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EFFECT OF HYDROLYZED VEGETABLE PROTEIN,
AUTOLYZED YEAST PROTEIN AND BUTYLATED
HYDROXYANISOLE ON THE STABILITY OF
PALM OIL IN A MODEL SYSTEM

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Mark E. Ukhun

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L. R. Dugan Jr.
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EFFECT OF HYDROLYZED VEGETABLE PROTEIN, AUTOLYZED YEAST PROTEIN AND BUTYLATED HYDROXYANISOLE ON THE STABILITY OF PALM OIL IN A MODEL SYSTEM

By

Mark E. Ukhun

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ABSTRACT

EFFECT OF HYDROLYZED VEGETABLE PROTEIN, AUTOLYZED YEAST PROTEIN AND BUTYLATED HYDROXYANISOLE ON THE STABILITY OF PALM OIL IN A MODEL SYSTEM

By

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The objective in this work was to demonstrate the antioxidant properties of hydrolyzed vegetable protein (HVP) and autolyzed yeast protein (AYP) in a model system composed of varying levels of either the hydrolyzed vegetable protein or autolyzed yeast protein, the antioxidant butylated hydroxyanisole (BHA), carboxymethyl cellulose (CMC), palm oil and distilled water. The system was freeze-dried and allowed to oxidize under controlled conditions.

The rate of oxidation at 60^oC was monitored by measuring weight gains in the model system and by determining absorbance attributable to diene conjugation at 233 nm in the extracted oil every two days.

Under the conditions of this experiment, butylated hydroxyanisole, hydrolyzed vegetable protein and autolyzed yeast protein were found to improve the stability of palm oil in the model system. The degree of stabilization conferred by these antioxidants was dependent on their concentration in the model system. Antioxidant effectiveness was in the order BHA > AYP > HVP.

Combinations of these antioxidant compounds led to greater stabilization of the palm oil but only negative synergism could be demonstrated with BHA and HVP combinations. The greater stabilization was also observed to be concentration dependent.

Good correlation between the weight gain and absorbance due to diene conjugation at 233 nm was obtained when both methods were used to monitor the rate of oxidation of the palm oil in the model system.

Dedicated to the only God of the universe

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INTRODUCTION

Today's grocery shops are filled with a formidable number of foods prepared as convenience foods while others are for institutions. Whether it is at the shop level or at the home level, including institutions, it is important that such foods retain their qualities if they are to remain acceptable over a considerable and reasonable period of time. To achieve this, many of them, which contain fats or lipids, are stabilized with antioxidants, of which only a few are approved by law.

Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ), all of which are phenolic, are among others which are used in food stabilization. Given the fact that many of the antioxidants in use are phenolic, synthetic, and that consumers are beginning to point accusing fingers at them, it is desirable, if not imperative, that alternative sources of antioxidants such as natural and non-phenolic antioxidant substances be searched for.

There are reports in the literature of certain proteins and amino acids interacting with known antioxidants to impart stability to fats or oils in model systems. Working with a soup and gravy base, Bishov *et al*. (1967) reported that hydrolyzed vegetable protein had antioxidant properties. In a study by Koch _et al_. (1971) linoleate salts of lysine and arginine were shown to exhibit resistance to autoxidation.

A potential exists for interaction between proteins and lipids in a food system with intermediates such as hydroperoxides, peroxy- and oxy-radicals, and carbonyl compounds entering into a variety of interactions to possibly account for the changes observed in such a system. However, because of the complex nature of the interactions involved and because of a number of factors such as pH, ionic strength, temperature, light, etc. which can influence results, it has not yet been possible to apply such results in a standard way to produce formulations.

The present study was undertaken to

(i) determine if two proteins, hydrolyzed vegetable protein (HVP) and autolyzed yeast protein (AYP), have any antioxidant activity in a model system, the lipid component of which is palm oil.

(ii) find out if synergism exists between these proteins and the phenolic antioxidant BHA.

(iii) find out the effect of varying the concentrations of the protein and the phenolic antioxidant, individually and when used in combination.

Palm oil was chosen, despite its known relative stability, because of its increasing importance in industries, especially in tropical Africa, in food processing and in other processes involving the incorporation of oil. Moreover, room exists for improving its stability.

LITERATURE REVIEW

Palm Oil

Useful applications of this oil have ranged from a hand cream and medicine in 18th century Britain to a lubricant in the modern tin plate manufacturing industry in the United States. Most palm oil today is, however, consumed as edible oil, especially in shortening and margarine, while advances in fractionation methods have expanded its use in the manufacture of salad oils.

From the chemical standpoint, refining is one of the most important steps in the processing of palm oil and it includes:

(1) Neutralization: This involves treating crude oil with an alkali such as caustic soda, to effect a nearly complete removal of the free fatty acids. These acids (and a small amount of neutral oil) are saponified - converted into soap - by the process.

(2) Bleaching: This requires either heating the oil or treating it with bleaching earth to remove carotenoid pigments, which impart the red color characteristic of crude palm oil. The carotenoid pigment converts to vitamin A in man and other animals; hence removal of this pigment to meet consumer preferences for clear cooking oil or white shortening also reduces nutritive value of palm oil.

(3) Deodorization: This is accomplished by steam heating the relatively non-volatile crude oil to remove relatively volatile taste and odor substances, while maintaining low pressures to prevent atmospheric oxidation of the oil.

(4) Winterization: The major triglycerides, or fat molecules, in palm oil are oleo-dipalmitin - melting temperature of 35°C (96°F) - and palmitodiolein - melting temperature of 19°C (66°F). Therefore, at room temperature the latter group of triglycerides normally will melt while the former remains solid, separating the oil into liquid and solid fractions. Palm oil thus can also be fractionated through the winterization process. Chilled crude-oil runs onto a conveyor belt, which suspends the solid portion (stearin) while allowing the liquid portion (olein) to separate. Stearin serves as a pastry shortening and cocoa butter substitute, while olein is more suitable as a cooking and salad oil.

Stability of Lipids

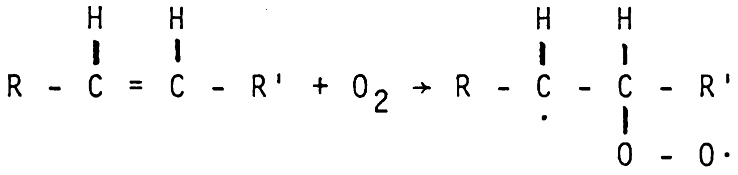
Dugan (1976) has described stability as the capability of a fat, oil, or fatty food to maintain a fresh taste and odor during storage and use and it is related to composition of the lipid moiety, the nature and degree of stress on the system, the presence or absence of pro-oxidants and antioxidants, and the effectiveness of packaging. He added that fats with substantial unsaturation in the fatty acids are usually unstable or moderately unstable and foods containing them reflect this instability; but that vegetable oils usually tend to be more stable than some of the animal fats, such as lard, even though the total unsaturation of the vegetable oils may be greater because natural antioxidants are usually present in the vegetable oils. Smith and Dunkley (1962) and Waters (1971) have also referred to the promoting effects of light, especially of short wave length, heat and metal catalysts, especially iron and copper, in the autoxidation of the lipid components of foods.

Because of its comparatively high level of palmitic and oleic acids, palm oil is regarded as stable when compared to many other oils of plant origin.

The stability of the lipid or fat component of a food is, of course, related and relatable to the level of deterioration of that food. Lipolysis is a term which refers to the hydrolysis of ester linkages of lipids resulting from enzymes, from thermal stress, or from chemical action. A major cause of food deterioration is also attributable to fat rancidity as a result of oxidation - "oxidative rancidity". Lipolytic rancidity is thought to pose less of a flavor problem than oxidative rancidity because the former develops off-flavor only in those fats which contain short-chain fatty acids (less than C_{14}).

Mechanisms of Lipid Oxidation

The reaction of oxygen with unsaturated fatty acids in lipids constitutes the major means by which lipids or containing foods deteriorate. Oxidation of fat is frequantly alluded to as autoxidation because the rate of oxidation increases as the reaction proceeds. Although this process, which involves the uptake of atmospheric oxygen, is common to most foods containing plant or animal tissue, the mechanisms involved may vary (Tappel, 1953, 1955; Lea, 1962). Dugan (1976) has described the oxidation process as proceeding through a free-radical chain reaction mechanism involving three stages: (1) initiation, formation of free radicals; (2) propagation, free-radical chain reaction, and (3) termination, formation of non-radical products. In the initiation stage an unsaturated hydrocarbon loses a hydrogen to form a radical: $RH \rightarrow R\cdot + H\cdot$ and oxygen may add at the double bond to form a diradical:



$$R - \overset{H}{C} = \overset{H}{C} - R' + O_2 \rightarrow R - \underset{\cdot}{\overset{H}{C}} - \underset{O - O\cdot}{\overset{H}{C}} - R'$$

It is also possible for oxygen in the singlet state to interpose between a labile hydrogen to form a hydroperoxide directly ($RH + O_2 \rightarrow ROOH$).

During propagation, the chain reaction is continued by $R\cdot + O_2 \rightarrow ROO\cdot$ and $ROO\cdot + RH \rightarrow ROOH + R\cdot$ to form peroxy-radicals, hydroperoxides, and new hydrocarbon radicals. The new radical formed then contributes to the chain by reacting with another oxygen molecule.

When two radicals interact, termination occurs:

$R\cdot + R\cdot \rightarrow RR$

$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$

$$RO\cdot + R\cdot \rightarrow ROR$$

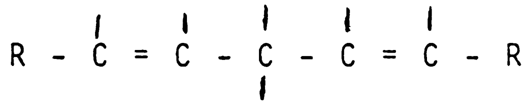
$$ROO\cdot + R\cdot \rightarrow ROOR$$

$$2RO\cdot + 2ROO\cdot \rightarrow 2ROOR + O_2$$

Because of the high energy necessary for the rupture of a CH bond (about 80 Kcal) and for diradical formation at the double bond, it is thought that energy reducing phenomena such as by metal activation, enzyme catalysis or photo-oxidation might be involved (Farmer and Sutton, 1943; Bolland and Koch, 1945).

Oxidation of Monoenoic Acids: When a monoene is oxidized, hydrogen is abstracted, during the initiation stage, from either of the carbons α to the double bond, so that two radicals are possible, each of which can assume two forms through resonance. When oxygen and hydrogen are added at each radical site, hydroperoxides are formed.

Oxidation of Polyenoic Acids: Oxidation of polyenes is initiated more readily and proceeds more rapidly than oxidation of monoenes. Most natural polyunsaturated fatty acids have 1,4-pentadiene structures:



$$R-\overset{|}{C}=\overset{|}{C}-\underset{|}{\overset{|}{C}}-\overset{|}{C}=\overset{|}{C}-R$$

Because the radical formed by the abstraction of hydrogen from the methylene group having contiguous double bonds on both sides is unstable, the electrons redistribute to form a conjugated double bond system. Thus a radical is formed at either the 1 or the 5 carbon of the original pentadiene system.

Oxidation of Saturated Acids: Severe conditions are necessary for the oxidation of saturated acids and the hydroperoxide group generally forms on the carbon β to the carboxyl or ester group. The hydroperoxide then changes to a keto group, giving rise to a β-keto acid.

Hydroperoxides are readily decomposed by high-energy radiation, thermal energy, metal catalysis, or enzyme activity, with the means depending on the system in which they exist. The oxidation then becomes more complex after the development of a quantity of ROOH with the formation of more free radicals which add to the chain process (Dugan, 1961). Farmer and Sutton (1943) have also made mention of the role of thermal instability in the decomposition of hyderperoxides.

The decomposition of a hydroperoxide can take place by a monomolecular as well as by a bimolecular mechanism (Mabrouk and Dugan, 1960: Lundberg, 1962) as shown below:

Monomolecular

$$ROOH \longrightarrow RO\cdot + \cdot OH$$

Bimolecular

$$2ROOH \longrightarrow 2RO\cdot + H_2O$$

Mabrouk and Dugan (1960) reported a lower activation energy for the bimolecular than for the monomolecular mechanism for hydroperoxide breakdown while lower hydroperoxide concentration and higher temperatures were said to promote monomolecular decomposition. The proliferation of radicals causes acceleration of oxidation without requiring new initiation events.

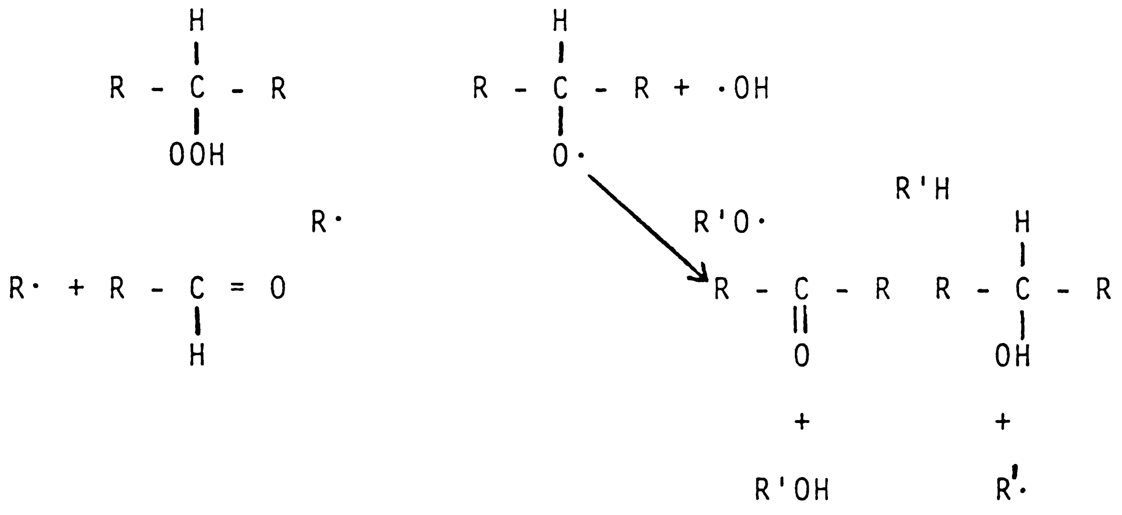
In his own contribution to the explanation of the autoxidation process, Baddings (1960) has stated that:

"(1) The rate of autoxidation is dependent on the energy required for the rupture of an α-CH bond.

(2) The reaction can be accelerated by light, trace metals, biological catalysts and radical-forming products such as benzoyl peroxide.

(3) Autoxidation is inhibited by compounds which react with free radicals to form non-radical products. Consequently, the free radical chain reaction may be interrupted and the formation of chain initiating free radicals can be prevented. A number of compounds, such as certain phenols, inhibit oxidation when present in small amounts.

(4) When a hydroperoxide decomposes to form $RO\cdot$ radicals, these in turn are capable of a series of reactions leading to several products which can be isolated from oxidizing lipid system; and the following reactions are apparently involved. Some of these products are radicals and they are capable of continuing in the chain propagation process." Others, such as the hydroxy acids, keto acids, and aldehydes, are commonly found in oxidizing lipid systems. The aldehydes, many of which are short chain, and the short-chain acids derived by further oxidation of those aldehydes,



are largely responsible for the off-flavor and odors characteristic of stale or rancid foods.

Lipid Oxidation in Food Systems: It is known that in a refined fat or oil, oxidation usually proceeds through autocatalytic processes, reaction rate increasing with time as a result of product formation which catalyzes the reaction; but as Dugan (1961) and Lundberg (1962) pointed out, in foods the processes are more complex and factors such as proteins, carbohydrates, moisture, salt and enzymes influence the rate and course of the oxidation process. In consonance with this, Lea (1962) also stated that minor food components such as the natural aroma, flavor and coloring substances which make food attractive, as well as nutritionally important vitamins can also increase the susceptibility of a food to autoxidative deterioration.

Lipid-Protein Interactions: The interaction between lipids and protein and/or amino acids in food systems is increasingly being investigated especially in model systems. The exact mechanism has not yet been fully established. Possibly, the binding of lipids to proteins or vice-versa may depend on such factors as configuration, multiple attachment sites, and/or the matching of polarity between opposing groups in much the same manner as for the combination of enzyme and substrate.

Chapman (1969) referred to metal ion, electrostatic and hydrophobic binding as being important in the interaction between lipids and proteins. In consonance with this, Gurd (1960) suggested that the interactions might be between similar types of functional groups in the two classes of compounds, such as between non-polar hydrophobic residues of the fatty acid moieties of lipids and the similar residues of certain of the side chain groups of proteins while other interactions may involve polar or charged groups. Primary covalent linkages, such as ester bonds were regarded as being of little importance.

The consequences for the food system of interaction between proteins and lipids can be classified into those affecting the protein component of the food and those affecting the lipid component of the food.

The effects of lipid oxidation during freeze-drying are well known: changes in rehydration properties, texture, off-flavor development and therefore the limitation of the shelf-life of the food are some of these. These observations prompted Koch (1962) to speculate that interaction between the products of lipid oxidation and proteins does take place during the processing and storage of dried foods.

In their studies on a gelatin-linoleate system in the dry state, Zirlin and Karel (1960) observed that gelatin-lipid reactions lead to the scission of protein coupled with cross-linking.

Other workers have shown that oxidation of lipid-protein systems often leads to browning and copolymerization of oxidized lipids and proteins as depicted by losses in the chemical and physical properties of protein. Narayan and Kummerow (1958) have referred to complex formation in the presence of oxidized lipids with the reaction being environment dependent and different proteins responding differently. Complex formation was attributed to secondary bonds such as hydrogen bonding.

The isolation of fluorescent compounds with $-C=N-$ functional groups from an oxidizing system consisting of methyl linoleate and coho salmon myosin and the observation that the ε-amino groups of myosin were destroyed led Braddock and Dugan (1973) to speculate a cross-linking reaction between amino groups and the products of fatty-acid oxidation. Lysine, methionine and histidine were found to be the most susceptible to such destruction.

Solubility changes and the loss of protein-amino-acids were reported by Roubal and Tappel (1966a) as being the result of enzyme and protein damage from peroxidizing lipids with polymerization of proteins with polyunsaturated lipids occurring as oxidation progresses; while radical attack, rather than aldehyde attack, on protein is mainly responsible for protein damage in a lipid-protein system (Roubal and Tappel, 1966b).

Working with carbonyl compounds, Schwenke (1975) stated that blocking of α-ε-amino groups alters the isoelectric point, electrophoretic properties, solubility and precipitation characteristics of proteins. This led to the postulation that radicals from oxidizing lipids play a role in protein destruction with the possibility that carbonyl-amine reactions, as in Maillard browning, form Schiff bases and secondary products.

The observations by Jarenback and Liljemark (1975) that linoleic acid hydroperoxides were much more effective than linoleic acid in reducing the amount of protein in a KCl-extract led them to suggest more extensive binding between the linoleic acid hydroperoxides and protein than between the linoleic acid and protein.

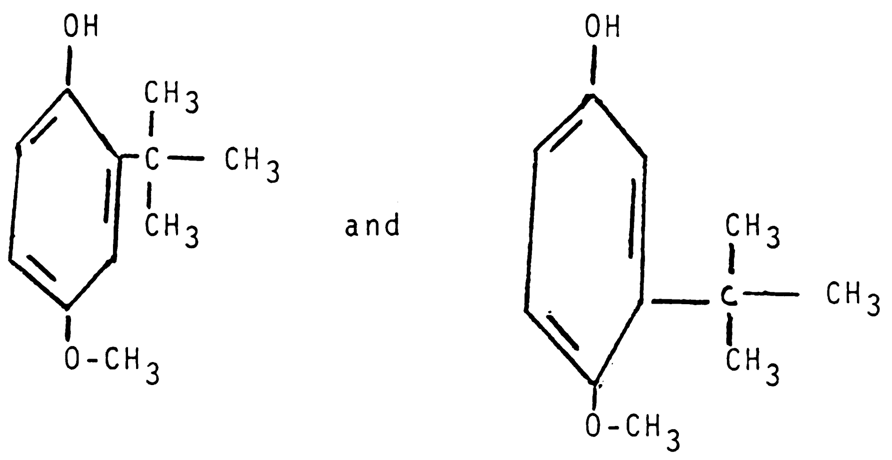
The other side of the picture of a protein-lipid system is the stabilizing effect certain proteins have on the lipid component of a food system. Most of the work in this area has been done using freeze-dried model systems.

Role of Antioxidant: Dugan (1976) has described an antioxidant as a "substance that is added to fats or fat-containing foods to retard oxidation and thereby prolong their wholesomeness and palatability." Antioxidants interfere with or delay the onset of the oxidative breakdown of fats and fatty foods (Blanck, 1955).

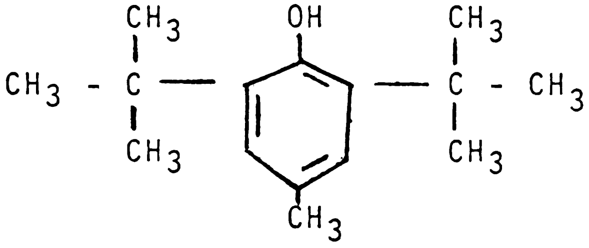
Ideally, an antioxidant should

(i) have no harmful physiological effects;
(ii) not contribute an objectionable flavor, odor, or color to the fat or food in which it is used;
(iii) be effective in low concentrations;
(iv) be fat soluble;
(v) persist following processing to provide effective protection to food in which it exists;
(vi) be readily available; and
(vii) be economical.

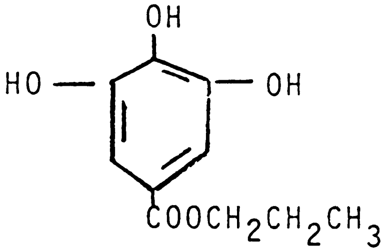
Butylated hydroxyanisole (BHA) (a mixture of 2-t-butyl-4 hydroxyanisole and its isomer 3-t-butyl-4-hydroxyanisole), butylated hydroxytoluene (BHT) which is 2,6-di-t-butyl-4-methylphenol, esters of gallic acid, and di-tert-butyl hydroquinone are commonly added to food since they are effective and they comply with safety regulations of the Federal Food and Drug Administration of the United States. These antioxidants are illustrated below:



(BHA)



(BHT)



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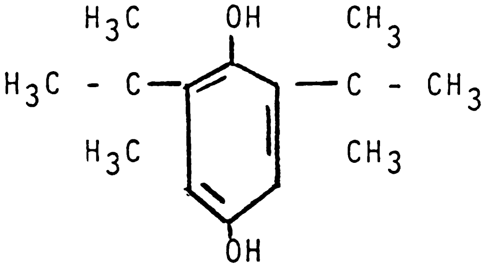

(TBHQ)

Many naturally occurring substances such as the tocopherols (α, β, γ, δ), lecithin or mixtures of phosphatides, flavones, sterols, and sulfhydryl compounds have some antioxidant properties.

BHA is synergistic with BHT and propylgallate, and with several antioxidants. This means that when used in combination these antioxidants are more effective than when used alone. However, the use of BHT with propyl gallate results in negative synergism, in which the keeping quality of a fat is less than expected from the sum of the effects of each used alone. Dugan and Kraybill (1956) also showed that the addition of BHA and the tocopherols beyond optimum levels resulted in negative synergism.

Mechanism of Antioxidant Action

Cort (1974) has indicated that the phenolic antioxidants act as electron or hydrogen donors thereby quenching electron mobility and interrupting the free-radical chain reactions.

In food systems, the most effective antioxidants function by interrupting the free-radical chain mechanism (Shelton, 1959) while those used in gasoline, lubricants, rubber, and other applications may function as peroxide decomposers (Dugan, 1976). Antioxidants, such as ascorbic acid, function by being preferentially oxidized and they afford relatively poor protection.

An antioxidant AH reacts with radicals produced during autoxidation according to the scheme postulated by Uri (1961):

$$R\cdot + AH \rightarrow RH + A\cdot$$

$$RO\cdot + AH \rightarrow ROH + A\cdot$$

$$ROO\cdot + AH \rightarrow ROOH + A\cdot$$

$$R\cdot + A\cdot \rightarrow RA$$

$$RO\cdot + A\cdot \rightarrow ROA$$

This scheme shows that antioxidants interfere with the free-radical, chain oxidation process and that reaction products of antioxidant molecules and oxidized lipid molecules may appear among the final products. Relatively little evidence exists in support of the latter statement. It is also possible that the antioxidant is oxidized directly by oxygen. Tappel (1962) has reported that tocopherol is partly oxidized to tocoquinone.

Some antioxidants are effective in prolonging the keeping time of both fats and oils and the foods containing them. Such antioxidants are known as "carry through antioxidants" since they "carry through" or survive the thermal stresses, steam distillation, and pH effects of processing to give longer shelf-life to the finished food (Dugan, 1962).

Proteins and Amino Acids as Antioxidants

In some protein-lipid systems it would appear that the destructive effects to proteins is matched by a stabilizing effect to the lipid component of the system. Bishov and Henick (1972) reported an antioxidant effect of AYP and HVP in a freeze-dried model system of lipid, carboxymethyl cellulose, the protein, and distilled water. Earlier work by Bishov *et al*. (1967) had indicated improved fat stability in dehydrated proteinaceous food mixes of chicken flavored soup and gravy mix; while the universality of synergism between protein hydrolyzate and phenolic antioxidants was shown by Bishov and Henick (1975).

The ability of protein to impart stability in such a system was also found to vary with the type of protein, method of preparation, pH, and experimental condition. For example, in their work as reported above, differences in the stabilizing effect between the AYP and HVP were attributed to the milder conditions of autolysis in comparison to those of hydrolysis, which gave better stabilizing potential to the former. HVP is obtained by hydrolyzing soy protein with an acid followed by alkaline neutralization. The salt which may be thus formed is also thought to lower its antioxidant effectiveness. AYP, on the other hand, is obtained with minimum heat treatment since the yeast cells are self-digesting.

In consonance with the above, Hayes et al. (1977) has hinted, with respect to soy flour antioxidant, that there is no clear cut, generalized relationship between concentration of added soy flour and the degree of stability obtained. He added that the nature of other product ingredients, the character of the fat or oil involved and whether the soy flour is full fat or defatted all probably complicate the outcome.

The antioxidant activity of soy flour has been ascribed to its natural component; such as isoflavones which Walz (1931) isolated. Amino acids and peptides are others. Soybeans normally contain small quantities of peptides and amino acids which are present as a result of incomplete protein synthesis or possibly because of some protein degradation (Smith and Circle, 1972a). Another class of compounds associated with the antioxidant activity of soy flour has been the phospholipids and Smith and Circle (1972a) have indicated that only part of the phospholipids are removed in commercial extraction of soybean flakes.

In their studies with methyl linoleate in freeze-dried models, Karel et al. (1966) found that certain amino acids including histidine, β-amino-butyric acid, lysine and cysteine had substantial antioxidant activity and that the nature of this activity was different from that observed with propyl gallate since the main effect of the amino compounds was to prolong the induction period and to affect the initial rate of oxidation. No effect was present in the more rapid, bimolecular phase of oxidation, whereas propylgallate had an inhibiting effect in the latter stage.

As a follow-up to this, Tjho and Karel (1969) reported that histidine in low concentration has an antioxidant effect in the very early stages of oxidation followed by a slight pro-oxidant effect in the later stages.

Working with herring oil, Marcuse (1960) observed that while histidine had an antioxidant effect, cysteine was pro-oxidant. Marcuse (1961) also reported on a study of the antioxidant effects of several amino acids added to aqueous solutions of linoleate at pH 7.5 and concluded that:

(1) Histidine, alanine, methionine and lysine reduced oxygen absorption by linoleate by as much as 50-80%.

(2) Each amino acid had an optimum concentration for the antioxidant activity and at high concentrations showed an activity inversion; becoming pro-oxidant rather than antioxidant in its action.

(3) The antioxidant activity depended on pH, the presence of other antioxidants or synergists, and the state of oxidation of linoleate.

(4) Since antioxidant properties were shown in the absence of tocopherol and other antioxidants, amino acids

may act as primary antioxidants.

Then in 1962, Marcuse observed that the antioxidant effect is enhanced, and the pro-oxidant effect is lowered by an addition of phosphate.

It has been indicated here earlier that carbonyl-amine reactions might be important in the lipid-protein interactions which, more often than not, lead to protein disintegration while promoting the stability of the lipid component. There is, however, no doubt that many possibilities exist for explaining the mechanism behind the stabilizing effect of protein and/or amino acids in lipid protein systems. Working on the stability of amine salts of linoleic acid, Dorlores and Lopieks (1971) suggested that the carboxylic acid group of the fatty acid may form a salt with one of the amine groups of the basic amino acids, leaving the other amine group available to affect the double bond area of the fatty acid. If the latter interaction is a charge-transfer complex, it is a very weak one involving only partial donation of electrons and little bond distortion. It would appear that the protective effect is specifically associated with the solid state.

The rigid crystalline lattice of lysinium linoleate could act as a barrier to the diffusion of oxygen and oxidizer intermediates, and to the propagation of chain reaction. On the other hand, studies of the oxidation in solution by Koch _et al_. (1971) suggest that the salt complex also prolongs the induction period and retards the rate of autoxidation. Therefore, one might speculate that initially the solid complex physically prevents oxygen from entering the reaction. After the oxygen succeeds in attacking the organic substrate, the oxidation would be terminated by a mechanism in which the basic amino acid acts as an oxygen scavenger or a free-radical chain terminator (Dorlores and Lopieks, 1971).

Role of Water in Fat Stability

Water is known to retard lipid oxidation in many dehydrated and low moisture food products (Stephens and Thompson, 1948). On the other hand, removing water from a food by freeze-dehydration results in a product that has a porous, sponge-like matrix. The porosity of the dehydrated food permits ready access by oxygen to the components of the food, thereby facilitating oxidative changes of food lipids.

Several hypotheses have been advanced to explain the protective effect of water in retarding lipid oxidation. For example, while Halton and Fisher (1973) suggested that water has a protective effect due to retardation of oxygen diffusion, Uri (1956) postulated that water lowers the effectiveness of metal catalysts such as copper and iron. Salwin (1959) mentioned that water is attached to sites on the surface, thereby excluding oxygen from these sites. Lee (1958) indicated that water had protective effects because it promotes nonenzymatic browning, and browning can result in the formation of antioxidant compounds. Finally, the formation of hydrogen bonds between water and hydroperoxides and the consequent retardation of hydroperoxide decomposition was also thought by Lee (1958) to at least partially account for the stabilizing effect of water on lipids.

Evaluation of the State of Oxidation

Measurement of the state of oxidation of a lipid or a lipid-containing food is very complicated and uncertain. Measurement of peroxide value is useful to the stage at which extensive decomposition of hydroperoxides begins. Measurement of carbonyl compounds is useful provided secondary reactions and volatilization have not occurred to a significant extent. The pattern of oxidation may be changed by the reaction of aldehydes with amino groups from proteins, amino acids, and amino-lipids, or by the nature of the stress on the system. This, in turn, causes different carbonyl species to form. Measurement of acid-type carbonyls is not revealing if used alone. A composite of peroxide, TBA, carbonyl type, and acid determination provides perhaps the best indication of the state of oxidation; yet the data are so variable that no oxidized system has been very well characterized or defined (Dugan, 1976).

Link and Formo (1961) have also indicated that although various physical methods, such as polarography and absorption spectroscopy are highly useful in elucidating reaction mechanisms and identifying products, these must normally be augmented by methods of degradation, separation, and chemical analysis.

Ultraviolet (UV) Absorption Method. The ultraviolet spectrum is particularly useful in composition studies on fats and in the analysis and identification of fatty materials. Spectral examination has become indispensable in studying and following chemical reactions of fatty materials, especially cis-trans and position isomerization, oxidation and polymerization. The spectra of fats can indicate whether they have been mishandled, since oxidation and polymerization products have characteristically different spectra from those of fresh materials (Swern, 1964). He has also stated that unsaturated fatty materials absorb light in the ultraviolet region of the spectrum. This region is between 200-400 mμ. In the case of non-conjugated and saturated materials, the absorption is weak and general and cannot be used for analytical purposes.

In describing the application of UV absorption to oxidation studies, Mehlenbacher (1960) also indicated that the oxidation of polyunsaturated fatty acids produces peroxides and the position of the double bond shifts to a conjugated form. Conjugated linkages give rise to characteristic and intense absorption bands within the spectral range of 200-400 nm, while the absorption of isolated bouble bonds within the same region is very weak. This, he added, is the basis for the ultraviolet absorption method for determining the state of oxidation.

However, many workers including Rusoff et al. (1945), Pitt and Morton (1957) have reported ultraviolet spectral data on numerous non-conjugated compounds.

In addition, when ultraviolet absorption methods indicate the presence of small quantities of conjugated compounds in natural fats, the results must be carefully interpreted because non-conjugated polyunsaturated components may have undergone conjugation as a result of autoxidation or other mishandling (Swern, 1964). He had earlier reported (1961) that the oxidation of polyunsaturated fatty acid is accompanied by increased ultraviolet absorption and that the magnitude of change is not easily related to the degree of oxidation because the effects upon the various unsaturated fatty acids are different in quality and magnitude as depicted in the analysis of a sample containing dienoic, trienoic, tetraenoic and pentaenoic groups where the total diene content was due not only to linoleic acid, but also to each of the more unsaturated acids. Therefore, according to Swern, the spectral change for a given substance should be used as a relative measure of oxidation, rather than its measurement.

Early workers such as Dann and Moore (1933) and Moore (1937), showed that linoleic and linolenic acids are readily isomerized to conjugated dienoic and trienoic acids respectively by heating them with an alcohol or ethylene glycol solution of potassium hydroxide at elevated temperatures. Mitchell, Kraybill and Szcheile (1943) applied this isomerization procedure to the direct quantitative determination of the linoleic and linolenic acid content of fats.

In his study on the shelf-life stability of peanut butters during long and short-term storage, Angelo _et al._ (1975) found good correlation between the spectrophotometric determination of conjugated diene hydroperoxides and the peroxide value determinations, over four and twelve week periods of storage. The spectrophotometric estimation of conjugated hydroperoxides was stated to require smaller samples, to be quicker, more accurate and simpler than the method of peroxide value determination. The non-requirement of additional reagents and non-dependence upon a chemical reaction or color development were also stated as obvious advantages over the method of peroxide value determination. Vitamin A, which has a characteristic maximum at 325 mμ can be determined in fats and in vitamin A concentrates by absorption in the ultraviolet region of the electromagnetic spectrum. α,β-unsaturated carbonyl compounds can be determined in autoxidized fats. Ultraviolet absorption has been used fairly extensively in studying the autoxidation of drying oils since the conjugation of polyunsaturated components parallels oxygen absorption (Swern, 1964).

Oxygen Uptake. From a practical standpoint, atmospheric oxidation of fats may be more or less sharply differentiated into that occurring in highly unsaturated oils, which is accompanied by polymerization, and is generally useful in the preparation of protective coatings (Privett, 1959); and that occurring in less unsaturated materials, which leads to the development of rancidity and is the source of most of the spoilage of edible fats and oils (Riemenschneider, 1955). Both types of oxidation, however, follow from the same types of reaction between oxygen and the unsaturated constituents of fats (Swern, 1964).

Oxygen normally exists in a triplet state but it has been established that electrophilic singlet oxygen reacts directly with olefinic molecules. Since RH and ROOH are in singlet states, singlet oxygen could react without change of spin and with conservation of energy. Conversion of oxygen to the singlet state can be accomplished by photosensitization in the presence of suitable sensitizers, such as chlorophyll, or possibly by the heme pigments myoglobin or by their derivatives. Thus, trace amounts of pigments or other sensitizers may be responsible for the initial formation of hydroperoxides in some systems (Dugan, 1976).

The rate of oxygen uptake by an oxidizing system may be monitored by the use of the oxygen bomb test, which involves placement of a sample in a bomb-like device, the addition of a fixed amount of oxygen, and the monitoring of pressure at constant temperature. The time at which oxygen uptake accelerates as determined by a decrease in pressure, corresponds to the stability of the sample (Dugan, 1976).

Another method which involves the uptake of oxygen is the use of manometric respirometers such as the Barcroft-Warburg apparatus, and Gilson differential respirometer which have automatic recording devices. The sample is added to reaction vessels and along with reference flasks are connected to manometers and agitated periodically with the temperature held constant (Gilson, 1963). The progress in oxidation is monitored by pressure changes in the chamber which contains pure oxygen or air (Lundberg, 1962). Correlations have been made between the development of peroxides and the first presence of odors typifying rancidity (Dugan, 1976).

Working with beef, Tappel (1962) observed that only 50% of the oxygen absorbed during the initial oxidative deterioration was attributable to lipid peroxidation. Possible oxidation of the -SH groups of the protein was suggested to account for the other 50% and thereby introducing errors into the estimations of the extent of oxygen uptake. The great care needed in oxygen uptake studies and its relative usefulness when large numbers of samples are involved for a relatively short period of time under closely controlled conditions have been referred to by Stuckey (1968).

Literature also exists which reports measurement of oxygen uptake by weight gain studies in which weight changes are directly correlated with the uptake of oxygen. For example, Fukuzumi _et al_ (1976) used the method to study the antioxidant effect of phenothiazine derivatives on methyl linoleate.

EXPERIMENTAL

Materials and Equipment

Hydrolyzed Vegetable Protein: Hydrolyzed vegetable protein (HVP) was obtained from A.E. Staley Mfg. Co., Illinois.

Autolyzed Yeast Protein: Autolyzed yeast protein (AYP) was also obtained from A.E. Staley Mfg. Co., Illinois.

Carboxymethyl Methyl Cellulose (CMC): Carboxymethyl Cellulose (CMC) was supplied by Polyscience, Inc., Warrington, Pennsylvania.

Palm Oil: This was obtained from A.E. Staley Mfg. Co., Decatur, Illinois.

Butylated Hydroxyanisole (BHA): This antioxidant was obtained from Eastman Chemical Products, Inc., Tennessee.

Chemicals: The chemicals used in this work were of analytical grade.

Solvents: All the solvents with the exception of isooctane were also of analytical grade.

Spectrophotometer: A Beckman DU-2400 spectrophotometer from Beckman Instruments, Inc., Fullerton, California, was used in this work to monitor absorbance changes.

Vacuum Oven: A vacuum oven, Model 29, Precision Scientific Instruments, Chicago, Illinois, equipped with a Cenco Hyvac-4 vacuum pump, Cenco Instrument Corporation, Chicago, Illinois, was used for the determination of the moisture content of the freeze-dried samples.

Electrical Oven: An ordinary electrical oven without any vacuum was used in the Schaal oven-test.

Methods

Determination of Iodine Number of Palm Oil

The Hanus Method as outlined in the Official Methods of Analysis of the AOCS (1974) was employed in the iodine number determination of palm oil.

Determination of Free Fatty Acid

The official AOCS (1974) method was also used in the determination of the free fatty acid content of the palm oil.

Preparation of Methyl Esters for Gas-Liquid Chromatography

Metcalfe *et al*. (1966) has described a method for rapidly preparing methyl esters from lipids.

4 ml of 0.5 N methanolic NaOH was added to approximately 150 mg of palm oil and the mixture heated on a steam bath until the fat globules were in solution. This was then followed by boiling the soaps with 5 ml of BF_3-methanol for 2 minutes to give a quantitative conversion of the fatty acids to methyl esters. Enough saturated NaCl was added to the methyl esters, which were then readily withdrawn with a syringe.

Gas-Liquid Chromatography

Gas liquid chromatography of the palm oil methyl esters obtained as described above was run with a F and M Scientific (Hewlett Packard) dual column, temperature programmed gas chromatograph equipped with a flame ionization detector. The columns were packed with 10% SP 2340 on Supelcoport (100-120 mesh) as a solid support. The column temperature was 100°C at 4°C/min.; detector temperature was 250°C. Inlet temperature - 200°C, chart speed was 0.25"/min.; nitrogen pressure 60 psi and air, hydrogen pressure was 25 psi.

Esters were identified by comparing their retention times with those of standard mixtures of known fatty acid methyl esters while peak areas were calculated by multiplying peak height by the width at half-height from which the relative percentage composition of the fatty acids could be determined as shown below:

% composition of fatty acid

$$= \frac{Ax}{Ax + Ay + Az} \times 100$$

(where Ax, Ay, Az are individual peak areas).

Peroxide Value

Peroxide value (PV) of the palm oil was determined by the AOCS official method, Cd 8-53, 3rd Ed. (1974).

Moisture Determination

The moisture contents of the model systems after the freeze-drying process were determined by the procedure enumerated in the A.O.C.S., 13.003, 9th ed. (1960).

Percentage Recovery

The percentage recovery of the palm oil component of the freeze-dried samples were monitored by dissolving 1.6 g of the freeze-dried sample in 50 ml respectively of chloroform and chloroform:methanol 50:50 mixture for 24 hours. The whole mixture was then separated into a solvent fraction containing the dissolved palm oil and a residual fraction containing the carboxymethyl cellulose and any other undissolved component, by filtration through a Whatman filter paper number 1. This extraction was made twice for each 1.6 g sample.

The solvent containing the dissolved palm oil was then slowly evaporated on a steam bath under a hood leaving the palm oil.

The percentage recovery was calculated as

$$\frac{x}{y} \times 100,$$

where x = weight of palm oil obtained after extraction with either chloroform alone or with chloroform:methanol mixture (50:50). y = calculated weight of palm oil in 1.6 g of freeze-dried sample.

Purification of Iso-octane

To obtain accurate results from the absorbance measurements, the iso-octane used in this work had to be purified before use, using the AOCS (1974) method.

Preparation of the Model Systems

Each of the model systems was prepared by adding in succession to a blender cup 800 ml of distilled water, the antioxidant material (HVP, AYP, BHA), carboxymethyl-cellulose (CMC), and 10 g of palm oil.

10, 20, 30, and 40% (oil weight basis) each of either AYP or HVP were used in this study while the BHA was used at levels of 0.005, 0.01, and 0.02% (oil weight basis). In each of the model systems, the amount of CMC used was such that the CMS:palm oil ratio was 1:1. A control model system in which any antioxidant material was excluded was also prepared.

The entire mixture was then blended for 2 min. and thereafter transferred to a freeze-dry tray and frozen thus for 24 hours after which it was freeze-dried for another 48 hours.

Weight Gain Studies

For the purpose of monitoring weight gain by the Schaal oven test, 1.6 g of the freeze-dried material was placed in a petri-dish (5.5" in diameter and 3/4" high) and kept in an oven at a temperature of 60°C. This was done in duplicate for each model system used.

Since Eubank and Gould (1942) have hinted that the arrangement of samples inside the oven is a factor affecting results, all the freeze-dried samples were kept at the same positions in the oven throughout the period of this study. Weights were taken every two days for a 27-day period in the case of the model systems containing HVP as the antioxidant material and for 43 days in the case of those containing AYP as the antioxidant material. The period for the BHA was either 27 or 43 depending on whether it was used along with HVP or AYP.

Extraction of Palm Oil from Freeze-dried Materials

To be able to take absorbance values for the system undergoing oxidation in the oven at 60°C, it was necessary to extract the palm oil from the system.

Folch _et al_. (1957) described a method for the extraction of lipid from a tissue which involves the use of chloroform-methanol (2:1, v/v). However, because no great differences as indicated by weight gain and absorbance measurements were observed when chloroform was used alone in the extraction procedure, the latter was used in subsequent extractions.

Therefore, to achieve extraction in this work, 1.6 g of the freeze-dried sample undergoing oxidation in the oven was disintegrated into 50 ml of chloroform and left for 24 hours to achieve sufficient extraction. This was followed by separation of the chloroform containing the extracted lipid and the undissolved residual matrix through a Whatman filter paper number 1. To further separate the chloroform from the palm oil, slow evaporation under a hood was done until the characteristic pungent smell of chloroform was no longer detectable. The residue on the filter paper was again treated with 50 ml chloroform and refiltered into the original filtrate to give higher recovery of the palm oil. Thus, two washings were done for each 1.6 gm of freeze-dried sample.

Measurement of Absorbance Changes

Having extracted the palm oil from 1.6 g each of the oxidizing system as described above, the absorbance due to diene conjugation as oxidation progressed in the oven was monitored every two days by placing 10 mg of the oil, accurately weighed, into 30 ml test tube into which 10 ml of purified iso-octane (2,2,4-trimethyl pentane) was poured and mixed thoroughly in a Fisher mini shaker. The mixture was then filtered through a Whatman No. 1 filter paper and absorbance values taken in duplicate on a Beckman DU spectrophotometer at 233 nm using purified iso-octane as blank.

Absorbance measurements were obtained for a period of 27 and 43 days which were respectively used for the systems containing HVP and AYP as antioxidants while that for BHA was either for 27 or 43 days depending on whether it was used along with HVP or with AYP.

RESULTS AND DISCUSSION

The peroxide value of the palm oil used in this study was found to be 2. This value is low enough to enable us to assume that the sample had not undergone extensive deterioration before use. Reservations about the use of peroxide values in measuring the state of oxidation in a lipid system exist as indicated by Labuza (1969) who maintained that in lipid-protein systems, possible interactions between the protein and lipid hydroperoxide during autoxidation can occur and thereby diminish the quantity of measured hydroperoxide. Similarly Dugan (1976) has stated that measurement of peroxide value is useful to the stage at which extensive decomposition of hydroperoxides begins.

The fresh palm oil contained no protein material and had not yet attained the stage at which extensive decomposition of hydroperoxides occurs. The peroxide value of 2 also apparently reflects the well known stability of palm oil and perhaps, the processing efficiency of the supplying company, as indicated by the high oleic and palmitic acids shown in Table 1, and by the low free fatty acid content and iodine value shown in Table 2.

To keep the sample fresh and to maintain the low peroxide value, it was stored throughout the period of this

Table 1. Fatty Acid Composition of the Palm Oil

Fatty Acid	Percentage
Myristic acid $C_{14:0}$	Trace
Palmitic acid $C_{16:0}$	47.50
Stearic acid $C_{18:0}$	4.82
Oleic acid $C_{18:1}$	37.26
Linoleic acid $C_{18:2}$	8.39
Hexadecenoic acid $C_{16:1}$	2.50

Table 2. Characteristics of the Palm Oil

	Value
Peroxide Number	2
Free Fatty Acid	0.05%
Iodine Number	43

study in a refrigerator at 0^oC.

Moisture Content: The moisture content of the freeze-dried model system was determined and found to be 3.5%.

The moisture content is important because this work involved a freeze-dried model system. To be a true dry model system, the moisture content had to be as low as possible. In addition, it was reasonable to attempt to eliminate substances or compounds extraneous to the antioxidants or the palm oil in the model system which might obscure an assessment of the effectiveness of the antioxidants used in this study. For example, as if water were an antioxidant in the conventional sense, it retards lipid oxidation in many dehydrated and low moisture food products according to Stephens and Thompson (1948). Finally, removing as much water as possible from a food system by freeze-drying results in a product that has a porous, sponge-like matrix, the porosity of which permits oxygen ready access to the components of the food, thereby facilitating oxidative changes.

Percentage Recovery

Table 3 shows the percentage lipid recovered using chloroform alone and chloroform:methanol 50/50 v/v mixture in extracting the palm oil from the autoxidizing freeze-dried model system. The results indicate that no great differences in percentage recovery occurred between the two solvents. On the basis of this, the chloroform solvent

Table 3. Percentage Recovery of Palm Oil from the Freeze-Dried System Using Chloroform Alone and a Chloroform:Methanol Mixture (50:50 v/v)

Time (days)	% Recovery of oil with $CHCL_3$ alone	Absorbance	Weight (gm)	% Recovery of oil with $CHCL_3$:CH_3OH (50:50 v/v)	Absorbance	Weight (gm)
1	95.821	0.035	3.521	96.103	0.034	3.521
3	96.014	0.036	3.521	96.012	0.035	3.521
5	95.498	0.034	3.521	06.001	0.036	3.522
7	95.782	0.035	3.521	95.869	0.034	3.521
9	96.116	0.035	3.521	95.921	0.034	3.521
11	96.019	0.072	3.550	96.013	0.058	3.548
13	95.994	0.084	3.598	96.011	0.080	3.596
15	96.210	0.194	3.596	95.921	0.195	3.599
17	96.022	0.233	3.589	95.764	0.212	3.599

Values = means of duplicate samples differing not more than 0.5%.

alone was used in the extraction of palm oil from the model system throughout the period of this study.

Folch _et al_. (1957) described a method for the extraction of lipid from tissues which involved the use of chloroform-methanol (2:1 v/v), apparently because this gave a higher percentage recovery than the use of chloroform alone. This contrasts with the findings of this study. The difference might be accounted for by the high porosity of our freeze-dried samples and their low thickness (about 1") which permitted extensive and intensive soaking of the samples and allowing greater dissolution of the palm oil in the chloroform. In addition, it is possible that under our experimental conditions, interactions between the lipid and protein component of the model system and/or other components were such that the solubility of the oil in normal lipid solvent such as chloroform was not seriously altered. The 5-6% of the unrecovered palm oil may have been lost through such interactions.

The same Table 3 shows that absorbance and weight gain changes were not much different when either solvent was used in extraction. The probability therefore that subsequent absorbance and weight gain changes recorded throughout this study may have been different if a chloroform:methanol mixture had been used was ruled out.

Effect of BHA on the Stability of Palm Oil

One way by which the stability of palm oil in the model system was followed was by determination of weight changes. Weight changes reflect changes in the rate of oxygen absorption which itself reflects the susceptibility of the system to autoxidation, other factors being constant.

Table 4 shows the percentage weight gain observed with a freeze-dried model system which contained palm oil, carboxymethyl cellulose (CMC), and distilled water, without any antioxidant and others containing various levels of the antioxidant BHA in the system. The results are in considerable agreement with the conventional theory regarding autoxidation: an initial lag phase during which there is no remarkable gain in weight attributable to autoxidation followed by a log phase in which increased oxygen absorption leads to logarithmic increases in the rate of autoxidation attributable to a free radical mechanism. Finally there is a termination phase during which non-radical and non-reactive species are formed. Dugan (1976) refers to the three phases as initiation, propagation and termination, and these are depicted in Figure 1.

Of interest too is the fact that even after 9 days of storage at 60°C, the palm oil in the control model system had virtually no increase in weight. Just what this means in terms of stability is shown when this is compared to the induction period of only 8 hours which Chahine _et al_. (1974) obtained in studies with crude whale oil, stored at 40°C. Cort _et al_. (1975) obtained induction periods of 12, 15, 6 and 6 for corn, peanut, sunflower and safflower oils, respectively, when they were stored at 45°C. The

Table 4. Weight Gain, Increase in Absorbance and Induction Periods of Palm Oil for Various Levels of Butylated Hydroxyanisole in the Model Systems

	Control Model System	System with 0.005% BHA	System with 0.01% BHA	System with 0.02% BHA
Weight Gain in 27 days (%)	11.13	8.13	5.87	0.75
Induction Period by Weight Gain (Days)	11	17	21	-
Increase in Absorbance in 27 Days	0.252	0.133	0.094	0.008
Induction Period by Increase in Absorbance (Days)	11	17	21	-

- No detectable end of induction period within the 27-day test period.

Values = means of duplicate samples differing not more than 0.5%

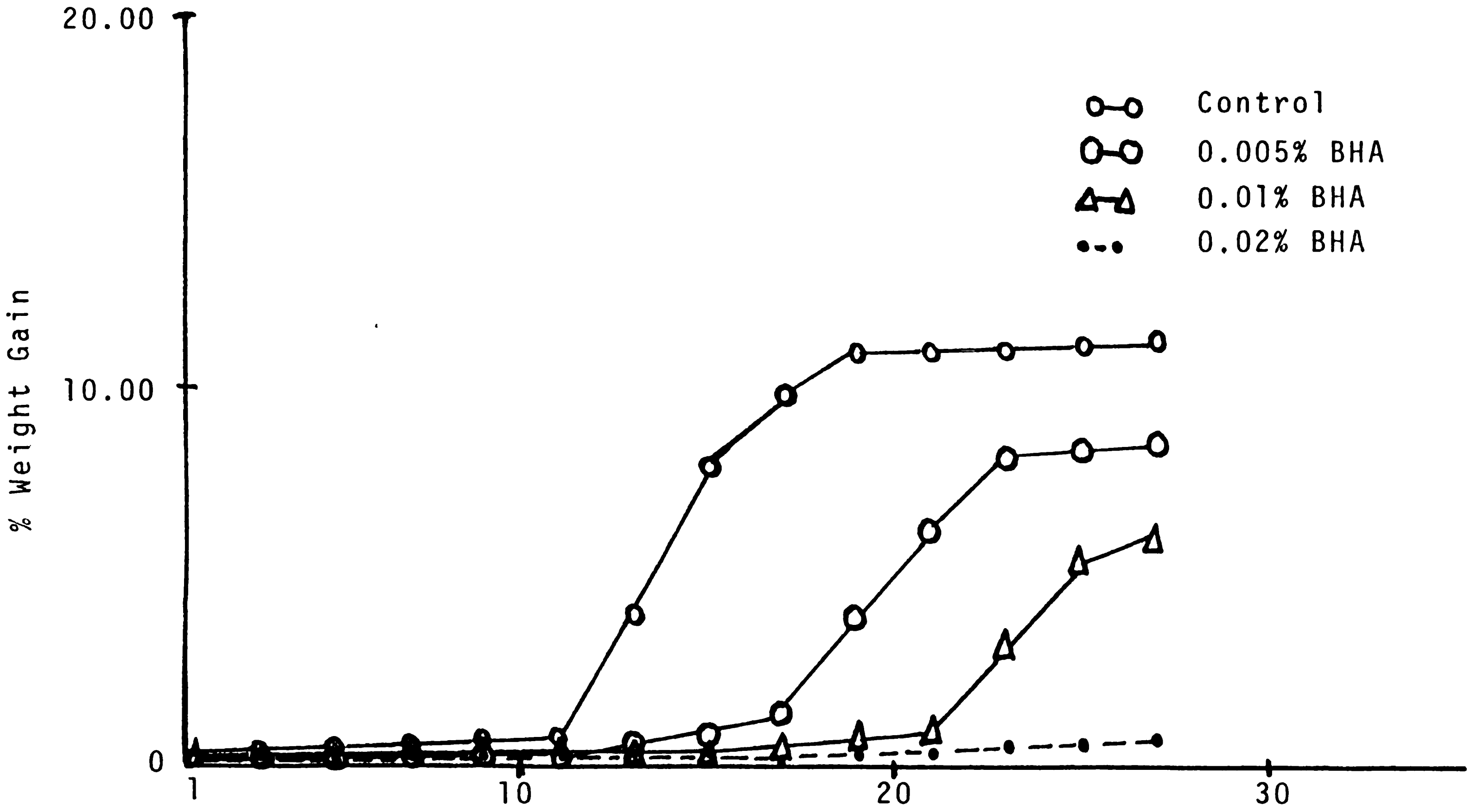

Figure 1. Weight changes in model systems of BHA, CMC and palm oil, stored at 60^{o}C for 27 days.

comparatively long induction period obtained here for the unprotected palm oil in the model system may have been due to the inherent stability of palm oil which is well known. For example, in the southern part of Nigeria where the oil is the most popular cooking oil in the kitchen and where temperatures can typically run as high as 65°C, a bottle of oil, even exposed, can last a week without any appreciable or detectable level of deterioration in terms of flavor, odor or aroma characteristics.

In any case the BHA still had beneficial effects on the stability of the palm oil in the model system. Its effect was to increase the induction period which meant improved stability. The effect of concentration was also apparent: as the level of BHA increased from 0.005% to 0.01 and to 0.02% (oil weight basis) in the freeze-dried system, longer induction periods were recorded. In fact, at the 0.02% level, no detectable end of induction period was attained in the 27 day experiment which indicates the antioxidant potency of the BHA in such a system. The antioxidant effect of BHA is well known and the results obtained here harmonize with those of other workers such as Sherwin _et al_. (1975) who demonstrated that BHA improved the stability of crude safflower seed oil and cotton-seed oil. Cort _et al_. (1975) reported that BHA incorporated into corn, peanut, sunflower and safflower oils at 0.02%, improved the stability of these oils while a similar stabilizing effect was observed by Chahine _et al_. (1974) in their work with crude whale oil. In contrast to these, Astudillo *et al*. (1968) observed no stabilizing effect of BHA when introduced into irradiated olive oil. This contrasting observation emphasizes the importance of altered state of the oil as may have been induced by irradiation, on the effectiveness of an antioxidant.

Table 4 also shows the results when absorbance measurements were made. The results confirm the observations made with the weight gain studies - that, among other things, BHA is a potent antioxidant for palm oil in the freeze-dried model system.

The percentage weight and absorbance increases shown in Table 4 are found to have a negative correlation with the inclusion of BHA in the system and with increasing levels of BHA. The values throw some light on the level of autoxidative deterioration to be expected in a freeze-dried food system containing palm oil with BHA as the antioxidant over a 27 day period of storage at 60°C. The values could be expected to be lower if the food or oil alone were stored at room temperature or at refrigeration temperature as happens most of the time.

The mechanism of BHA antioxidant activity in this system is thought not to be different from that described by Dugan (1976) - that, like any other phenolic antioxidant, the BHA acts to interrupt the free-radical chain mechanism involved in autoxidation.

The effectiveness of BHA in improving the stability of palm oil is also considered here to be of special significance to tropical countries where palm oil is used commonly as a cooking oil or where it may find its way into other palm oil based products. Specifically, in Nigeria, the oil is sold in open vats where accessibility of atmospheric oxygen is unhindered and at temperatures typically tropical. If an antioxidant like BHA could be used with palm oil in Nigeria, the already stable oil could be made even more stable such that even with long exposure to atmospheric oxygen and elevated temperatures, its wholesomeness can still be guaranteed. This is even more pertinent when it is realized that not necessarily every home in that country has a refrigerator or cooling system where the oil or products based on it are stored.

Effect of HVP on the Stability of Palm Oil

As can be seen in Table 5, the HVP increased the keeping time of the palm oil in the system, based on the induction period of the control sample compared to those containing HVP and based on the percentage weight gain attained throughout the twenty-seven day storage period at 60°C. The stabilizing effect attributable to the HVP observed here is in agreement with the findings of other workers; like Bishov and Henick (1972) who reported an antioxidant effect of HVP in a freeze-dried model system of corn oil, carboxymethyl-cellulose, the protein and distilled

Table 5. Weight Gain, Increase in Absorbance and Induction Periods of Palm Oil for Various Levels of HVP in the Model Systems

	Control Model System	System with 10% HVP	System with 20% HVP	System with 30% HVP	System with 40% HVP
Weight Gain in 27 Days (%)	11.13	10.13	8.13	4.38	2.00
Weight Gain Induction Period (Days)	11	16	17	21	-
Increase in Absrobance in 27 Days	0.252	0.175	0.130	0.092	0.019
Increase in Absorbance: Induction Period (Days)	11	13	16	21	-

- No detectable end of induction period within the 27 day test period.

HVP = Hydrolyzed vegetable protein.

Values = means of duplicate samples differing not more than 0.5%.

water. Earlier work by Bishov and co-workers (1967) had indicated improved fat quality and stability in dehydrated proteinaceous food mixes of chicken flavored soup and gravy mix.

What is apparent in Table 5 too is the fact that increasing levels of HVP in the freeze-dried system gave longer induction periods and lower weight gain illustrating the effect of HVP concentration on its stabilizing ability. Many other workers have reported such concentration effects on lipid stability. For example, Lips _et al_. (1949) tried levels of 4, 12 and 20% of defatted soybean flour as an ingredient in ration biscuits and stored at 43.4°C and noted that peroxide values were significantly reduced as the concentration of the soy flour component was increased. Similarly Neil and Page (1956) found that inhibition by soy flour of peroxide development in both frozen, raw, ground pork and in frozen ground pork, precooked before freezing was also concentration dependent. The concentration effect reported in this study however contrasts with the observation of Sylvester _et al_. (1942) who could not demonstrate such increasing effectiveness with soy flour. Similarly, Overman (1948) found that lard in frozen pastry containing full fat soybean flour in proportions of 5, 10, 15 or 20% kept about equally well, as indicated by peroxide numbers. Hayes _et al_. (1977) have struck a compromise by stating that there is no clear-cut, generalized relationship between concentration of added soy flour and the degree of antioxidative stability obtained and that the nature of other product ingredients, the character of the fat or oil involved and whether the soyflour is full fat or defatted all probably complicate the outcome.

It might also be pointed out here that although 10, 20, 30 and 40 percent of HVP all gave longer induction periods when compared to that obtained with the control samples, the induction periods obtained with the 10-20 percent levels of HVP did not differ greatly from each other. The 30% was quite effective but only the 40% HVP really distinguished itself from the other percentage levels as was evidenced by the fact no detectable and obvious end of induction period was recorded throughout the 27-day period of storage at 60°C. This would tend to be consonant with the findings of Overman (1947) with soybean flour fortification of frozen pastry. Compared on the basis of the final weight gain values attained over the period of the study, clear differences existed among the different levels of protein incorporated into the model system. Since Dugan (1976) has pointed out that the end of the induction period marks the beginning of rapid and measurable deterioration of a lipid sample during autoxidation, the former basis of comparison appears more practical and realistic.

Of equal interest is the fact that a ten percent level of HVP in the system did not, at least when compared to other conventional antioxidants, significantly increase the induction period or lower the weight gain recorded; yet food products covered by the Food, Drug, and Cosmetic Act are allowed to contain a total of 0.02% BHA and BHT based upon the fat content of the food, while food products covered under the Meat Inspection Act and Poultry Act generally can be treated with up to 0.01% of an individual antioxidant and a combined total of not more than 0.02% of all approved antioxidant based upon the weight of fat. The important point here is that these rather low levels are very effective in prolonging very significantly the induction periods of those foods where they are used. This then helps to place the relative effectiveness of natural antioxidants like HVP in the correct perspective vis a vis the synthetic phenolic antioxidants such as BHA and BHT approved for food use. In fact, Bishov and Henick have reported 10% HVP to be equivalent to 0.02% BHA (oil weight basis) in this system.

Table 5 also illustrates the results when oxidation was measured by taking absorbance values and here again, similar observations are made as discussed above: effectiveness of HVP in the system which is concentration dependent and the high level of the protein (10%) which, although showing some effectiveness, is minimal compared to the synthetic, phenolic antioxidants.

The same Table 5 also indicates the maximum weight gain and absorbance values attained over the period of the study, and therefore enables us to predict, within reasonable limits, the maximum level of spoilage to be expected in such

Table 6. Weight Gain, Increase in Absorbance and Induction Periods of Palm Oil for Various Levels of AYP in the Model Systems

	Control Model System	System with 10% AYP	System with 20% AYP	System with 30% AYP	System with 40% AYP
Weight Gain in 43 Days (%)	10.38	9.75	6.00	2.88	1.38
Weight Gain Induction Period (Days)	11	19	20	31	-
Increase in Absorbance in 43 Days	0.246	0.150	0.120	0.079	0.018
Increase in Absorbance: Induction Period (Days)	11	17	19	29	-

- No detectable end of induction period within the 43 day test period.

AYP = Autolyzed yeast protein.

Values = Mean of duplicate samples differing not more than 0.5%.

a system over the test period by noting that stability is negatively correlated with high absorbance and weight gain values.

Effect of Autolyzed Yeast Protein (AYP) on the Stability of Palm Oil

When the induction period of the control model system is compared with those containing AYP, as shown in Table 6, an antioxidant effect is observed for this protein.

The effect was also found to be dependent on the levels of AYP in the system which were 10, 20, 30 and 40% (oil weight basis) respectively with the induction periods increasing with increasing levels. The induction periods were approximately 19, 20, 31 days respectively, for the 10, 20 and 30 percent levels of AYP. There was even no discernible end of the induction period for the 40 percent AYP in the system. This indicates the potency of this protein at such high levels but since the induction period for the control system used along with the other model systems containing various levels of HVP was approximately 11 days, it is obvious that even at levels of 10 and 20 percent AYP there was no very remarkable improvement in the stability of the system. Only the systems containing 30 and 40 percent levels of AYP showed very remarkable improvement in stability. The limited literature on the stabilizing effect of AYP appears to suggest that very remarkable improvement in stability can be obtained by using 5-10-25 percent levels of the protein in the model system. For example, Bishov and

Henick (1972) obtained 24 hours as the induction period for a control system that was composed of corn-oil and CMC, mixed in distilled water while the induction period went up to as high as 145 hours when 10% AYP was introduced into the model system. Differences in the effectiveness of antioxidants in different systems, depending on the nature and type of oil, pH, metal concentration, temperature and other such factors are well recognized (Marcuse, 1961).

If, on the other hand, one were to look at the results shown in the same Table 6 from the point of view of the final weight gain values attained by the systems, then it would be clear that only the 10% AYP did not give a remarkable improvement in stability - still contrasting with the findings of Bishov and Henick but harmonizing somehwat with the observation recorded for the HVP in this work.

When the absorbance values shown in the Table are looked at, the same general observations can be made on the effect of the protein on the stability of the model systems containing, among other things, palm oil. It can also be looked at as indicating the maximum degree of deterioration one can expect from a food system which simulates the condition of this experiment.

The present state of knowledge on just how proteins like AYP and HVP act makes it difficult to pinpoint any specific mechanism involved in their stabilizing effects as reported in this work. Smith and Circle (1972) have hinted that such effects may be ascribed to the peptide and amino acid profile of the protein. Karel and Tannenbaum (1966) found that the amino acids acted only to prolong the induction period of autoxidizing lipids whereas phenolic antioxidants also reduced the rate of the rapid oxidation phase. Then in 1975 Karel _et al_. noted that a free-radical chain termination mechanism could arise from the presence of proteins while Togashi _et al_. (1961) in their work with cottonseed oil autoxidation in model systems which contained gelatin suggested that convolutions in protein, by reducing the surface area of lipid molecules, may exert a protective effect. In their own contribution, Koch _et al_. (1971) reported that the basic amino acids were unique in being able to protect a lipid material against deterioration due to autoxidation. Tumerman and Webb (1965) with their work on casein point to the possibility of complex polymer formation helping to reduce the rate of autoxidation of a lipid either alone or as in a model system.

It is obvious therefore that no concensus has been reached on how proteins act as lipid stabilizers. Yeast protein is known to be high in lysine making the observation of Koch _et al_. (1971) as stated above attractive as far as our work here is concerned, but instead of ascribing the stabilizing effect of HVP and AYP to just the amino acid and peptide factors as done by Bishov and Henick (1972) it is here being suggested that one or more of the factors stated may provide the explanation to the protective effects observed in this study.

Effect of the Combination of HVP or AYP and BHA on the Stability of Palm Oil

Studies were made of the effects of combining HVP and AYP each with BHA at various levels in the model system and the results obtained in the weight gain measurements are shown in Tables 7 and 8.

It can be seen that improved stabilities are obtained with combinations of either HVP or AYP with BHA. That is, such combinations gave what were considered to be longer induction periods than control samples and samples which contained only either AYP, HVP and BHA.

Such increases in stability can also be seen to be relatable and related to concentration. For example, while the induction period for the model system containing a combination of 0.005% BHA and 10% HVP was 21 days, that for the system with 0.005% BHA and 20% HVP was 23. It was 23 days for a 0.005% BHA and 10% AYP while it increased to 27 days when 0.005% BHA and 20% AYP was used. The same observations were made throughout the studies when the levels of combinations of either the AYP or HVP used with the antioxidant BHA were increased from 10 to 20, 30, and 40 percent or when that for BHA used either with AYP or HVP was increased from 0.005 to 0.01 to 0.02 percent (oil weight basis).

The phenomenon of greater stability when antioxidant substances are used in combination than when used individually, as observed in this work, is referred to as synergism which comes from the Greek word "synergus" and which,

Table 7. Induction Periods for Combinations of Hydrolyzed Vegetable Protein (HVP) and Butylated Hydroxyanisole (BHA) in the Model System

Model System	Induction Periods in Days (Weight Gain Method)	Induction Periods in Days (Absorbance Method)
Control	11	11
0.005% BHA + 10% HVP	21	21
0.005% BHA + 20% HVP	23	23
0.005% BHA + 30% HVP	25	25
0.005% BHA + 40% HVP	-	-
0.01% BHA + 10% HVP	23	23
0.01% BHA + 20% HVP	25	25
0.01% BHA + 30% HVP	25	27
0.01% BHA + 40% HVP	-	-
0.02% BHA + 10% HVP	-	-
0.02% BHA + 20% HVP	-	-
0.02% BHA + 30% HVP	-	-
0.02% BHA + 40% HVP	-	-

\- no detectable end of induction period within the 27 day test period.

Table 8. Induction Periods for Various Combinations of Autolyzed Yeast Protein (AYP) and Butylated Hydroxyanisole (BHA) in the Model Systems

Model System	Induction Periods in Days (Weight Gain Method)	Induction Periods in Days (Absorbance Method)
Control	11	11
0.005% BHA + 10% AYP	23	25
0.005% BHA + 20% AYP	27	27
0.005% BHA + 30% AYP	29	31
0.005% BHA + 40% AYP	-	-
0.01% BHA + 10% AYP	25	23
0.01% BHA + 20% AYP	31	31
0.01% BHA + 30% AYP	35	35
0.01% BHA + 40% AYP	-	-
0.02% BHA + 10% AYP	-	-
0.02% BHA + 20% AYP	-	-
0.02% BHA + 30% AYP	-	-
0.02% BHA + 40% AYP	-	-

- no detectable end of induction period within the 43 day test period.

Webster's Dictionary defines as working together. Mahon and Chapman (1953) reported synergistic effect when two phenolic antioxidants were used together. Dugan and Kraybill (1954) also reported synergistic effect when BHA and BHT were used together while Olcott and Mattill (1936) noted the same phenomenon with some organic acids, when used with phenolic antioxidants.

It has to be pointed out here that as depicted in Tables 7, 8, 9 and 10 synergism in the sense implied by the above workers could be demonstrated only with combinations of AYP and BHA. Specifically, this harmonized with the report of Bishov and Henick (1974, 1975) who observed a synergistic effect when BHA and AYP were used together in a freeze-dried model system the lipid component of which was stripped corn-oil. In contrast the HVP used in combination with BHA resulted in negative synergism first reported by Dugan and Kraybill (1954) in their work with BHA and PG. In a separate study Dugan and Kraybill (1956) hinted that the addition of BHA and the tocopherols beyond optimum level resulted in negative synergism in which the resulting stabilizing effect was less than would be expected from their combination. It is also obvious that even the strong synergism between AYP and BHT reported by Bishov and Henick (1974, 1975) could not be demonstrated in our work; the best we obtained was weak and not the strong one mentioned. Dahle and Nelson (1941) have pointed out that such discrepancies are manifestations of the fact of an antioxidant or

Table 9. Synergism in Model Systems of HVP, BHA, Palm Oil and CMC, Stored at 60°C for 27 Days.

Model System	Synergism Based on Induction Period (Weight Gain Method)	Synergism Based on Induction Period (Absorbance Method)
0.005% BHA + 10% HVP	0	3
0.005% BHA + 20% HVP	-1	0
0.005% BHA + 30% HVP	-6	-6
0.005% BHA + 40% HVP	-	-
0.01% BHA + 10% HVP	-4	-1
0.01% BHA + 20% HVP	-5	-3
0.01% BHA + 30% HVP	-8	-6
0.01% BHA + 40% HVP	-	-
0.02% BHA + 10% HVP	-	-
0.02% BHA + 20% HVP	-	-
0.02% BHA + 30% HVP	-	-
0.02% BHA + 40% HVP	-	-

- no detectable end of induction period within the 27 day test period.

Table 10. Synergism in Model Systems of AYP, BHA, Palm Oil and CMC, Stored at 60°C for 43 Days.

Model System	Synergism Based on Induction Period (Weight Gain Method)	Synergism Based on Induction Period (Absorbance Method)
0.005% BHA + 10% AYP	2	2
0.005% BHA + 20% AYP	1	2
0.005% BHA + 30% AYP	2	-4
0.005% BHA + 40% AYP	-	-
0.01% BHA + 10% AYP	-5	-5
0.01% BHA + 20% AYP	1	1
0.01% BHA + 30% AYP	3	2
0.01% BHA + 40% AYP	-	-
0.02% BHA + 10% AYP	-	-
0.02% BHA + 20% AYP	-	-
0.02% BHA + 30% AYP	-	-
0.02% BHA + 40% AYP	-	-

- No detectable end of induction period within the 43 day test period.

combinations of antioxidant for one system having less effect on another system and that variance in levels of primary antioxidants and synergists from product to product, with varying consequent synergistic effects, may be involved. True, the lipid materials, the temperature, the percentage and combinations of HVP or AYP were not the same for both studies.

To give a clearer perspective of what one can expect from a combination of antioxidants under the conditions of this study, a histogrammic illustration of results is shown in Figures 2 to 13: positive synergism for AYP and BHA combination, and the concentration dependence of such synergisms, if such considerations are based on the weight gain and absorbance increases attained by the model systems over the 43 and 27 day periods. It would appear also that the extent of synergism in each case is reflective of the antioxidative potency of the HVP, AYP and BHA.

The mode of action of synergism in these studies, like the whole concept, generally is not clearly understood. Dugan (1957) has postulated that synergists may work as metal scavengers, peroxide decomposers and sparing agents, as in the interaction of phenolic antioxidants or the interaction of other agents with phenolic antioxidants. The possibility of any of these to have operated in our system cannot be ruled out.

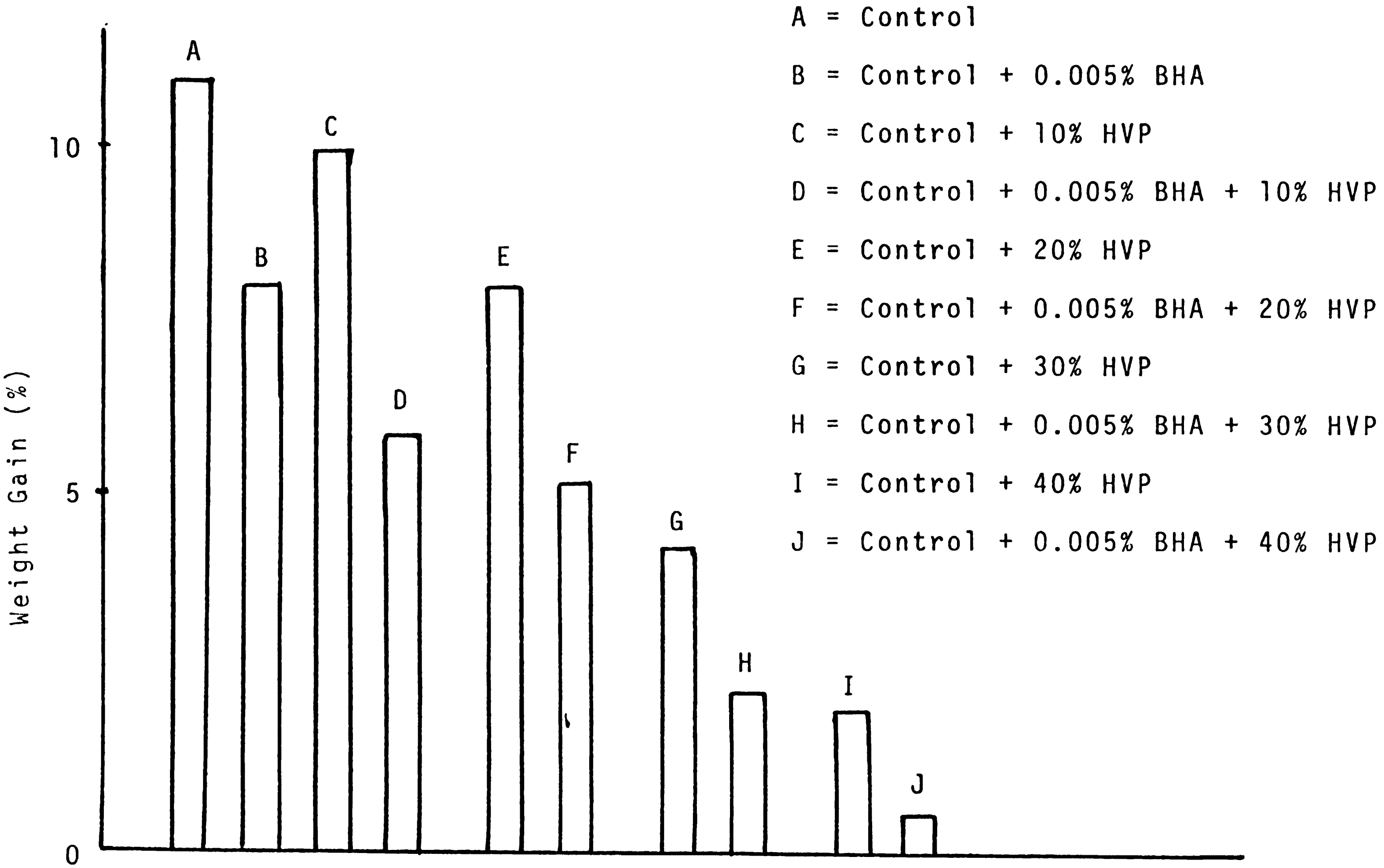

Figure 2. Weight gain in freeze-dried model systems of CMC, HVP, BHA and palm oil stored at 60^{o}C for 27 days.

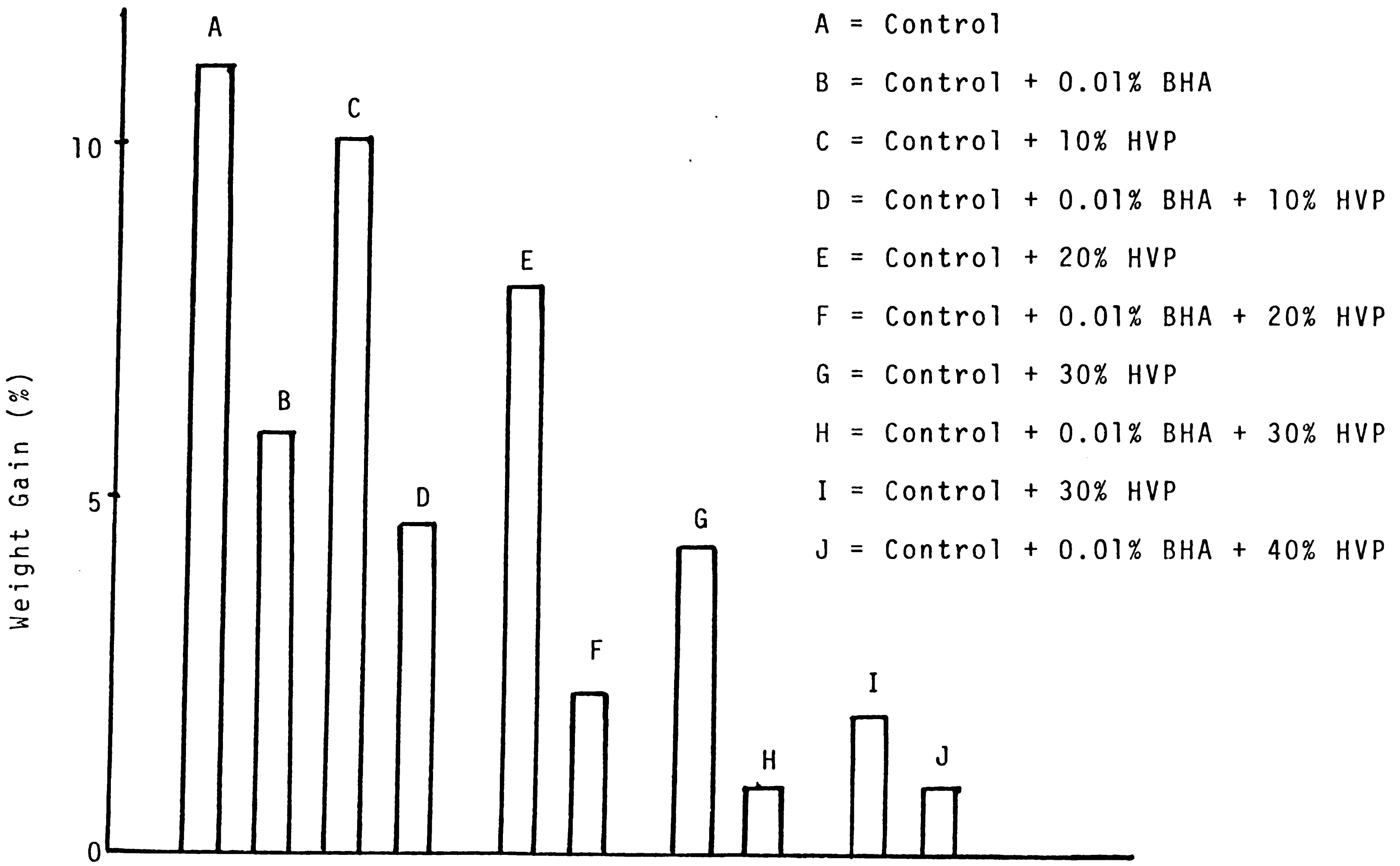

Figure 3. Weight gain in freeze-dried model systems of CMC, HVP, BHA and palm oil stored at 60^{o}C for 27 days.

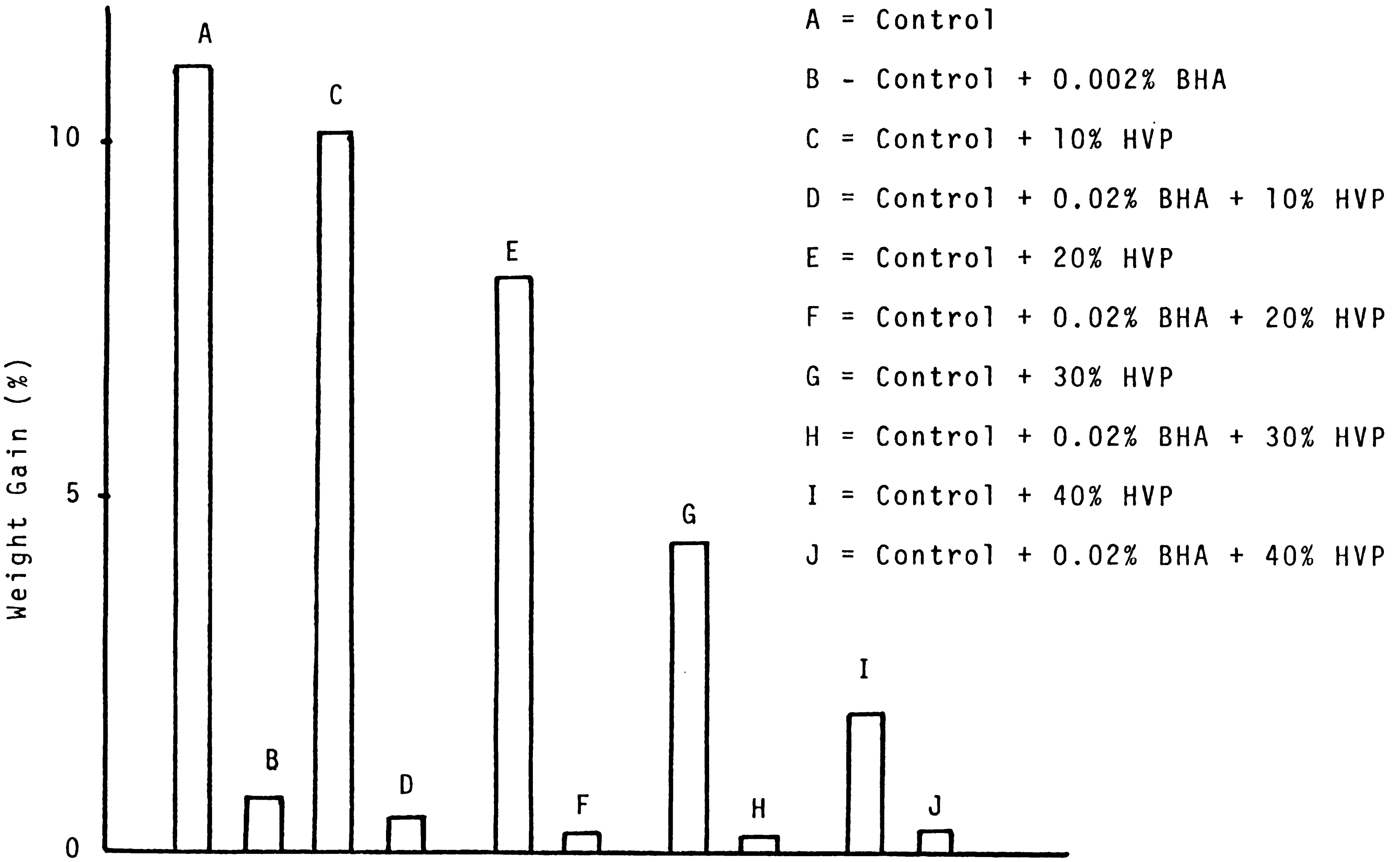

Figure 4. Weight gain in freeze-dried model systems of CMC, HVP, BHA and palm oil stored at 60°C for 27 days.

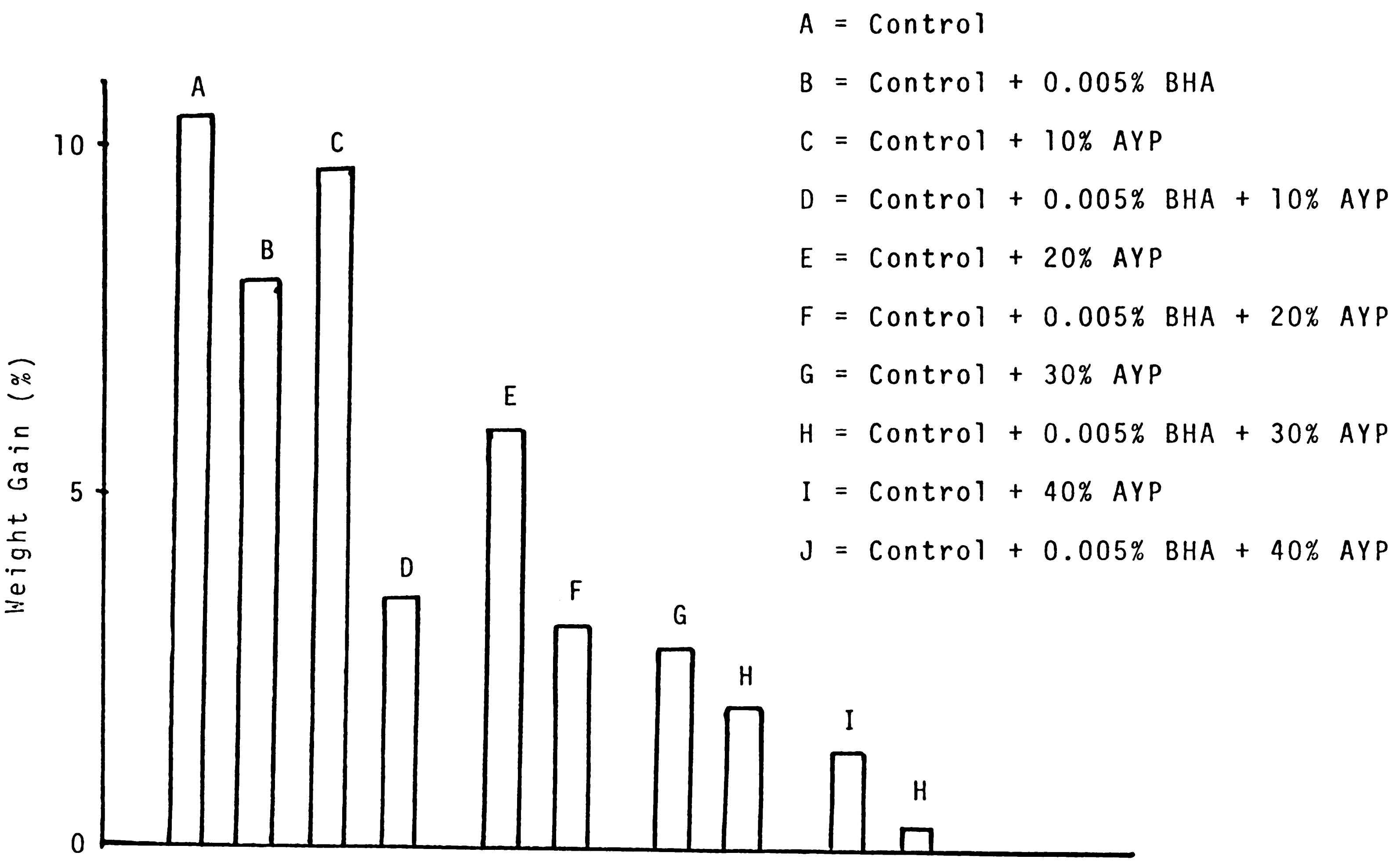

Figure 5. Weight gain in freeze-dried model systems of CMC, AYP, BHA and palm oil stored at 60°C for 43 days.

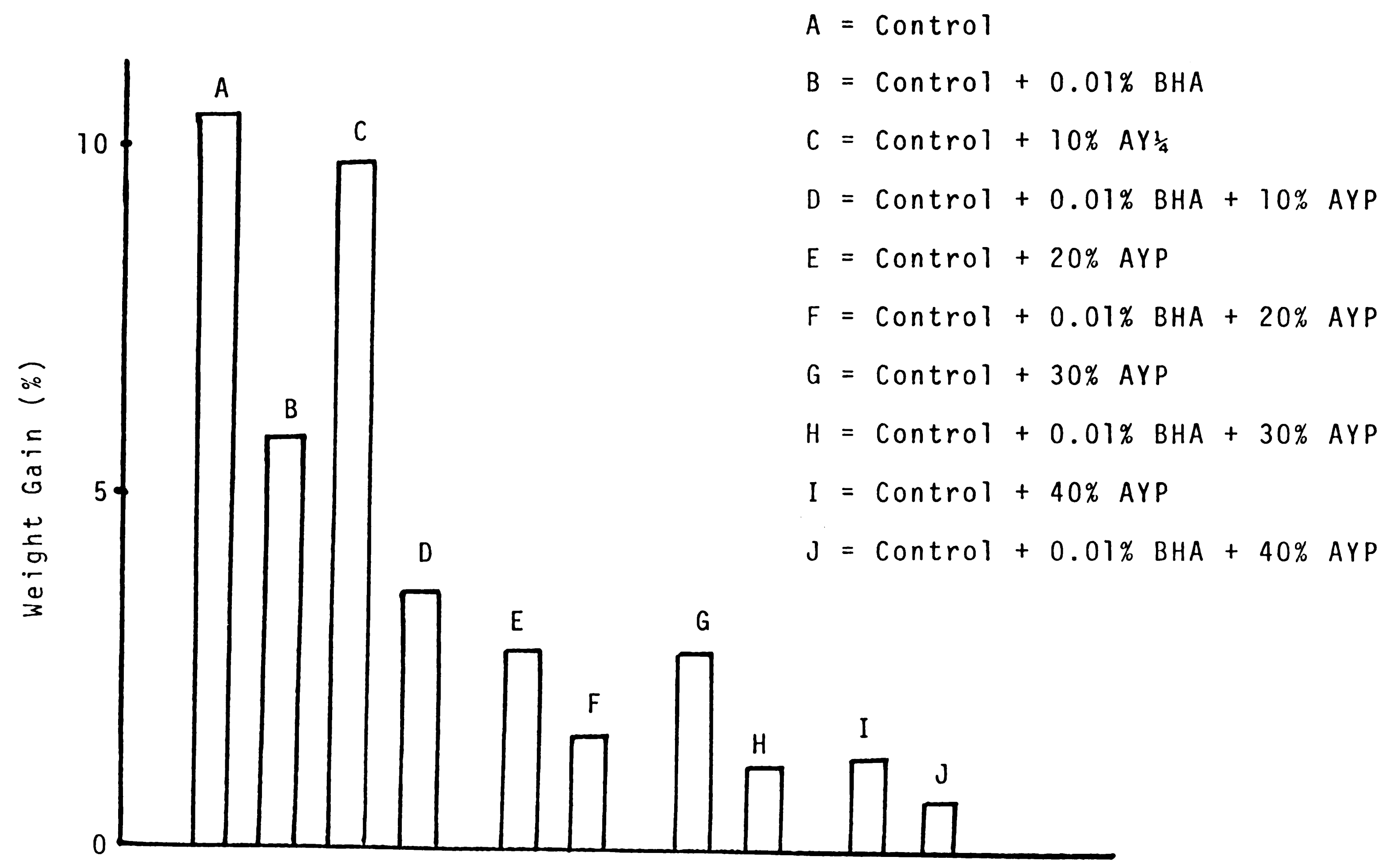

Figure 6. Weight gain in freeze-dried model systems of CMC, AYP, BHA and palm oil stored at 60°C for 43 days.

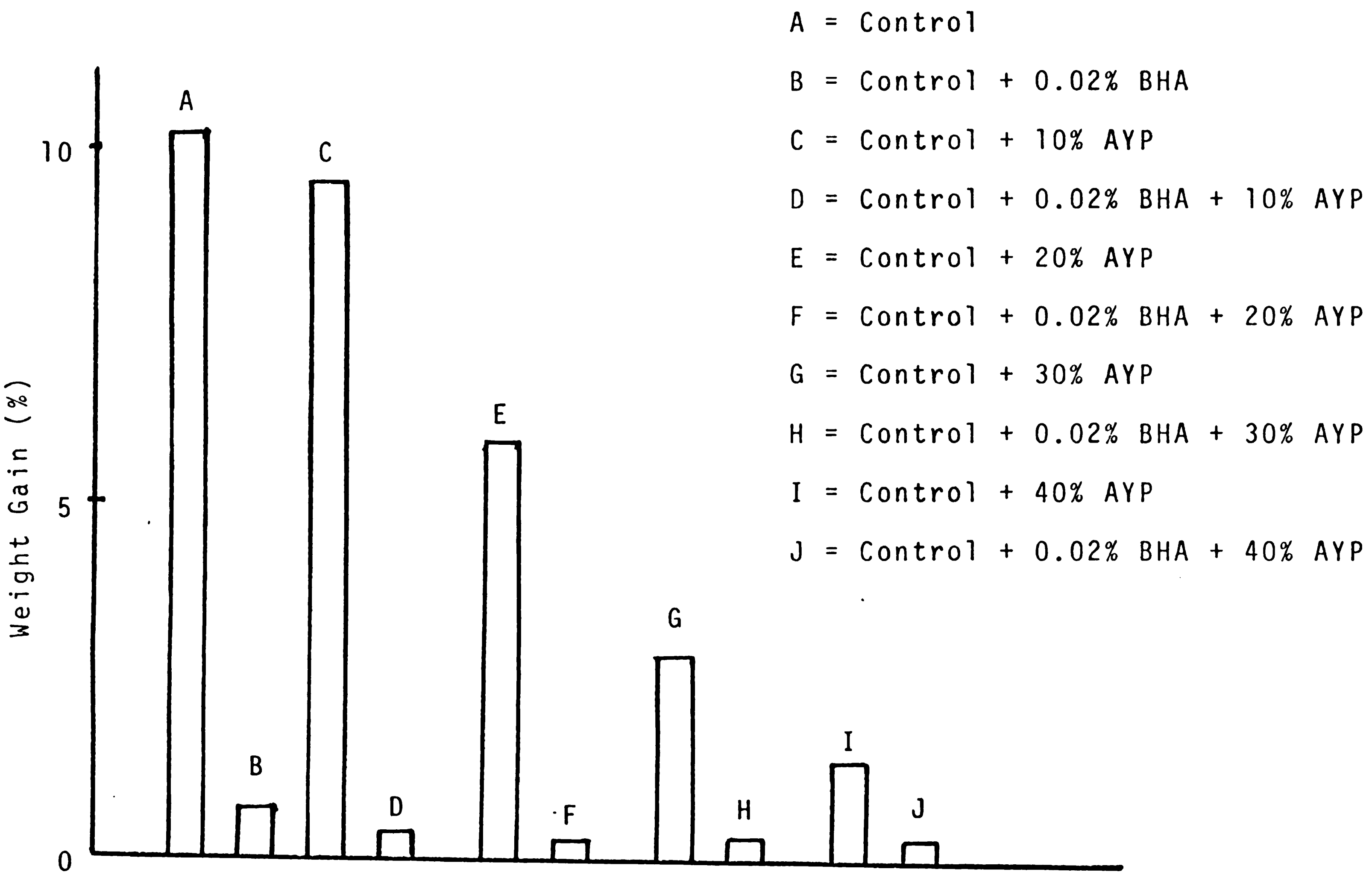

Figure 7. Weight gain in freeze-dried model systems of CMC, AYP, BHA and palm oil stored at 60^{o}C for 43 days.

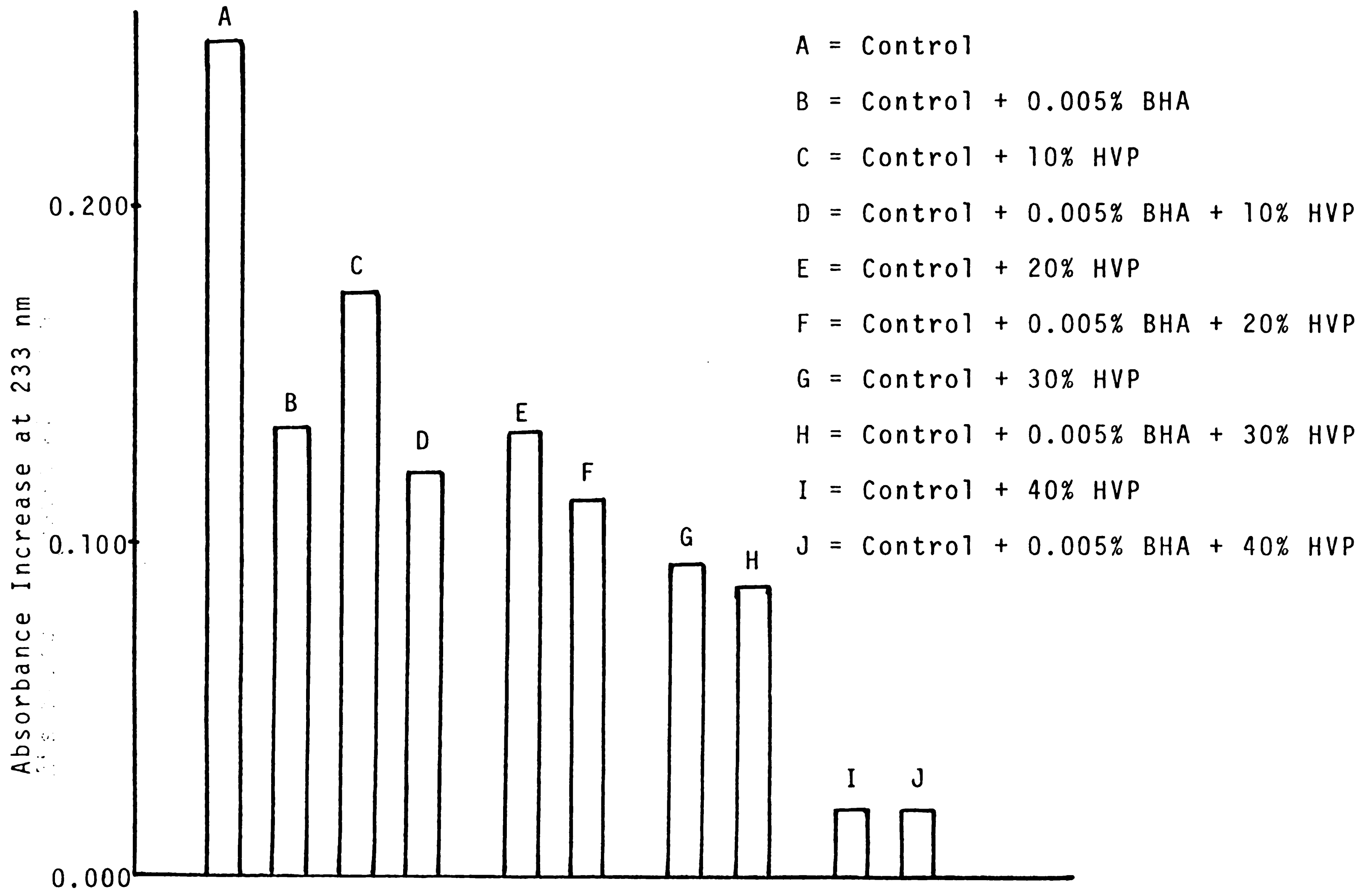

Figure 8. Absorbance increases in model systems of CMC, HVP, BHA and palm oil stored at 60^{0}C for 27 days.

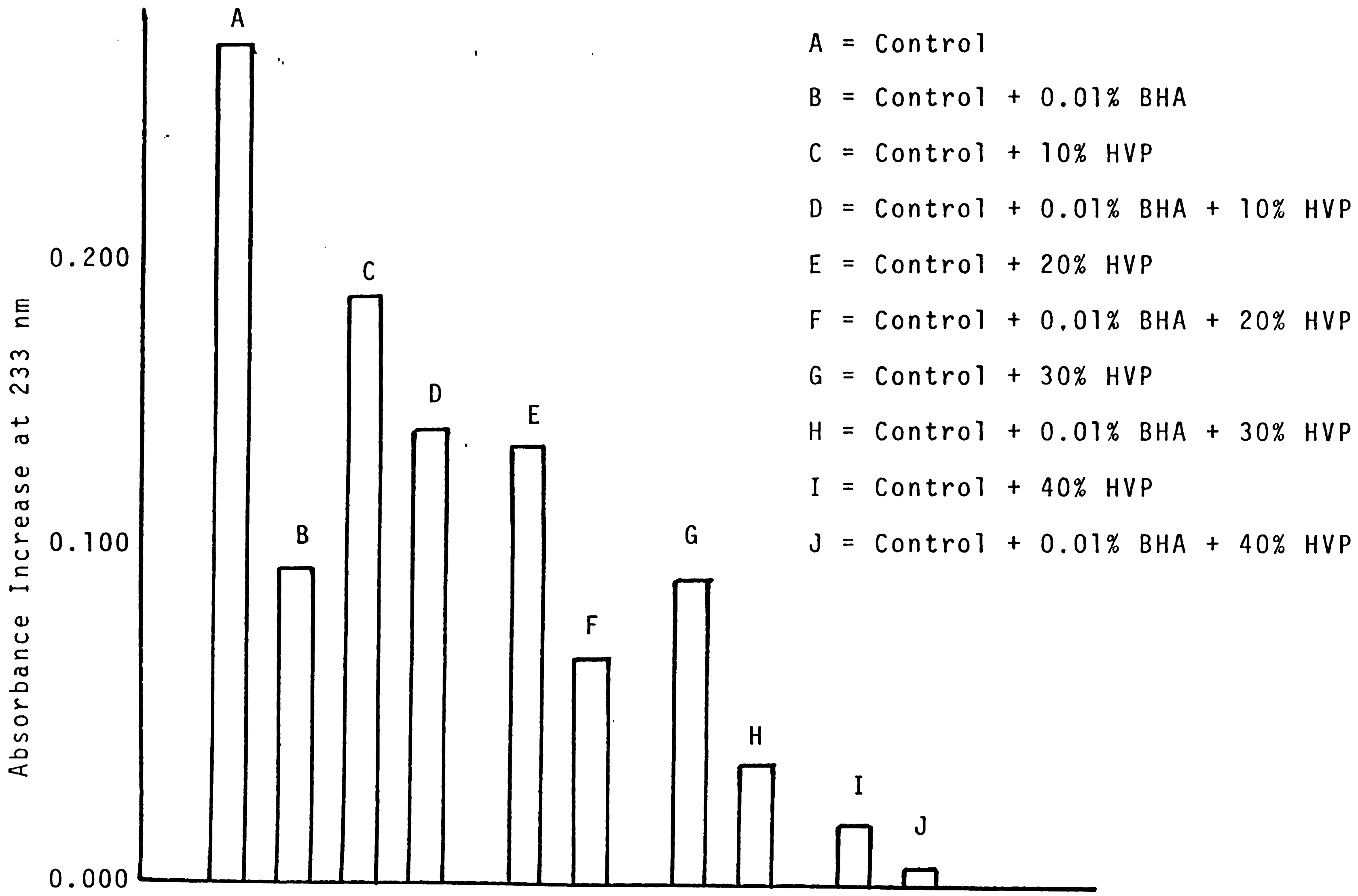

Figure 9. Absorbance increases in freeze-dried model systems of CMC, HVP, BHA and palm oil stored at 60°C for 27 days.

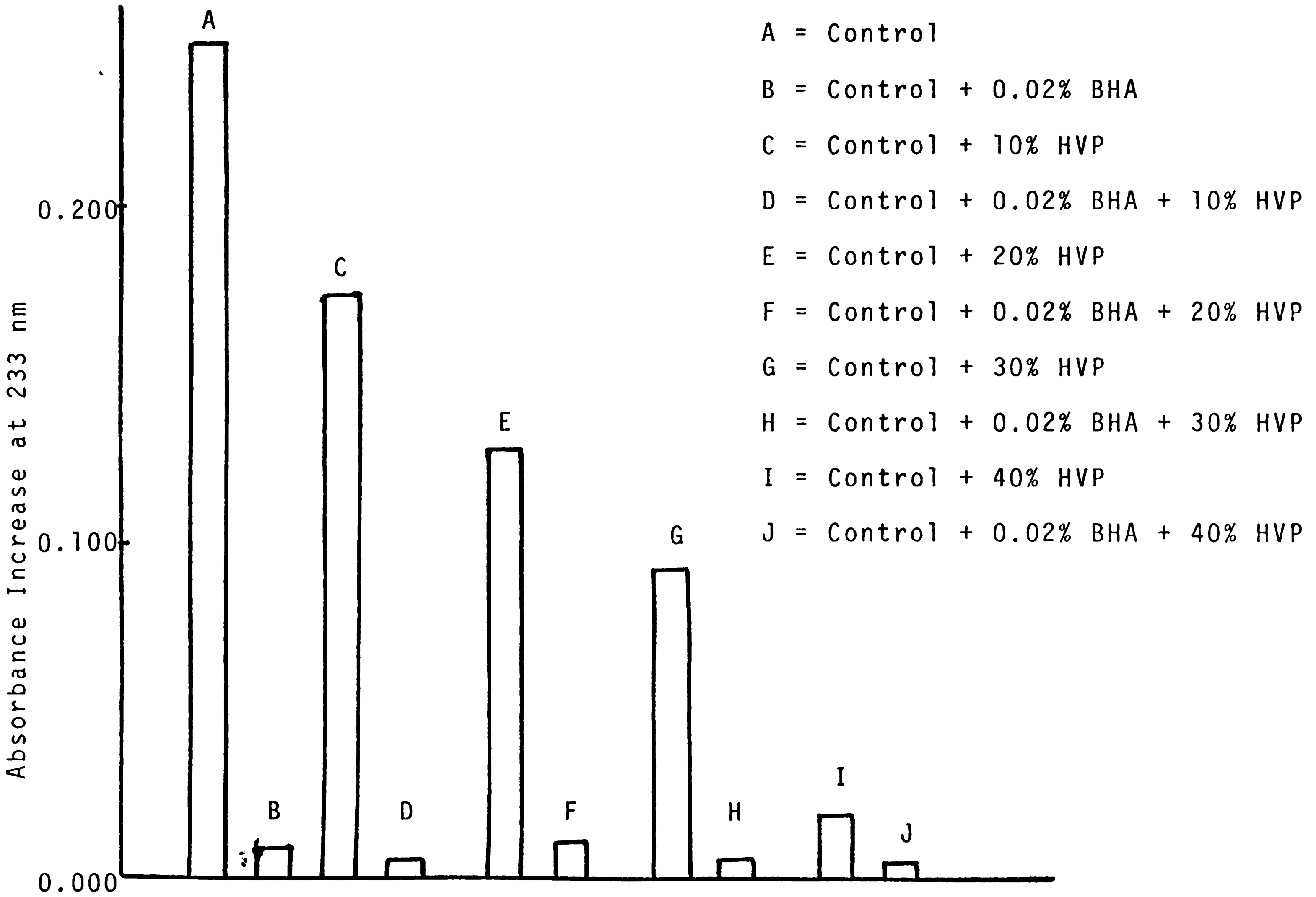

Figure 10. Absorbance increases in freeze-dried model systems of CMC, HVP, BHA and palm oil stored at 60^{0}C for 27 days.

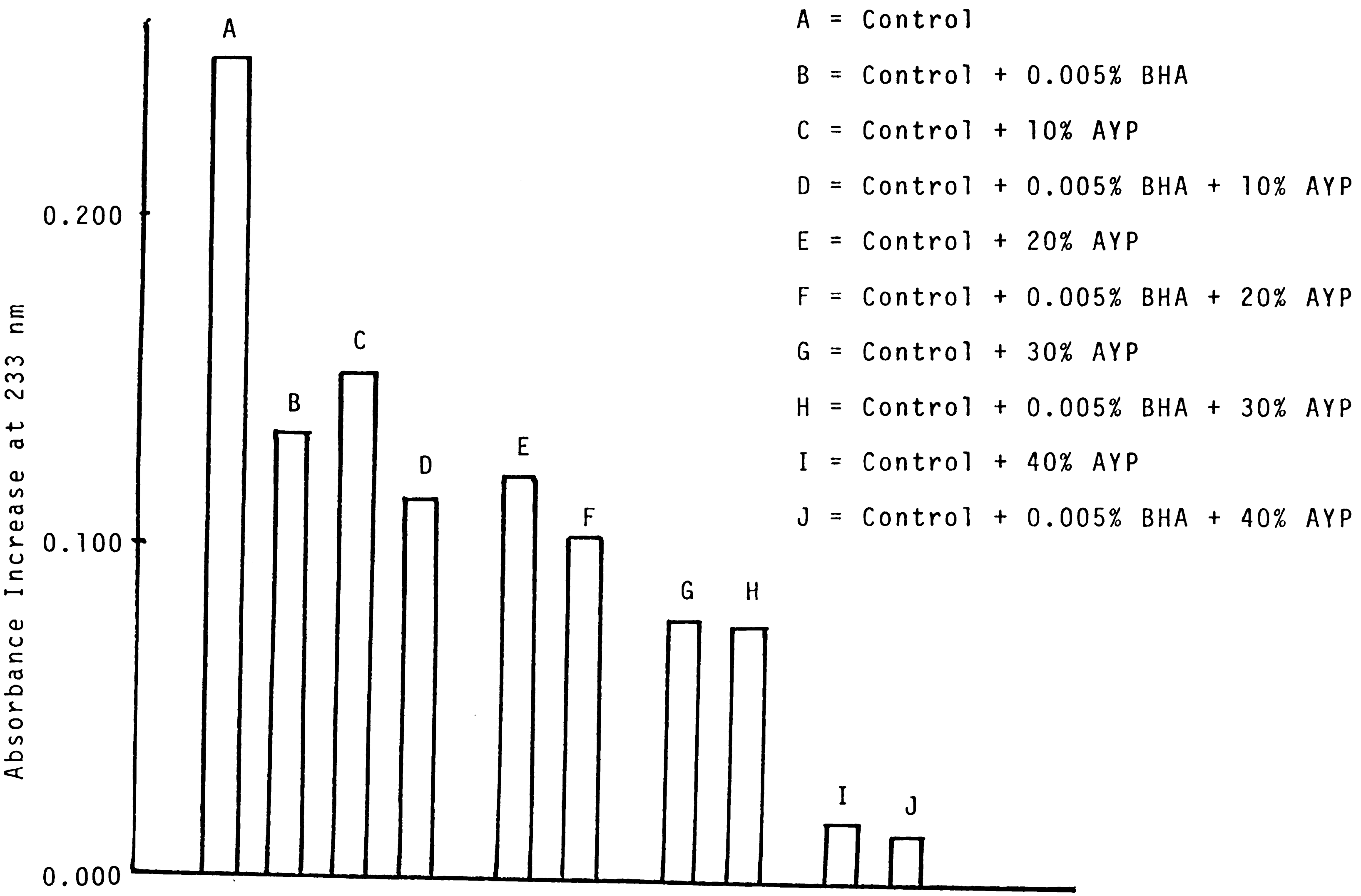

Figure 10. Absorbance increases in freeze-dried model systems of CMC, AYP, BHA and palm oil stored at 60°C for 43 days.

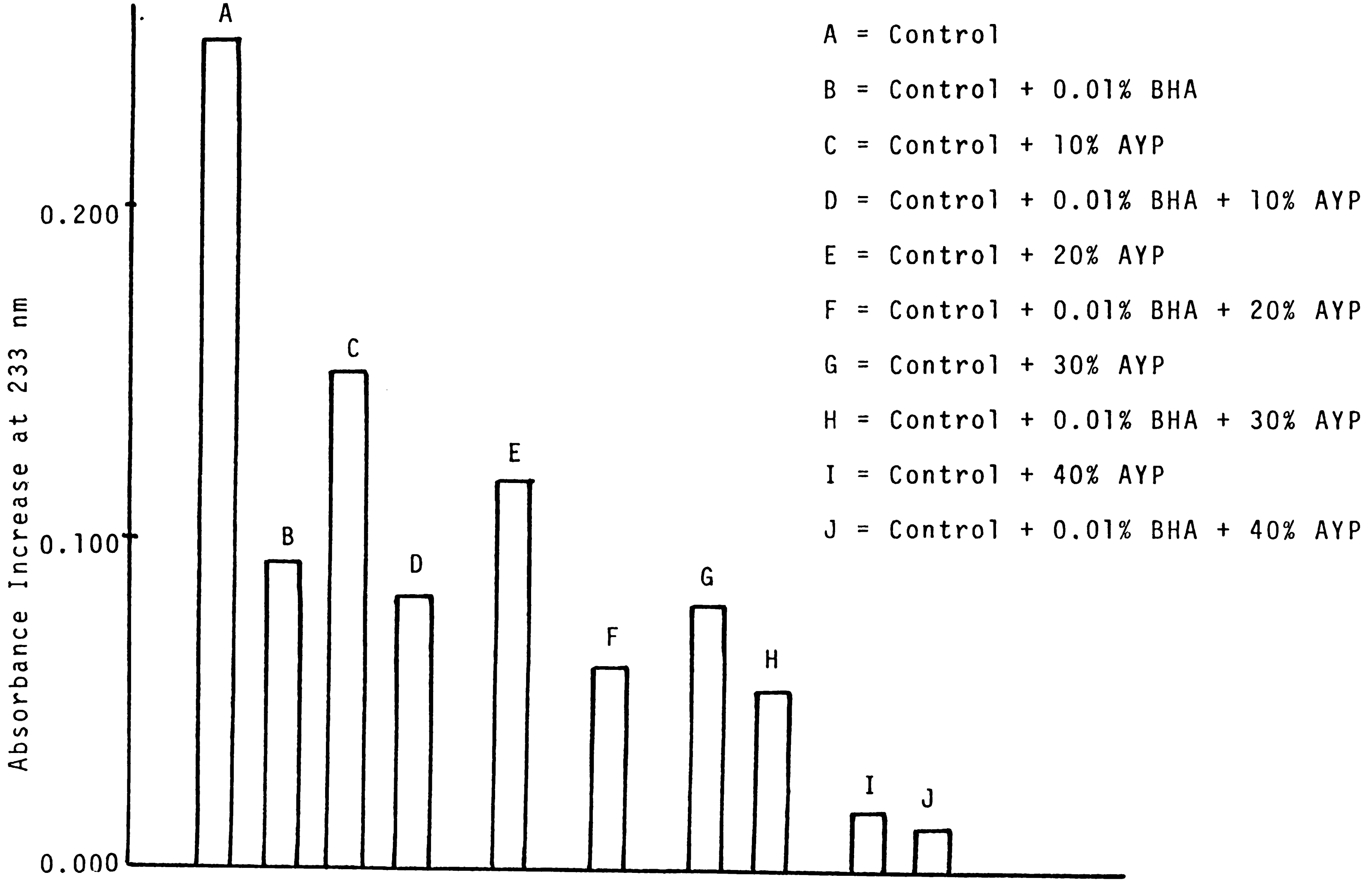

Figure 12. Absorbance increases in freeze-dried model systems of CMC, AYP, BHA and palm oil stored at 60°C for 43 days.

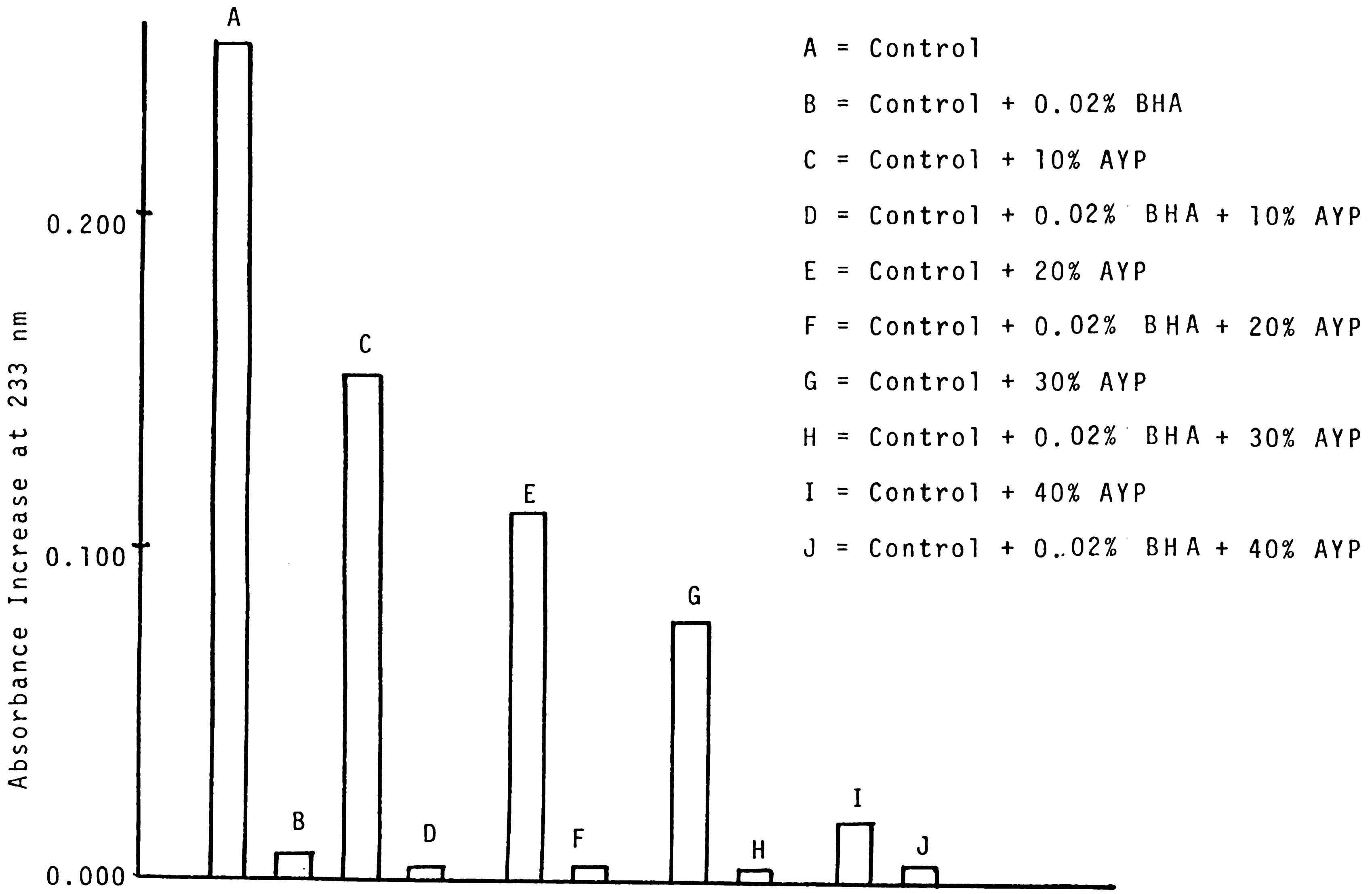

Figure 13. Absorbance increases in freeze-dried model systems of CMC, AYP, BHA and palm oil stored at 60°C for 43 days.

Comparison of the Antioxidant Effects of BHA, HVP and APY in Palm Oil

A look at all the results recorded in this work and already referred to in the discussion clearly indicate that BHA was more effective in providing improved stability to the palm oil components in the model system than AYP which in turn was more effective than HVP. There are numerous reports which indicate that given the same experimental conditions all antioxidants are not equally effective. BHT or BHA were reported by Cort *et al*. (1975) to be inferior to TBHQ in improving the stability of a lipid system. Sherwin and Luckadoo (1970) made a similar observation. As a more striking contrast, Marcuse (1960) working with herring oil observed that while the amino acid histidine had an antioxidant effect, cysteine was a pro-oxidant under the same conditions of activity. The observation in this study indicating differential activity among HVP, AYP and BHA is therefore in agreement with other works.

Since the working conditions in these studies were maintained the same as much as possible throughout the period of experimentation, any differences in activity among the antioxidant BHA, AYP and HPV can only be accounted for by the intrinsic differences among them and/or differences in their preparations. First BHA is a synthetic antioxidant specifically prepared to provide improved stability in lipid systems prone to autoxidation while AYP and HVP are regarded as natural products or products of natural products which, in any case, are beginning to receive more serious attention as antioxidants.

The greater effectiveness of AYP over HVP has been postulated by Bishov and Henick (1972) to be due to the milder conditions of autolysis of AYP compared to the hydrolytic conditions involved in the preparation of HVP - conditions which are regarded as more severe and therefore more destructive to the functionality of the HVP. The salt which is produced by the neutralization of the HVP during its preparation may also be pro-oxidative and therefore lower the antioxidant effectiveness of the HVP. Even with similar conditions of preparation one would reasonably expect differences in effectiveness as antioxidants attributable to differences in their composition such as in amino acids, which have been reported by many workers to have antioxidant effects (Marcuse, 1960, 1962, Karel _et al_., 1966, Smith and Circle, 1972, Scarborough and Watts, 1949) and in peptide profile (Bishov and Henick, 1972, 1975).

According to Dugan (1962) some antioxidants are effective in prolonging the keeping time of both fats and oils and the foods containing them and he described such antioxidants as "carry through antioxidants" since they "carry through" or survive the thermal stresses, steam distillation and pH effects of processing to give longer shelf-life to the finished food. The procedure which was used in this study to prepare the model system which involved mixing the CMC, HVP or AYP, palm oil and BHA in distilled water by mechanical agitation in a blender, followed by freezing for 24 hours and by freeze-drying for another 48 hours and finally holding at 60°C for periods of 27 and 43 days could be assumed to amount to food processing. In view of this, the improved stability observed with the BHA and HVP and AYP after all these stresses is tantamount to "carry through" properties for these compounds. It might also be stated here that differences in effectiveness reported in this study among the above three antioxidants may have been due, among other things, to the differences in extent to which the stress conditions of processing employed in the study altered their functionalities.

Comparison of Weight Gain Measurements to Absorbance Values

In measuring the extent of autoxidation in a lipid or in estimating its state of autoxidation it is the usual practice to use more than one method for the purpose of comparison which can inspire more confidence in the results obtained. This is because the whole concept of oxidation or autoxidation is such a complex thing. In this study, therefore, the absorbance due to diene conjugation and weight gain studies attributable to oxygen uptake were used to measure oxidation.

Labuza *et al*. (1969) reported that after 24 hours peroxide value determinations became 10-20 times less than the expected values and therefore suggested that the value is not a useful method for the measurement of oxidation in foods containing protein, especially under accelerated conditions.

Angelo *et al*. (1975) noted that the mechanism which causes the peroxidation of polyunsaturated fatty acids produces conjugated diene hydroperoxides and that both peroxide value and diene conjugation measure the same products of oxidation which are hydroperoxides. On account of this, the measurement of diene conjugation by spectrophotometric measurement of absorbance at 233 nm as was done in this study did eliminate the need to take peroxide value measurement.

The tables and figures already referred to in this discussion so far indicate that, in general, the weight gain values were in considerable agreement with the absorbance values which indicate diene conjugation. Although Swern (1961) has noted that the diene conjugation as measured by absorption of ultraviolet radiation at 233 nm was in agreement with oxygen uptake (weight gain measurement) only in the initial stages of oxidation, in oils containing linoleate or more highly unsaturated systems, the results here show agreement at all stages of oxidation, to the extent involved in these studies. The suggestion of Corliss (1968) that although UV measurements of conjugated dienes do not indicate the precise stage of lipid oxidation and that these can be used in combination with quantitative determinations such as weight gain measurements hold correct in these studies.

Any specific differences which are observable from the results such as in the specific lengths of the induction periods, the maximum weight gain values and absorbance values and in the final weight and absorbance, might be accounted for by differences in the precision of both methods and certain physical phenomena one of which could have been oxygenation. Oxygenation makes it possible to have physical absorption of oxygen by a system and therefore increase in weight without a corresponding increase in UV absorption to parallel increased oxygen uptake as one would expect from oxidation (Campbell *et al*., 1974). It is, however, likely that the phenomenon was too subtle to measure for this study.

SUMMARY AND CONCLUSION

This work was done with the objective of probing the antioxidant effects of Butylated Hydroxyanisole (BHA), Hydrolyzed Vegetable Protein (HVP) and Autolyzed Yeast Protein (AYP) on palm oil in a model syste, composed of either HVP or AYP, BHA, CMC and palm oil.

Results indicated

a) that BHA, HVP and AYP had antioxidative effects on palm oil and therefore improved the stability of the palm oil in the model system;

b) that such improved stability was directly related to the concentration of each of these antioxidant compounds in the system;

c) that the order of effectiveness was BHA > AYP > HVP;

d) that combinations of the antioxidants gave greater stability but combinations of BHA and HVP gave only negative synergism which was also found to be concentration dependent;

e) good correlation between the weight gain measurements and absorbance due to diene conjugation in monitoring oxidation of palm oil in the model system.

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