abcd}	0.124^{bcdef}	0.146^{gh}	0.290^{n}	0.234^{lm}
Trolox C + BHT	0.108^{ab}	0.120^{bcde}	0.140^{efg}	0.255^{m}	0.233^{l}
Trolox C + BHA + CA	0.121^{bcdef}	0.127^{cdef}	0.138^{efg}	0.155^{hi}	0.128^{def}
Trolox C + BHT + CA	0.123^{bcdef}	0.131^{ef}	0.155^{hi}	0.165^{ij}	0.142^{fg}

*measured at the wave length of 233 nm and calculated at a dilution of 1:1000 (w/v)

^{ab}=values sharing common letters were not significantly different (by Duncan's New Multiple Range Test) $P < 0.01$

**antioxidant concentration = 0.01% except CA = 0.005%.

Standard Error of Means = 0.005.

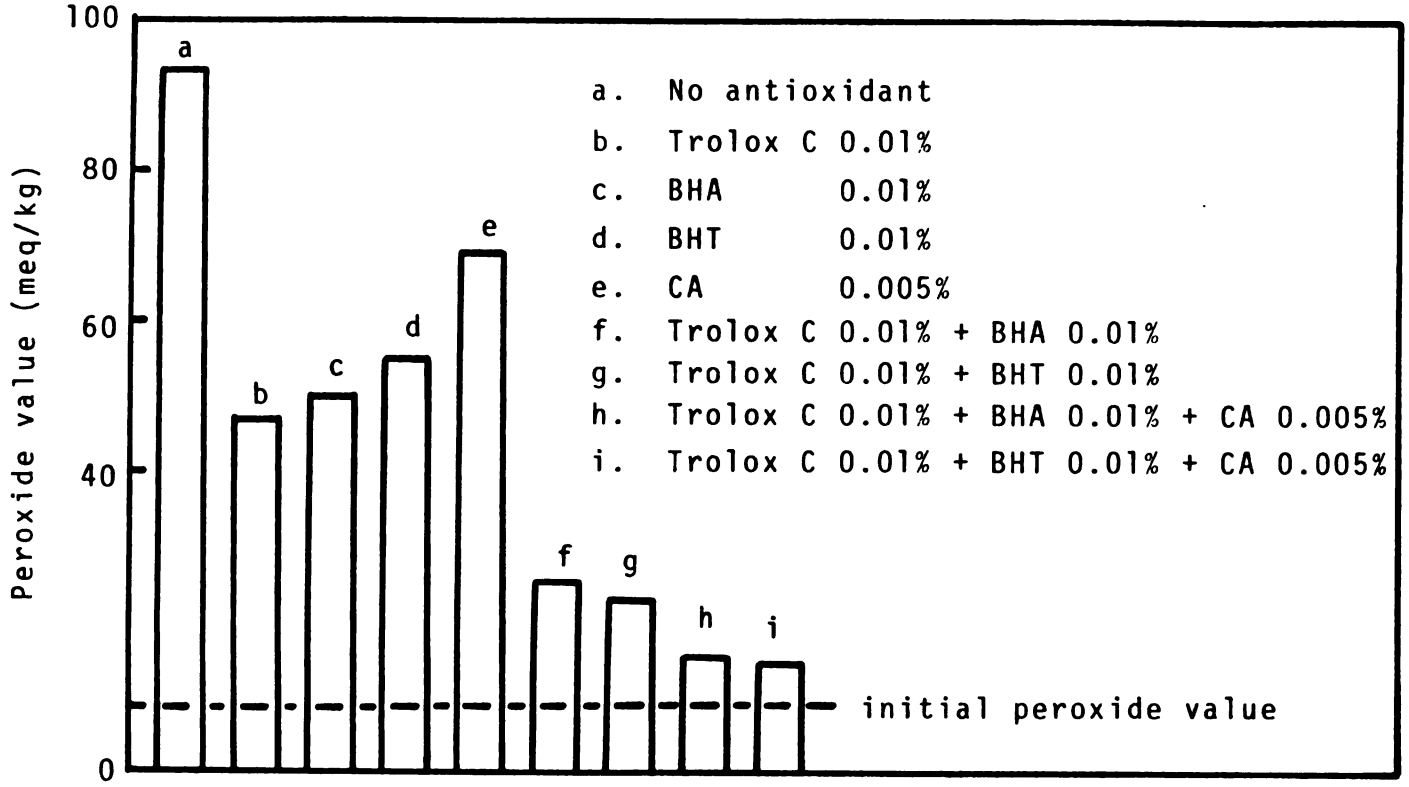

Figure 9. Stabilization of coconut oil stored at 63°C for six weeks (measured by PV).

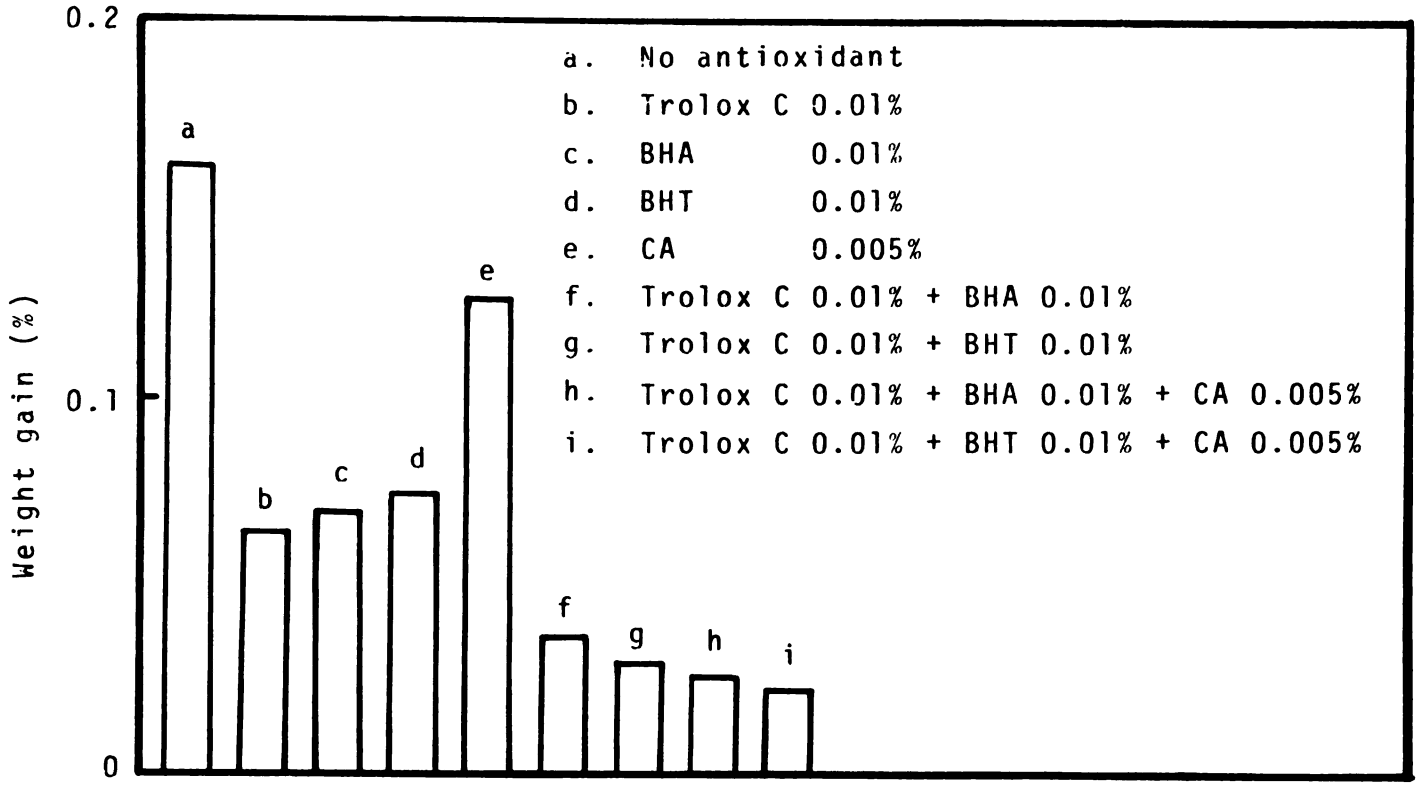

Figure 10. Stabilization of coconut oil stored at 63°C for six weeks (measured by weight gain).

diene absorption measured on coconut oil treated with various antioxidants. Statistical analysis of the data revealed highly significant effects from treatment ($P < 0.01$).

To test the difference between means, Duncan's New Multiple Range Test was employed. As indicated, there were significant differences between controls and antioxidant treated samples at a storage time of 2 weeks. Trolox C performed more effectively than BHA and BHT which in turn were more effective than citric acid. BHA had slightly better activity than BHT. Cort et al. (1975) showed that neigher TBHQ or Trolox C appeared very efficient in palm oil. They stated that in most palm oils, Trolox C in combination with ascorbic acid gave the highest AOM values. In the present study, as shown in Table 9, citric acid greatly enhanced the effectiveness of Trolox C in combination with BHA or BHT.

The peroxide value of the control sample reached the highest point at a storage period of six weeks then it decreased as the time increased. This phenomenon resulted from the fact that peroxides are intermediate product in the peroxidation of oil. The rate of peroxides formation was less than the rate of degradation at a storage period of six weeks. Figure 9 displays the stabilization of coconut oil stored for six weeks as measured by peroxide value, while Figure 10 exhibits that stabilization as analyzed by the weight gain method. Here it is seen that similar patterns exist.

Corn oil was oxidized more rapidly than peanut oil which in turn was more rapidly oxidized than coconut oil (Tables 7 to 9; Figures 5 to 10). The differences that were noted were ascribed to the fatty acid composition of these oils (Table 2). Holman and Elmer (1947) measured the oxidation rate of several unsaturated fatty acids and their esters. They concluded that an increase of one double bond in unsaturated fatty acid or ester caused the rate of oxidation to increase by a factor of two or more. Corn oil contains more linoleic acid than peanut and coconut oils. Thus the greater amount of this polyunsaturated fatty acid could explain why corn oil is more susceptible to autoxidation than peanut and coconut oils.

As mentioned, a similar trend of oxidative change was noted in coconut, corn and peanut oils as measured by PV and CDA. This phenomenon results from the fact that shifts of double bonds occur along with hydroperoxide formation during autoxidation. The amount of oxygen uptake was proportional to the peroxides produced. Privett et al. (1953) and Cannon et al. (1952) isolated about 90% conjugation in hydroperoxides while studying autoxidation of linoleate.

In this study, all antioxidants added to the vegetable oils retard oxidative deterioration during storage by controlling the progressive build up of peroxide value as well as diene conjugation and oxygen uptake. Trolox C is a tocopherol derivative and it acts as an electron donor which quenches electron mobility. Although BHA is better than BHT

when treated in corn and coconut oil, it is slightly less effective than BHT in peanut oil. This phenomenon has not been explained.

Citric acid when used alone showed a small inhibitory effect in the vegetable oils used. Lange (1950) reported that residual levels of tocopherols in various oil may occur at 83 p.p.m. in coconut oil, 48 p.p.m. in peanut oil and 90 p.p.m. in corn oil. These residual levels contribute to the oxidative stability of oils. The inhibitory effect of citric acid may be because of its function to quench the trace metals present thus the stability of oils can be established due to the naturally occuring antoixidant tocopherols.

The synergistic effect of citric acid upon oxidative stability of vegetable oils was high in the presence of Trolox C in combination with BHA or BHT. This may be due to the fact that citric acid chelates the trace metals such as Fe^{+++} and Cu^{++} found in vegetable oils and inhibits the conversion of Trolox C to its quinone form.

Trolox C in combination with BHA or BHT and citric acid displayed a negative synergism in corn oil but resulted in a positive synergism in peanut oil. Why this is negative in one oil and positive in another oil is not readily apparent.

Room Temperature Storage.

In this study, the effect of the antioxidants Trolox C, BHT, BHA and TBHQ at a concentration of 0.02% was examined

during a storage period of 24 weeks. The concentration of antioxidant permitted in food is usually not to exceed 0.02% based upon the fat and oil content. The results were based on the measurement of peroxide value and absorbance at 233 nm.

The peroxide values measured on coconut oil are presented in Table 10. The data indicate that all samples stored at room temperature ranging from 20 to 28^{o}C, had little change in peroxide value. At a storage period of 12 weeks, the control sample achieved a peroxide value of 9.2 meq/kg at which the difference between the peroxide value reached and the initial peroxide value was significant. This point was reached at a storage period of 12 weeks for BHA and BHT treated samples. Samples containing TBHQ required 16 weeks while Trolox C treated samples claimed 20 weeks. Thus Trolox C in coconut oil stored at room temperature exhibited a greater effectiveness than TBHQ which in turn was more effective than BHA and BHT.

Similar results were obtained based on conjugated diene absorption measurement as illustrated in Figure 11. The absorption due to diene conjugation increased throughout the storage period. The higher the conjugated diene absorption the lower the stability, thus coconut oil containing Trolox C displayed the greatest stability.

Results performed with peanut oil were exhibited in Table 11 and Figure 12. Analysis of variance of the data indicated a high treatment effect. As shown in Table 11, the control sample required 8 weeks to achieve a peroxide

Table 10. Mean peroxide value of coconut oil stored at room temperature (20-28°C) for 24 weeks.

Time (weeks)	Antioxidant*				
	Control	Trolox C	BHA	BHT	TBHQ
0	8.2^{a}	8.4^{abc}	8.5^{abcd}	8.5^{abcd}	8.4^{abc}
4	8.4^{abc}	8.3^{ab}	8.5^{abcd}	8.6^{abcd}	8.3^{ab}
8	8.5^{abcd}	8.4^{abc}	8.7^{abcd}	8.9^{abcde}	8.5^{abcd}
12	9.2^{bcdefg}	8.6^{abcd}	8.6^{abcd}	9.3^{cdefg}	8.4^{abc}
16	10.3^{h}	9.1^{abcde}	10.0^{fgh}	10.1^{gh}	9.0^{abcde}
20	11.8^{i}	9.0^{abcde}	11.3^{i}	11.4^{i}	9.8^{efgh}
24	16.7^{k}	9.4^{defgh}	13.3^{j}	12.7^{j}	11.2^{i}

*Antioxidant concentration = 0.02%

abcde = values sharing common letters were not significantly different (by Duncan's New Multiple Range Test) $P < 0.05$.

Standard Error of Means = 0.293.

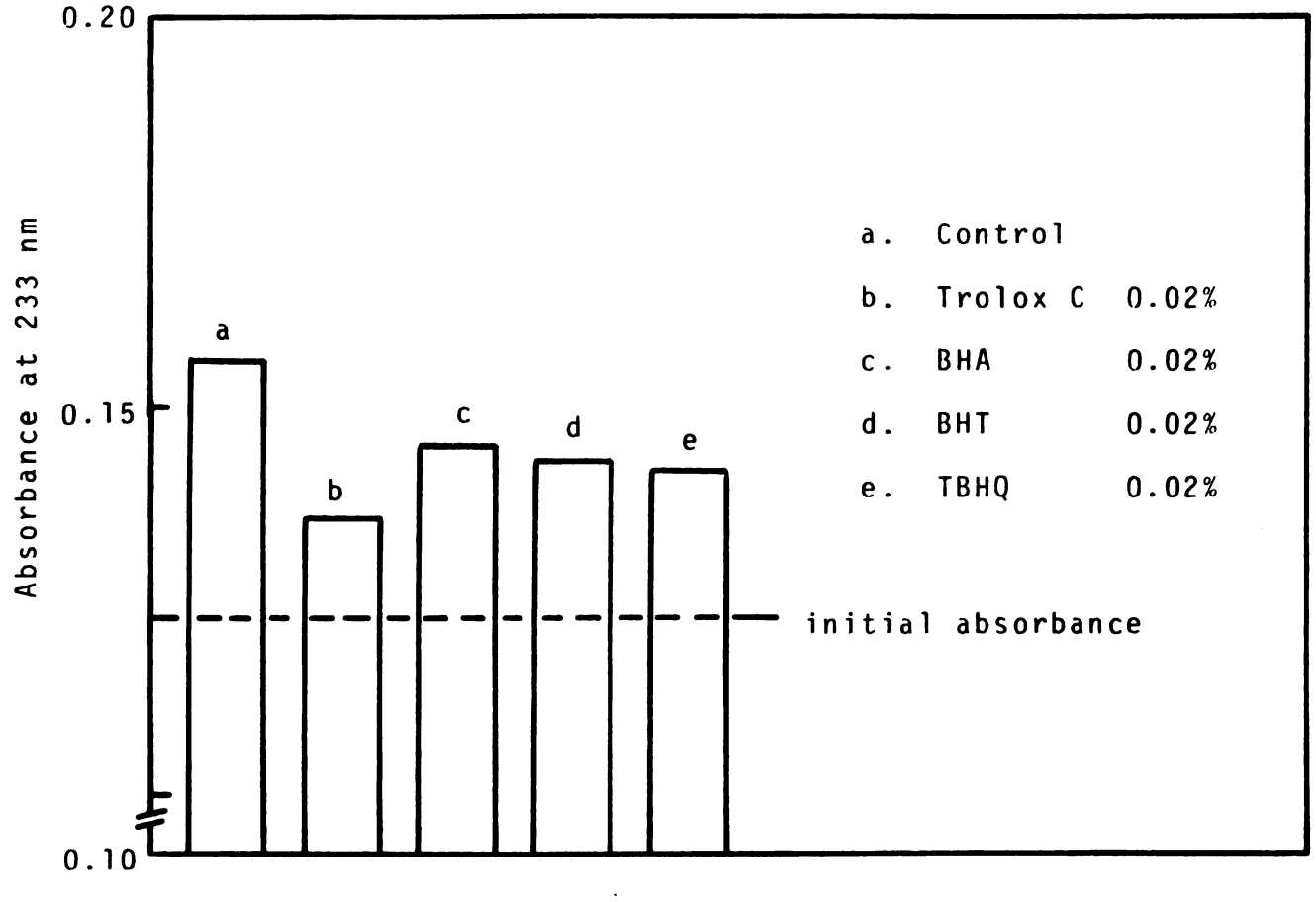

Figure 11. Stabilization of coconut oil stored at room temperature for 24 weeks (as measured by conjugated diene absorption)

Table 11. Mean conjugated diene absorption of peanut oil stored at room temperature (20-28°C)*

Time (weeks)	Antioxidant**				
	Control	Trolox C	BHA	BHT	TBHQ
0	0.210^{ab}	0.205^{a}	0.213^{abc}	0.208^{ab}	0.207^{a}
4	0.239^{abcdef}	0.217^{abcd}	0.236^{abcdef}	0.235^{abcdef}	0.213^{abcde}
8	0.289^{gh}	0.230^{abcde}	0.255^{efg}	0.244^{bcdef}	0.227^{abcde}
12	0.350^{jk}	0.240^{abcdef}	0.301^{hi}	0.252^{def}	0.247^{cdef}
16	0.415^{n}	0.250^{def}	0.369^{k}	0.310^{i}	0.251^{def}
20	0.594^{l}	0.304^{hi}	0.575^{l}	0.351^{k}	0.270^{jk}
24	0.835^{o}	0.318^{ij}	0.801^{o}	0.370^{m}	0.324^{ij}

*measured at 233 nm and calculated at a dilution of 1:1000 (w/v)

**antioxidant concentration = 0.02%

^{ab}values sharing common letters were not sifnificantly different (by Duncan's New Multiple Range Test) $P < 0.01$

Standard Error of Means = 0.008.

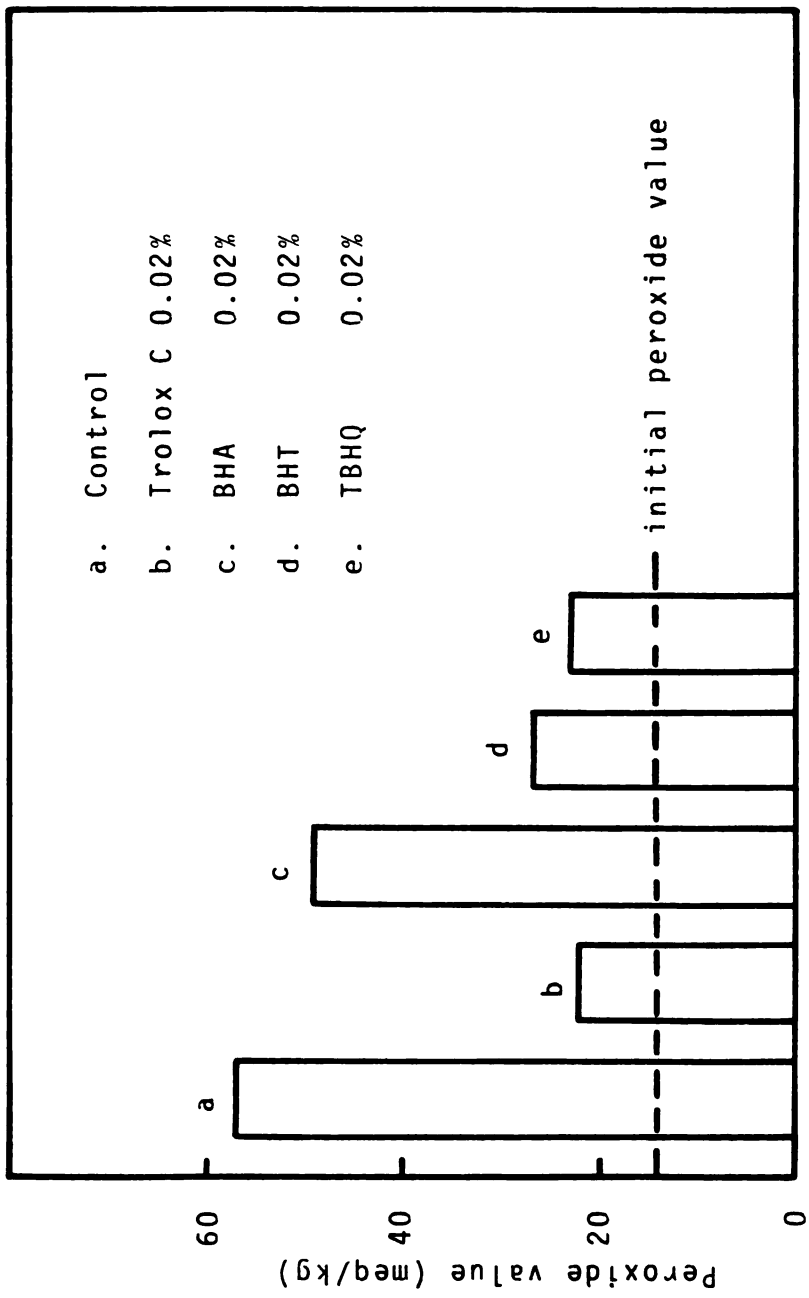

Figure 12. Stabilization of peanut oil stored at room temperature for 24 weeks (as measured by peroxide value).

value which was significantly different from the initial value. All antioxidant treated samples required various times to reach this point. Trolox C and TBHQ treated samples claimed 16 and 12 weeks respectively. It was interesting to note that BHA was not a good antioxidant for peanut oil. In peanut oil stored at room temperature, Trolox C was the best antioxidant compared to the other antioxidants used.

Figure 12 exhibits stabilization of peanut oil as measured by peroxide value. The peroxode value developed to various degrees throughout storage time depending upon antioxidant used. Trolox C was the most effective, followed by TBHQ, BHT and BHA with respect to the retardment of peroxide development.

Table 12 and Figure 13 display the results performed with corn oil. Statistical analysis by the F test denoted highly significant effects from treatment. As exhibited in Table 12, the difference between peroxide value achieved and the initial peroxide value measured in control, BHA and BHT treated samples were significant at a storage period of 8 weeks. Corn oil containing Trolox C required 16 weeks to reach this point while TBHQ treated samples claimed 12 weeks. It was noted that BHA and BHT were not as effective as antioxidants for corn oil as were Trolox C or TBHQ.

Figure 13 shows the stabilization of corn oil stored at room temperature for 24 weeks as observed by the peroxide method. The effectiveness of antioxidants in decreasing

Table 12. Mean conjugated diene absorption of corn oil stored at room temperature (20-28°C)*

Time (weeks)	Antioxidant**				
	Control	Trolox C	BHA	BHT	TBHQ
0	0.362^{abc}	0.350^{ab}	0.358^{abc}	0.347^{a}	0.350^{ab}
4	0.421^{def}	0.358^{abc}	0.389^{abcde}	0.371^{abcd}	0.356^{ab}
8	0.479^{gh}	0.389^{abcde}	0.420^{def}	0.410^{cde}	0.394^{abcde}
12	0.658^{k}	0.402^{bcde}	0.631^{k}	0.506^{hi}	0.410^{cde}
16	0.815^{m}	0.426^{ef}	0.715^{l}	0.581^{j}	0.434^{efg}
20	0.973^{o}	0.470^{fg}	0.822^{m}	0.754^{l}	0.536^{ij}
24	1.159^{p}	0.530^{i}	0.885^{n}	0.861^{n}	0.632^{k}

*measured at 233 nm and calculated at a dilution of 1:1000 (w/v)

**antioxidant concentration = 0.02%

^{ab}values sharing common letters were not significantly different (by Duncan's New Multiple Range Test) $P < 0.01$.

Standard Error of Means = 0.012.

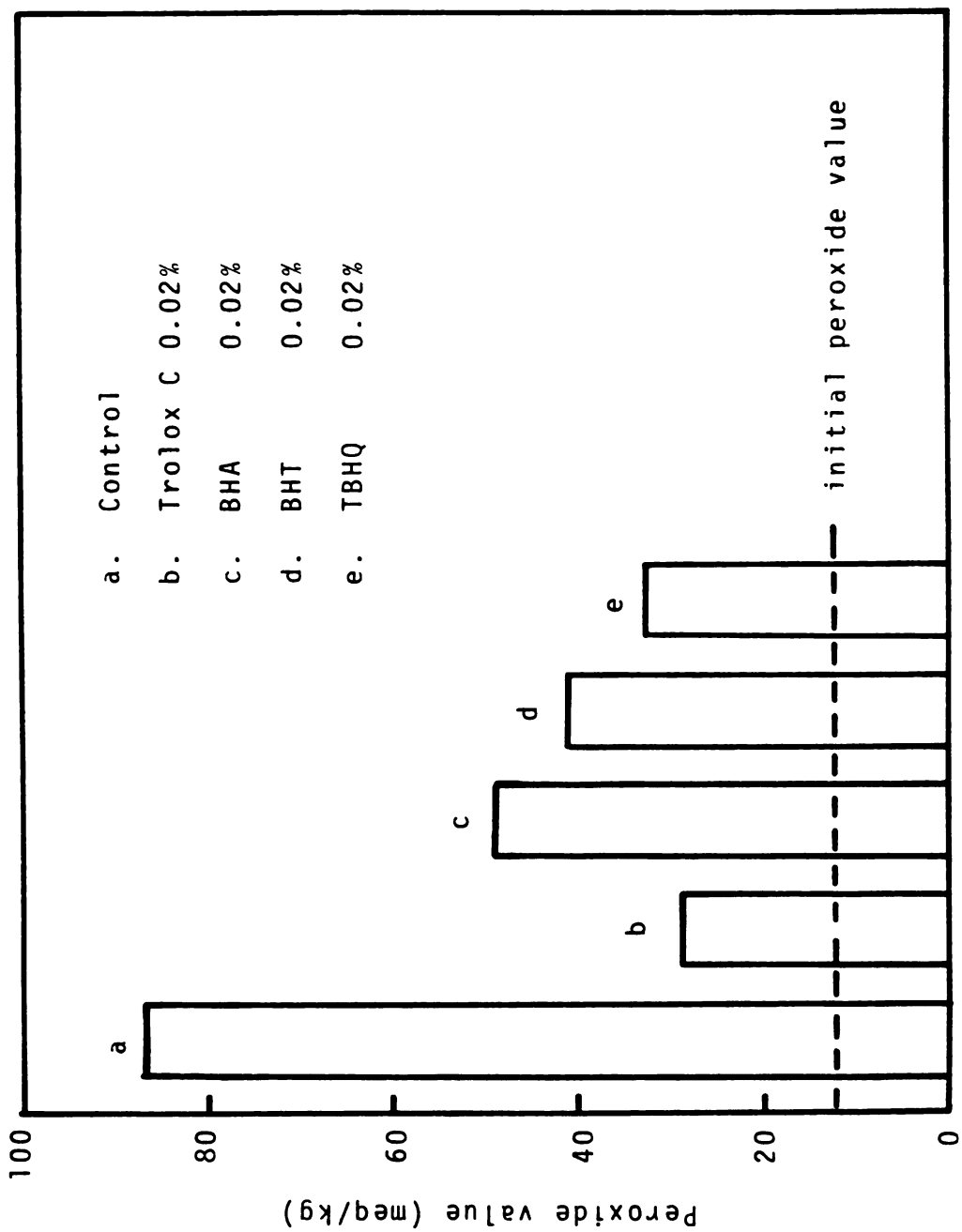

Figure 13. Stabilization of corn oil stored at room temperature for 24 weeks (as measured by peroxide value)

order was Trolox C, TBHQ, BHT and BHA with respect to inhibition of peroxide development.

As mentioned, all the oils stored at room temperature ranging from 20 to 28°C underwent oxidative changes during storage periods. These oxidative changes varied with the oils. Neither coconut nor peanut oils reached a peroxide value of 70 meq/kg during a storage period of 24 weeks, while only the control sample achieved this end point in corn oil. This phenomenon, of course, could be accounted for by the higher polyunsaturated fatty acid content of corn oil compared to that of peanut and coconut oil. The stability of control samples might be due to the residual tocopherols present in these oils.

The antioxidants added to vegetable oils inhibited oxidative deterioration during storage at room temperature. Both BHA and BHT seemed to be least effective as antioxidants in these vegetable oils. This finding was in agreement with the data reported by Berger (1975). Trolox C as well as TBHQ exhibited better activity than either BHA or BHT. The superiority of Trolox C over BHA and BHT was consistent with the finding reported by Cort et al. (1975). The greater efficacy of TBHQ compared to either BHA or BHT confirmed the data reported by Cort et al. (1975), Sherwin and Luckadoo (1970) and also Chahine et al. (1974).

It is well known that rancidity is a major problem in fats and oils. This problem is more significant in tropical countries such as Indonesia where the daily high temperatures

accelerate oxidative deterioration. As a consequence of this factor, rancidity occurs in tropical climates in a relatively shorter time than in a temperate climate. Besides, lack of advanced technology of storage, inappropriate handling and distribution system of food through marketing channel in most developing countries has emphasized the need of antioxidant treatment. The selection of an appropriate antioxidant for particular foods such as vegetable oils is highly important in order to obtain the best results. Trolox C seemed to be the most suitable antioxidant for vegetable oils.

SUMMARY

Investigations were made on the use of Trolox C, butylated hydroxyanisole, butylated hydroxytoluene, citric acid and tertiary butylhydroquinone in coconut, peanut and corn oils. The peroxide value, conjugated diene absorption and weight gain methods were used to follow the oxidative changes of the samples.

Excellent correlation was obtained between peroxide value and conjugated diene absorption. A good correlation was found between peroxide value and weight gain as well as between conjugated diene absorption and weight gain, but these were weaker than that between peroxide value and conjugated diene absorption.

The oxidative changes in the vegetable oils followed similar trends as evaluated by the three methods. The study suggested that shifts of double bonds occured along with hydroperoxides formation. The amount of oxygen uptake was proportional to the hydroperoxide produced.

Trolox C has been found to be the most effective among the antioxidants used in both natural aging and accelerated tests. The order of effectiveness in decreasing order was Trolox C, TBHQ, BHA or BHT and citric acid. As the concentration increased, the effectiveness was generally increased.

BHA, BHT and citric acid when used alone appeared to provide little additional stability to vegetable oils.

Trolox C, when used in combination with BHA or BHT, exhibited a negative synergism in both peanut and corn oils. However, Trolox C in combination with BHA or BHT and citric acid exhibited a small negative synergism in corn oil and displayed highly positive synergism in peanut oil.

The use of the highly effective antioxidant such as Trolox C for vegetable oils seemed to be applicable in tropical countries such as Indonesia.

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