EFFECTS OF CERTAIN PROTEINS ON LIPID OXIDATION

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ABSTRACT

EFFECT OF CERTAIN PROTEINS ON LIPID OXIDATION

By

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The study of lipid oxidation in foods has been approached using model systems comprised of some solid surfaces over which the lipid is dispersed either in dry state or in emulsion allowing interactions of the various components and, thus, simulating real foods. This simplified approach should generate valuable information concerning oxidative deterioration in dehydrated foods containing dispersed fat.

The oxidation rates of model dehydrated systems containing dispersed lipids are influenced by concentration of the components, type of medium on which the lipid is dispersed, and orientation of lipid film with respect to the medium on which it is dispersed.

This investigation was undertaken to find the role of proteins such as promine-D, sodium caseinate, as well as a polysaccharide microcrystalline cellulose "Avicel" when oxidizing lipids such as soybean oil, corn oil, and lard

are dispersed in the ratios of 0.5, 1.0, 5.0, and 10.0 percent on them.

The rate of oxidation of lipid was followed by measuring oxygen uptake with a Gilson differential respirometer for 120 hrs at 45°C and by determining absorbance due to diene conjugation at 233 nm after 48, 96, and 120 hrs of oxygen uptake studies. Changes in the solubilities of proteins were followed for a period by Biuret reaction at 540 nm.

The findings of this study were that:

(a) oxidation increased with an increase in unsaturation of lipids;

(b) there was a good correlation between oxygen uptake and diene value;

(c) oxidation occured more readily at the lower ratios of lipid to proteins;

(d) lipid oxidation occurred more readily on an Avicel surface than on protein surfaces;

(e) oxidation of lipids progressed less rapidly on casein than on promine-D; and

(f) little correlation was obtained between loss of protein solubility and lipid oxidation at the levels of oxidation achieved.

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INTRODUCTION

Interaction between proteins and lipids is a field of major concern in Food Science and biochemistry. Most of the work has concentrated on cellular and subcellular structures (especially membranes), on blood constituents, and on model system interactions in aqueous solution (Karel, 1973).

With the recent emphasis on the utilization of unconventional proteins for the fabrication of analogs which simulate conventional food products, an understanding of specific protein-lipid interaction, becomes important in achieving familiar accepted food characteristics (Castimpoolas and Meyer, 1971); for this knowledge is important for product formulation and for understanding the nutritional value of the product.

Besides, in food systems there is a potential for interaction between lipids and proteins (Koch, 1962). Many highly reactive intermediates are formed during autoxidation of unsaturated lipids. These reactive intermediates, including hydroperoxides, and peroxy and oxy-radicals, as well as a number of carbonyl compounds, are capable of entering into reaction with one or more of the functional groups within the protein molecules. As a consequence, one would expect a

variety of complex interactions to occur between proteins and autoxidizing lipids under suitable conditions. Such interactions could be expected to produce changes in protein solubility and cause cross-linking of protein molecules with consequent changes in protein quality.

Because of the complex nature of lipids and proteins, our knowledge of the properties of the lipid/protein system is not yet sufficient to be applied in any rational way to product formulation or improvement. Besides, one can also appreciate the fact that with a given lipid and protein, the interaction may vary, depending on the presence of other substances, ionic condition, pH, and temperature (Tria and Scanu, 1969).

Three methods may be used to study lipid/protein systems (Leslie, 1970).

First, we may try to study a naturally-occurring system in situ, but this method is fraught with experimental difficulty, and it depends on technical advances in instrumentation.

A second line of attack is to isolate pure lipoproteins and to establish their chemical and physical properties using analytical procedures and physical techniques. The major difficulties are the isolation of the lipoprotein systems and the establishment of criteria of purity. A further problem is the extrapolation of results obtained from the subfractions to the original large structures existing in natural systems.

A final approach is the preparation and study of model complexes between pure proteins and lipids. The advantages

of this approach are obvious. We start with components whose nature and properties are fairly-well understood, and this should help to understand what happens when these components are brought together.

In my study, attempts were made to find the role of proteins such as promine-D and sodium caseinate (with microcrystalline cellulose "Avicel" as a reference material) when oxidizing lipids, soybean oil, corn oil, and lard were dispersed in different ratios on them.

This simplified dry system approach, where lipids are dispersed on protein surfaces in different ratios allowing interaction between lipids and proteins, has been used to find:

(a) the effect of protein surfaces on lipid oxidation;

(b) whether proteins act as prooxidant or antioxidant; and

(c) the effect of changing the ratios of lipids dispersed on proteins.

The purpose was to derive a concept of oxidation of lipids when on different surfaces and thus obtain some insight into the question of lipid protein interaction in these systems.

The autoxidation of lipids dispersed on proteins and "Avicel" was followed manometrically. Diene conjugation and protein solubility studies were made to determine changes in lipids and proteins following oxygen uptake studies.

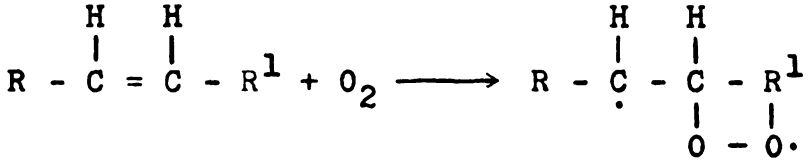
LITERATURE REVIEW

Mechanism of Lipid Oxidation

Autoxidation, defined as the spontaneous reaction between atmospheric oxygen and organic compounds, occurs under mild conditions although light, heat, the concentration of oxygen, moisture, and the presence of catalysts or inhibitors appear to affect the reaction with different results (Silbert, 1962). Among the constituents of foods susceptible to autoxidation are the unsaturated fatty acids in their various forms of combination and a considerable number of minor, but almost equally important, constituents. These latter include many of the natural aroma, flavor, and coloring substances which make food attractive, as well as a number of vitamins essential for adequate nutrition (Lea, 1962).

In a refined fat or oil the oxidation usually proceeds through autocatalytic processes, where reaction rate increases with time due to the formation of products which themselves catalyze the reaction, but in foods the systems and the processes become more complex. These complex systems bring into play the effects of moisture, salts, proteins, carbohydrates, enzymes, and pigments which exert an important effect on the rate and course of oxidative reactions (Dugan, 1961; Lundberg, 1962).

The autoxidation reactions have been described by Dugan (1976) as follows: "unless mediated by other oxidants or enzyme systems, oxidation proceeds 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. Hydroperoxides are the major initial reaction products of fatty acids with oxygen. Subsequent reactions control both the rate of reaction and the nature of products formed. In this initiation stage an unsaturated hydrocarbon loses a hydrogen to form a radical, $RH \longrightarrow R\cdot + H\cdot$, and oxygen adds at the double bond to form a diradical:



Alternately, oxygen in the singlet state can apparently interpose between a labile hydrogen to form a hydroperoxide directly ($RH + O_2 \longrightarrow ROOH$). Direct formation of hydroperoxides is not necessarily a free-radical chain mechanism, although it can initiate chain processes.

During propagation, the chain reaction is continued by $R\cdot + O_2 \longrightarrow ROO\cdot$ and $ROO\cdot + RH \longrightarrow ROOH + R\cdot$ 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.

$$R\cdot + R\cdot \longrightarrow RR$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

$$RO\cdot + R\cdot \longrightarrow ROR$$

$$ROO\cdot + R\cdot \longrightarrow ROOR$$

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

When no radicals are available for further reaction with oxygen, it is necessary for a new initiation reaction to occur if oxidation is to continue."

The oxidation process becomes more complex after the development of a quantity of ROOH, for the ROOH then decomposes either through thermal instability or through reaction with other materials to form more free radicals which then participate further in chain reactions (Dugan, 1961).

Besides the established fact that the majority of autoxidations proceed through free radical chain reaction and peroxides are the primary formed products, there are several other features of autoxidation. These have been described by Badings (1960) as follows:

(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, initiating new chains.

(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) The autoxidation pattern is complicated by the formation of secondary autoxidation products from dismutation of the hydroperoxides. The formation of dismutation products, such as the hydroxyacids, ketoacids, 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 these aldehydes, are largely responsible for the off flavors and odors characteristic of stale or rancid foods (Dugan, 1976).

Lipid Oxidation on Solid Surfaces

The study of lipid oxidation in foods has been approached using model systems comprised of some solid surfaces over which the lipid is dispersed either in dry state or in emulsion allowing interactions of the various components and thus simulating real foods. This simplified approach should generate valuable information concerning oxidative deterioration in dehydrated foods containing dispersed fat.

Bishov et al. (1961) reported that oxidation rates, at an elevated temperature, of model dehydrated systems containing dispersed lipids are influenced by concentration of the components, type of the medium on which the lipid is dispersed, and orientation of lipid film with respect to the medium on

which it is dispersed.

Togashi et al. (1961) mentioned that in dehydrated food the natural aqueous state of the component is upset, and there are new orientations of the lipid components in relation to other constituents. One important effect is creation of new surfaces upon which the lipid lies and spreads. Their study of oxidation of lipids in thin films suggested that as a fat is spread in thin layers, the rate of oxidation in air increases because of greater surface to volume ratio. Their model system study, using cottonseed oil spread on glass, showed that the oxidation rate is apparently independent of thickness for oil-thickness greater than about 10 mg/cm^2. For thinner films, the rate varied as the inverse of thickness reaching an apparent maximum at about 1 mg/cm^2.

Honn et al. (1951) studied the dependence of rate of oxygen consumption upon the oil/solid ratio. They found that when soybean oil was uniformly deposited on highly porous silica gel, the rate of oxygen consumption of a unit weight of soybean oil varied with the degree of dispersion of the oil upon the solid surface. They noted that the most rapid uptake of oxygen occurred at a critical oil/solid ratio characteristic of the surface area of the absorbant. At an oil/solid ratio above and below the critical ratio, the rate of oxygen absorption was markedly slower.

Bishov et al. (1961) have also studied the effect of various dispersing media on the rate of lipid oxidation. They reported that proteins such as egg white, ovalbumin, and

gelatin, decreased, while polymeric carbohydrates accelerated oxidation.

Togashi et al. (1961) explained that:

(a) hills and valleys in protein film reduces surface area of oil; and

(b) orientation effect of protein on oil reduces susceptibility to oxygen attack.

Labuza (1971) noted that the trace metal content of the system also accounted for the effect.

Tamsma et al. (1961) reported that the oxidation reaction also depended upon the physical state of the lipid--that is, whether it was emulsified or deposited on an exposed solid surface. In freeze-dried food the lipid is spread on a large porous matrix; thus, oxygen is readily available for reaction, and the food oxidizes even at low oxygen partial pressure.

Betts and Uri (1963) compared the rate of autoxidation between solid and liquid phase of the lipid and reported that autoxidation proceeded more rapidly in the solid phase than in the liquid phase. This was interpreted as due to a sharp reduction of the termination rate by free radical recombination ($ROO\cdot + ROO\cdot \longrightarrow$ Product), in comparison with rate determining propagation reaction ($ROO\cdot + RH \longrightarrow ROOH + R\cdot$) in the solid phase.

The lipid structure itself has some influence, since the way it is positioned on the surface of a solid phase can affect its contact with oxygen (Labuza, 1971). Triglycerides react more slowly than esters which are slower than free acids

(Labuza et al., 1969; Mabrouk and Dugan, 1960).

Lipid Protein Interaction

Gurd (1960) suggested that there is little evidence that lipids and proteins interact with each other through primary covalent linkages, such as ester bonds. The combination seems to be due more to interactions 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. Other interactions may involve polar or charged groups.

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 (Brunner, 1962).

According to Chapman (1969), electrostatic and hydrophobic binding and metal ion participation seem especially important in the binding of the lipid-protein molecule.

Studies of lipid-protein interaction in food systems have been made on model systems and on selected foods.

It has been observed that oxidation of lipids during storage of freeze-dehydrated food limits the shelf-life of food products. Quality changes such as off-flavor development and changes in texture and rehydration properties have prompted

the speculation that interaction between lipid oxidation products and proteins takes place during the processing and storage of dried foods (Koch, 1962).

Protein changes in meat and fish products cause physical changes which determine the eating qualities and functional properties of these products (Karel, 1973).

Studies on model systems containing lipids and proteins (Tappel, 1955; Narayan and Kummerow, 1958; Nishida and Kummerow, 1960; Desai and Tappel, 1963; Narayan and Kummerow, 1963; Narayan et al., 1964; Andrews et al., 1965; Roubal and Tappel, 1966 a,b; Zirlin and Karel, 1969; Roubal, 1971; Braddock and Dugan, 1973; Jarenback and Liljemark, 1975) have indicated that oxidation of lipid-protein systems often lead to browning and copolymerization of oxidized lipids and proteins as shown by losses in the chemical and physical properties of proteins.

Kummerow and group (as above) suggested that complex formation occurred in the presence of oxidized lipids, that different proteins responded differently, and that the reaction depended on environment. They attributed complex formation to secondary bonds, presumably including H-bonding.

Roubal and Tappel (1966 a) suggested that the damage to proteins and enzymes from peroxidizing lipids results in a loss of solubility, and constituent amino acids are destroyed. As oxidation progresses, polymerization of proteins occurs through reaction with peroxidizing polyunsaturated lipids (Roubal and Tappel, 1966 b).

Zirlin and Karel (1969) studied gelatin-linoleate interactions in the dry state and concluded that reactions of gelatin with lipid can lead to scission of protein as well as to cross-linking.

Roubal (1971) studied the role of free radicals in lipid protein mixtures with a low moisture content and hypothesized that radical attack and not aldehyde attack on protein is predominantly responsible for damage to proteins.

Braddock and Dugan (1973) reported isolation of fluorescent compounds, indicative of C = N functional groups from an oxidizing system consisting of methyl linoleate and coho salmon myosin. Similar compounds were also present in extracts from frozen and stored salmon. Furthermore, specific destruction of the ε-amino groups of myosin was observed, possibly a sign of cross-linking reactions between amino groups and fatty acid oxidation products. The amino acids most readily lost were methionine, lysine, and histidine. Jarenback and Liljemark (1975) indicated that linoleic acid hydroperoxides (LAHPO) were ten times more effective than linoleic acid in reducing the amount of protein in KCl-extracts from incubated myofibrils. These observations suggest that LAHPO are more efficient compared to linoleic acid in reducing the protein content of KCl-extracts from incubated myofibrils. LAHPO, by virtue of their reaction with amino groups, may be more extensively bound causing stronger interaction. This indicates the significance of involvement of lipid peroxides in changing protein extractibility

of myofibrillar protein.

It is apparent from these statements that lipid-protein interaction is of importance in food systems. The oxidizing lipids have been shown to bring about changes in proteins.

It may be that radicals derived from oxidizing lipids play a role in some of the damage to proteins. There is the possibility that carbonyl-amine reactions, as in Maillard browning, form Schiff bases and secondary products.

Schwenke et al. (1975) studied modifications of proteins by reaction with carbonyl compounds. They reported that blocking of α-ε-amino groups alters the isoelectric point, solubility, precipitation characteristics, and electrophoretic behavior of proteins. Nutritional value was related to the blocking of lysine.

Amino Acids as Antioxidants

Nearly all of the known amino acids have been reported to have prooxidative and/or antioxidative activity.

Marcuse (1960) examined, manometrically, the effect of amino acids on herring oil and reported that the antioxidative effect was strongest with histidine; it was prooxidative with cysteine. The tocopherol content of herring oil was 0.01 M. He suggested that amino acid may have antioxidative effects in the absence of tocopherol or other primary antioxidants. Amino acids are consequently able to act as primary antioxidants themselves; however, the effect is pH dependent.

Amino acids are usually supposed to function by chelating prooxidative trace metals. The prooxidative effect of copper traces is mainly due to copper in the free state in the fat phase. In the presence of amino acids, amino acid copper complexes are formed and transported into the water phase.

The antioxidative effect of different amino acids may be rather different (it is especially pronounced in the case of histidine and tryptophan). The antioxidative effect is enhanced, and the prooxidative effect is lowered by an addition of phosphate, or an emulsifier like tween (Marcuse, 1962).

Karel et al. (1966) studied the antioxidant activity of amino acids. Amino acids, including histidine, β-amino-butyric-acid, lysine, and cysteine, had substantial antioxidant properties, while methionine, phenylalanine, isoleucine, and argenine had no such activity.

Tjhio and Karel (1969) reported that histidine in low concentration has an antioxidant effect in the very early stages of oxidation followed by a slight prooxidant effect in the later stages. Thus, from these facts, one can say that the antioxidant property of amino acids varies with each amino acid, and that it depends on the pH, presence of other materials (metal, chelating agents or antioxidants) and the stage of oxidation.

Lipid Autoxidation Tests

There is no single approach to the problem of measurement of the autoxidation of a lipid or a lipid-containing food. 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. A composite of peroxide, TBA, carbonyl type, and acid determinations 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).

Various physical methods, such as polarography and absorption spectroscopy, are highly useful in elucidating reaction mechanisms and identifying products but normally must be augmented by methods of degradation, separation, and chemical analysis (Link and Formo, 1961).

Determination of Peroxide Value

The peroxide value is sometimes used as a measure of the extent of oxidation of an oil and is expressed as meq/1000 g of oil. This corresponds to the amount of peroxide undecomposed. An increasing value indicates that peroxides are being formed at a greater rate than they are

disappearing.

Labuza (1969) reported that peroxide value determination is not a useful technique for measurement of oxidation in foods containing proteins, especially if stored under accelerated conditions. Peroxide value determinations compared favorably in the first 24 hrs with actual manometric determinations corrected for protein oxidation after which it became ten to twenty times less than the actual manometric determinations.

Thiobarbituric Acid Test

The test involves, primarily, a reaction of thiobarbituric acid with malonic dialdehyde by heating in the presence of a strong acid to give a pink color (Lundberg, 1962). A feature of the TBA test is its comparative specificity for oxidation products of polyunsaturated fatty acids, which makes it particularly useful for food lipids rich in highly unsaturated acids (Lea, 1962). More recently, it has been shown that oxidation products besides malonic dialdehyde will give a positive reaction. This test supposedly gives a more reliable measure of the extent of oxidation in cases where substances other than fat are present and cause decomposition of peroxide (Lundberg, 1962).

It has been reported that TBA reagent is unstable in the presence of acid, peroxide and heat, i.e., under the test conditions (Tarladgis et al., 1962). The TBA test has been used successfully with stored fishery products, and

with lard and bacon. It has also been used less consistently to measure oxidized flavors of dairy products (Lundberg, 1962).

Ultraviolet Absorption Method

Oxidation of polyunsaturated fatty acids produces peroxides and the position of the double bonds 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 double bonds within the same region is very weak. This is the basis for the ultraviolet absorption method for determining oxidative effects (Mehlenbacher, 1960).

Swern (1961) reported that the oxidation of polyunsaturated fatty acid is accompanied by increased ultraviolet absorption. 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. For example, in the analysis of a sample containing dienoic, trienoic, tetraenoic or pentaenoic groups, the observed total diene content is not only due to linoleic acid, but it also contains contributions from each of the more unsaturated acids. Thus, the spectral change for a given substance should be used as a relative measure of oxidation. Probably, it is best applicable in the detection of oxidation, rather than its measurement.

Angelo et al. (1975) compared the peroxide value

determinations and spectrophotometric assay of conjugated diene hydroperoxides while studying shelf life stability of peanut butters during short- and long-term storage. They found good correlation between the two methods over 4 and 12 week storage periods. They mentioned that the conjugated diene hydroperoxides method requires smaller samples and is quicker, more accurate, and simpler than the peroxide value method, nor does it require additional reagents or depend upon a chemical reaction or color development.

Oxygen Uptake Studies

The principle of oxygen absorption has been employed as a measure of the susceptibility of fats to oxidation. Manometric respirometers such as the Barcroft-Warburg apparatus, and Gilson differential respirometer, with automatic recording devices are being used to estimate the oxygen uptake of samples. However, it has not been widely used in routine measurements of oxidative stability. Here, the experimental material is added to different reaction vessels. The reaction vessel and reference flasks are connected to manometers and agitated under controlled temperature (Gilson, 1963). The atmosphere in the chamber is either air or pure oxygen. As oxidation progresses, the amount of oxygen absorbed is indicated by the change of pressure determined manometrically (Lundberg, 1962).

In the study of oxidative deterioration of raw freeze-dried beef it was observed that only 50% of the oxygen

absorption, during the initial deterioration, could be accounted for as lipid peroxidation; the other 50% was apparently being used to oxidize the sulfhydryl groups of the protein (Tappel, 1962). Thus, other components of food systems can react with oxygen, making measurement of oxygen uptake erroneous. Oxygen uptake study normally requires considerable attention by trained personnel. It is quite valuable where large numbers of samples are to be compared for a relatively short period of time under closely controlled conditions (Stuckey, 1968).

EXPERIMENTAL

Materials and Equipment

Protein Sources

Promine-D. A general purpose, functional sodium soy proteinate was supplied by Central Soya, Chicago, Illinois.

Sodium-Caseinate. Sodium-caseinate was precipitated from fresh, twice-separated skim milk (0.06% butter fat), at pH 4.6, with 1 N hydrochloric acid at 30°C. After washing the precipitate with copious amounts of distilled water, the protein was redissolved by adding 1 N NaOH to bring the pH of the suspension back to 7.0. The precipitation and washing processes were repeated. The casein thus made was then freeze-dried at 100°F for 48 hrs at 5 mμ pressure.

Microcrystalline Cellulose

"Avicel". "Avicel" was supplied by the FMC Corporation, Marcus Hook, Pennsylvania.

Lipid Sources

ED-Soy Refined and Deodorized Soybean Oil. ED-soy refined and deodorized soybean oil was obtained from the A. E. Staley Manufacturing Company, Decatur, Illinois.

Corn Salad Oil. Corn salad oil was obtained from the

A. E. Staley Manufacturing Company, Decatur, Illinois.

Lard. Lard was obtained from the Meat Laboratory at Michigan State University, East Lansing, Michigan.

Chemicals

All chemicals used in this study were analytical reagent grade, with exception of Silica Gel (Type 60) which was obtained from Brinkman Instruments, Inc., Cantiague Road, Westbury, New York.

Solvents

All solvents (with the exception of anhydrous diethyl ether) were freshly redistilled before use.

Methanol was redistilled by adding 10 g of KOH and 25 g of zinc powder in 2 liters of methanol (A.O.C.S., 1974).

Chloroform was redistilled after washing with sulfuric acid. The sulfuric acid was dried by sodium sulfite.

Purification of Hexane

Hexane was purified by passing through a silica gel column according to Cd 7-58 A.O.C.S. (1974).

Gases

A purified grade of nitrogen was used throughout this study. Nitrogen was obtained from General Stores, Michigan State University, East Lansing, Michigan.

Gilson Differential Respirometer

A Gilson Differential Respirometer, Model GR-14

(refrigerated), from Gilson Medical Electronics, Inc., Middleton, Wisconsin, was used to measure oxygen uptake by the samples.

Spectrophotometer

A Beckman DU-2400 spectrophotometer from Beckman Instruments, Inc., Fullerton, California, was used for all of the absorbancy measurements.

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 moisture determination of proteins and Avicel.

Methods

Preparation of Reaction Mixture

Soybean oil, corn oil, and lard, each in the ratios of 0.5, 1.0, 5.0 and 10.0 percent, were dispersed on 2 g each of promine-D, Na-caseinate, and Avicel by adding appropriate quantities of an anhydrous diethyl ether solution of the lipid. The ether was later removed under the hood. To ensure the complete removal of the ether, a stream of N_2 gas was passed over the reaction mixture.

Moisture Determination

The moisture contents of promine-D, Na-caseinate and Avicel, were determined by the procedure outlined by the

A.O.A.C., 13.003, 9th ed. (1960).

Peroxide Value Determination

The peroxide value of soybean oil, corn oil, and lard used in this study was determined by the A.O.C.S. official method, Cd 8-53, 3rd ed. (1974).

Oxygen Uptake Studies

The rate of oxidation of the lipid-protein and Lipid-Avicel systems was followed by a Gilson differential respirometer (Gilson, 1963).

The respirometer is based on the principle that at a constant temperature and at a constant gas volume any change in the amount of gas can be measured by changes in its pressure. The rate of diffusion of a gas is dependent on the surface layer. The gas may be thought of moving across a surface film. A new surface is exposed to the gas by shaking.

Respirometer flasks with a capacity of 15 ml were charged with 2 g protein or Avicel with different lipids dispersed on them. Each respirometer flask contained one pellet of KOH (approximately 85%) in the center well to absorb any carbon dioxide evolved during oxidation. Experiments were conducted at 45°C for 120 hours in an atmosphere of air with a shaking rate of 150 oscillations per minute. Readings were taken after an interval of 12 hours and samples were removed after 48, 96, and 120 hours of oxygen uptake for diene conjugation and protein solubility studies. Each determination was made at least in duplicate.

The fluid in the manometer was boiled-kerosene (b.p. 175°-325°C) colored with Sudan red. Boiled kerosene was used because of its high boiling point, ease of operation, and non-interference with the experimental materials. To ensure a proper supply of oxygen in the system, the venting plug was opened to the air periodically.

The micrometer readings are given digitally in microliters. To convert to standard conditions, corrections were made for the following:

1. water bath temperature in degrees C = t
2. operating pressure (usually the same as barometric pressure) = P_b
3. pressure of water vapor = P_w

The micrometer readings were multiplied by the following to give microliters of dry gas at 760 milimeters Hg:

$$\frac{(273)\ (Pb\text{-}3\text{-}Pw)}{(t + 273)\ (760)} = \text{multiplying factor}$$

3 was subtracted to compensate for the specific gravity of Hg at room temperature

The microliter readings thus obtained were converted to moles of oxygen absorbed. Since 1 mole of any gas at standard conditions occupies 22.4 liters, each mole of oxygen gas can be considered as equal to 22.4 liters. Thus, 1 mole = 22.4 liters or 1 liter = 1/22.4 mole (1). Also, 1 microliter = 10^{-6} liter (11). Combining (1) and (11) 1 microliter = $1/2.24 \times 10^{-7}$ mole.

Extraction, Washing and Drying of Lipid from Reaction Matrix

Lipid extractions from the reaction matrix were made using the procedure of Folch et al. (1957) with 30 ml distilled chloroform-methanol (2:1, v/v). The lipid extracts were washed thoroughly with 20% volume of distilled water and twice with 20% volume of chloroform:methanol:water (3:48:47).

After each washing step, the contents were mixed vigorously for one minute and then centrifuged at 2000 rpm for 2 minutes. Following centrifugation, the upper phase containing all the nonlipid substances was removed.

After final washing, the lipid contained in the lower phase was dried by using purified compressed nitrogen gas.

Ultraviolet Spectrophotometric Determinations

The ultraviolet absorption of the lipid extracts at 233 nm were determined with a Beckman DU spectrophotometer with suitable dilutions of "spectrograde" hexane.

Diene conjugation = As/bc, where: As = observed absorbance at 233 nm; b = cell length in centimeters; and c = concentrations in grams/liters.

Extraction and Measurement of Soluble Protein Following Oxygen Uptake

After extracting the lipids from the lipid:protein matrix, the proteins were dried under a stream of nitrogen to remove residual solvent.

Solubility Study of Promine-D. 0.5 g of extracted promine-D was dispersed into 10 ml of 8 M urea:1 N sodium hydroxide

(1:1, v/v). The dispersion was shaken on a Burrell wrist-action shaker for one hour. Following shaking, the dispersions were clarified by centrifuging at 2000 rpm for 15 minutes in an IEC centrifuge. An aliquot (1 ml) of the supernatant was analyzed by the Biuret procedure (Lagget, 1967).

Solubility Study on Na-Caseinate. 0.5 g of extracted Na-caseinate was dissolved in 10 ml of 1 N NaOH. The rest of the procedure was the same as for promine-D (as above).

Preparation of Methyl Esters for Gas-Liquid Chromatography

Methylation of soybean oil, corn oil and lard was accomplished by a rapid procedure for preparing methyl esters from lipids based on a rapid saponification followed by esterification with BF_3-methanol (Metcalf et al., 1966). In this procedure, 4 ml of 0.5 N methanolic NaOH was added to approximately 150 mg of fatty material. This mixture was heated on a steam bath until the fat globules were in solution. This was followed by boiling the soaps with 5 ml of BF_3-methanol for 2 minutes. This procedure resulted in a quantitative conversion of the fatty acids to methyl esters. The methyl esters were extracted using a saturated salt solution.

Gas-Liquid Chromatography

Gas-liquid chromatography was accomplished with a F and M Scientific (Hewlett Packard) dual column, temperature programmed gas chromatograph.

Two coiled stainless steel columns (2 mm o.d. x 6 ft) were used for methyl ester separation. Both columns were packed with 10% SP 2340 on Supelcoport (100-120 mesh) as a solid phase. The operating conditions for this study were: column temperature - 170°C-200°C at 4°C/min; detector temperature - 250°C; inlet temperature - 220°C; chart speed - 0.25"/min; pressures for air, hydrogen - 25 psi each, and for nitrogen - 60 psi. All methylated samples were chromatographed using a flame ionization detector. Fatty acid methyl esters were identified by comparison of major peaks with those of chromatographic standards (Supelco methylesters combination of GL 40 and 50) passed through the same column under the identical condition. Peak areas were calculated by the triangulation method since the normal peaks approximate a triangle, the area can be approximated by multiplying the peak height times the width at half-height.

The composition of the samples were calculated by normalization of peak height for areas (without response factors), assuming that entire sample eluted.

$$\% \text{ composition} = \% X = \frac{Ax}{Ax + Ay + Az} X\ 100$$

(where Ax, Ay, Az represent individual peak areas)

RESULTS AND DISCUSSION

The Effect of Avicel, Promine-D and Na-Caseinate on Lipid Oxidation

The three different substances used in this study to compare their relative effect on lipid oxidation contrasted greatly with each other.

Oxygen uptake studies were conducted for 120 hours at 45°C in a Gilson Differential Respirometer to measure the susceptibility of soybean oil, corn oil, and lard to oxidation, when spread in different ratios on Avicel, promine-D and Na-caseinate.

Oxygen uptake was chosen to measure the rate of oxidation because it seemed more suitable and reliable compared to other measures of lipid oxidation.

Labuza et al. (1969), while studying linoleate oxidation in model systems, found that peroxide value determinations compared favorably in the first 24 hrs to the actual manometric determinations corrected for protein oxidation; after that, it became 10-20 times less than the actual manometric determinations. They suggested that peroxides reacted with the protein through a free radical mechanism and became united to the protein, indicating that the peroxide value

determination is not useful technique for the measurement of oxidation in foods containing proteins, especially if stored under accelerated conditions.

In the study of oxidative deterioration of raw freeze dried beef it was observed that only 50% of the oxygen absorption, during the initial deterioration, could be accounted for as lipid oxidation. The other 50% was apparently being used to oxidize the sulfhydryl groups of the protein. This situation changed with highly unsaturated fatty acids and more lipid peroxidation concurrent with sulfhydryl oxidation was obtained (Tappel, 1962).

The Gilson differential respirometer permitted the measurement of oxygen uptake under closely controlled conditions.

Figures 1, 2 and 3 show the amount of oxygen absorbed by soybean oil, corn oil and lard, when dispersed in the ratio of 0.5, 1.0, 5.0, and 10.0 percent on Avicel. The amount of oxygen absorbed increased with an increase in unsaturation of the lipids used. Soybean oil, the most highly unsaturated of the lipids used (Table 1), took up the most oxygen. This was followed by corn oil, which was followed in turn by lard. Also, the amount of oxygen absorbed increased gradually with time; this was more pronounced in the case of the lower lipid to Avicel ratio. The lesser the amount of dispersed lipid, the higher was the amount of oxygen absorbed per gram of each lipid used. It can also be noted that the amount of oxygen absorbed was greater for each lipid when they were spread on

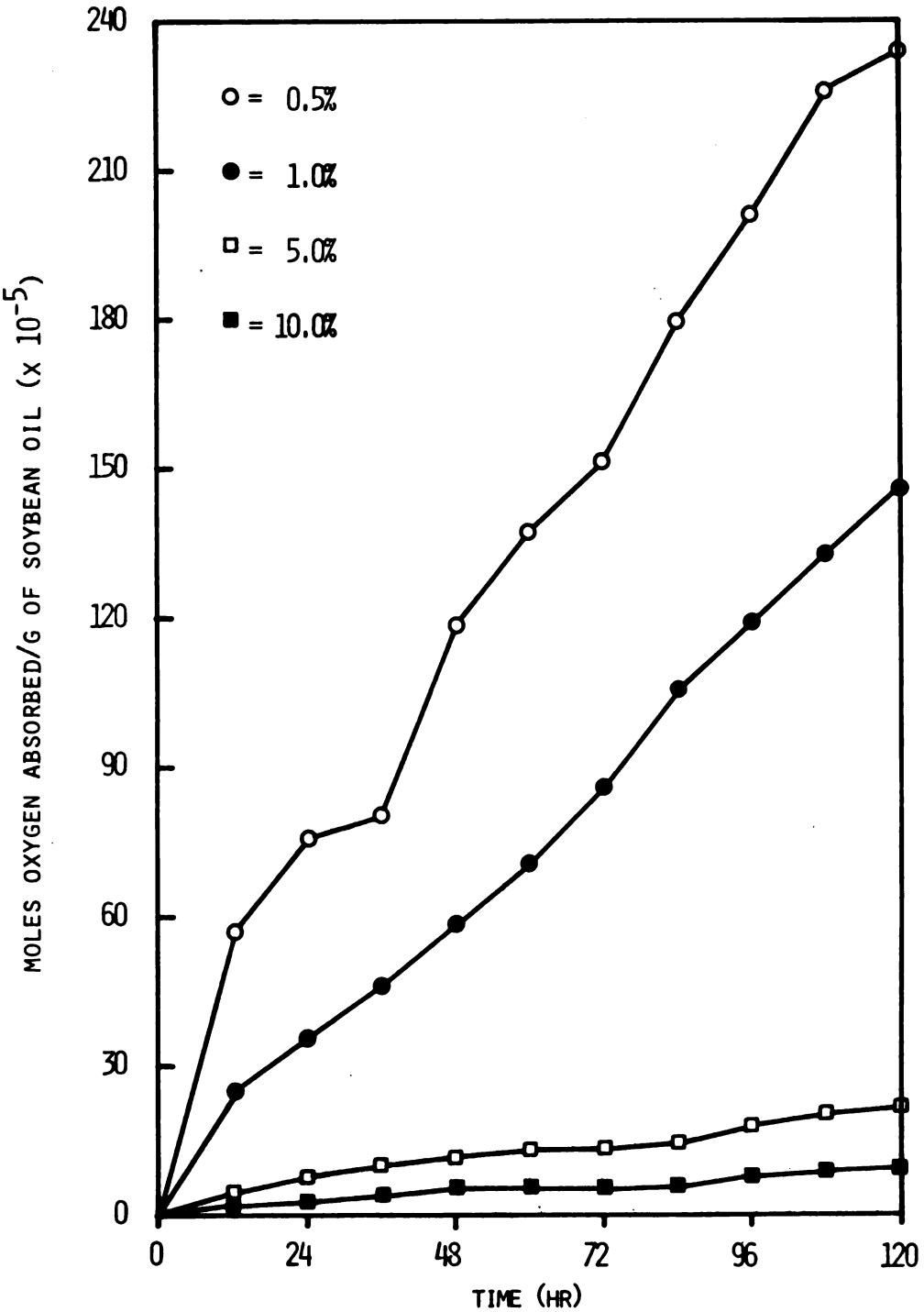


FIGURE 1. OXYGEN UPTAKE BY SOYBEAN OIL DISPERSED ON AVICEL. AT 45°C.

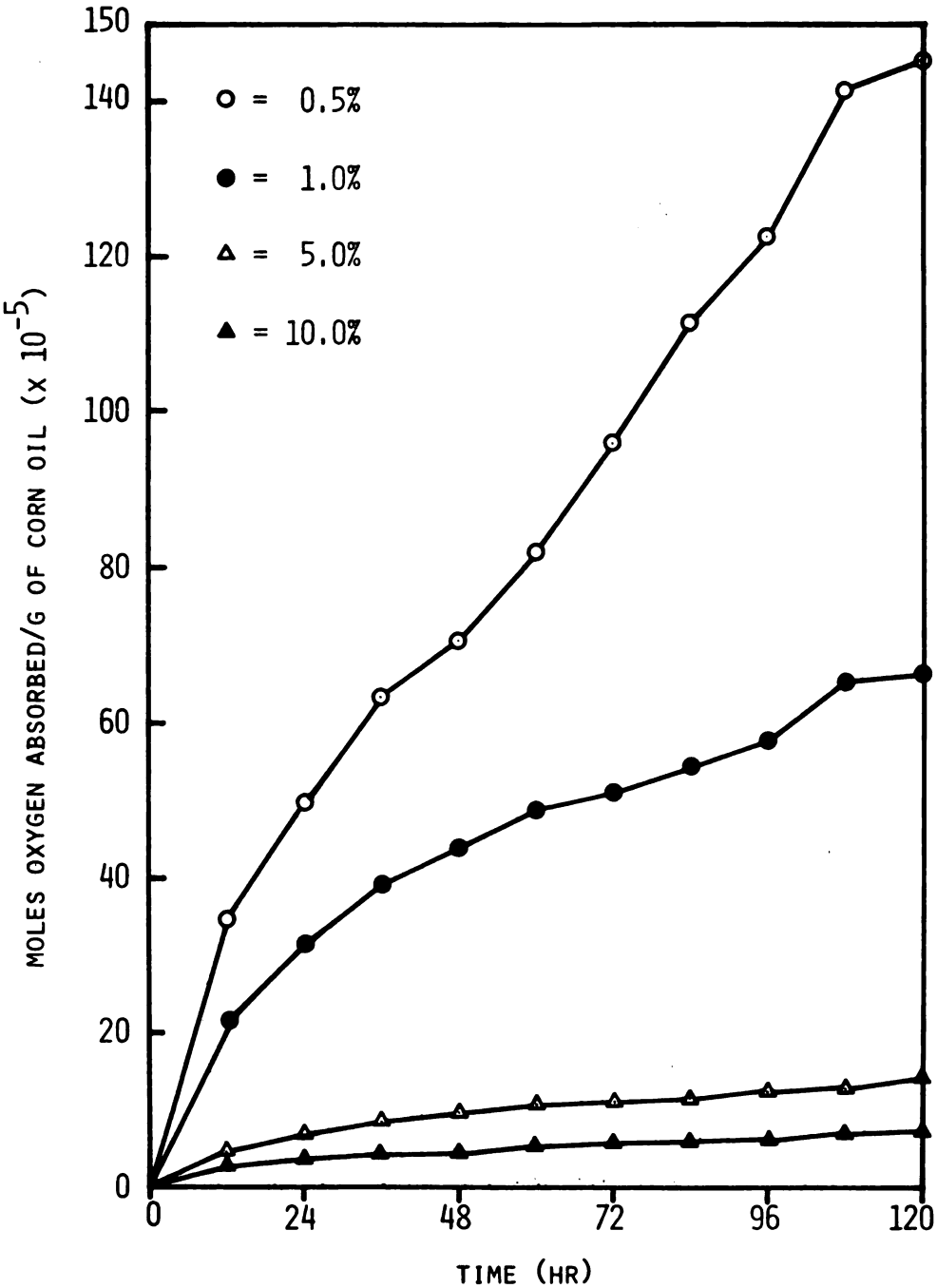


FIGURE 2. OXYGEN UPTAKE BY CORN-OIL DISPERSED ON AVICEL. AT 45^{o}C.

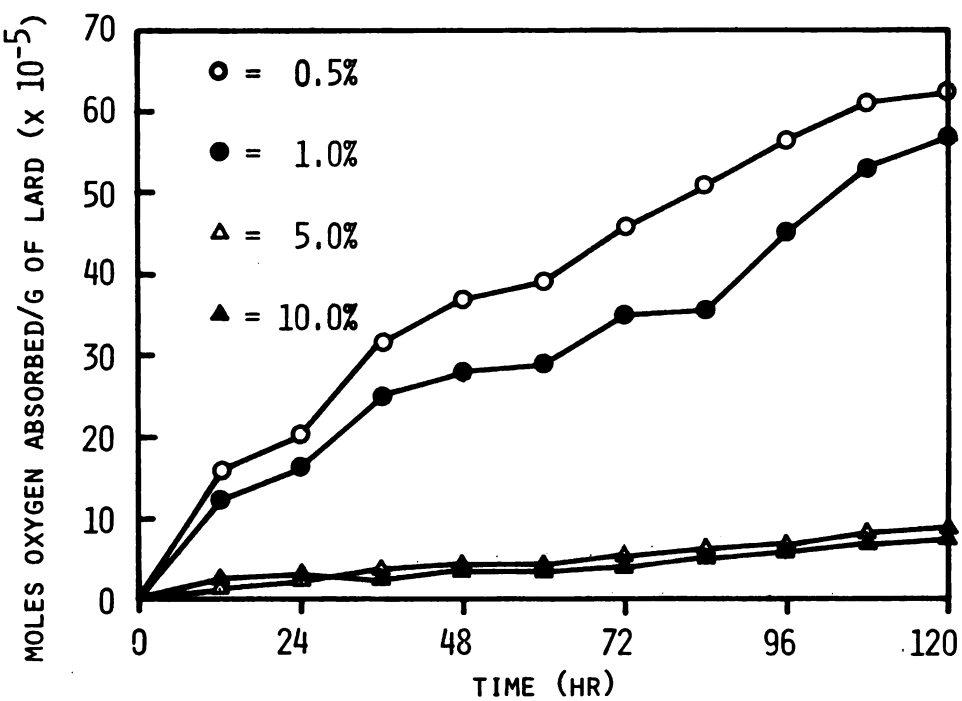


FIGURE 3. OXYGEN UPTAKE BY LARD DISPERSED ON AVICEL. AT $45^{o}C$.

Table 1. Fatty Acid Composition of Lipids Used.

Lipids	Fatty Acid[a]				
	16:0	18:1	18:2	18:3	20:4
Soybean Oil	18.2	28.0	49.1	4.8	-----
Corn Oil	20.8	31.0	48.2	----	-----
Lard	28.3	62.2	9.4	Trace[b]	Trace

[a]Fatty acid composition was determined by gas chromatography of the methyl esters. The notation used to describe fatty acids is number of carbon atom:number of double bonds.

[b]Trace denotes trace amounts.

Avicel than on promine-D (Figures 4, 5 and 6) or on Na-caseinate (Figures 7, 8 and 9).

The greater oxygen absorption in the presence of Avicel could be due to its microcrystalline structure which provided a better surface orientation to lipid molecules and facilitated efficient penetration and diffusion of gaseous oxygen into the lipid-Avicel matrix.

Avicel, a trade name for microcrystalline cellulose, is made by mild hydrolysis of native cellulose with hydrochloric acid, and is used as a non-nutritive additive (bulking agent) and as an absorbant for oil-based foods.

Upon hydrolysis with hydrochloric acid, uniform subunits consisting of particles of colloidal dimensions are formed, which chemically are still cellulose, but of lower chain length, and under the electron microscope look like bundles of very tiny needles (Battista and Smith, 1962). These cellulose molecules are set together with hydroxyl groups which create such forces as hydrogen-bonds between the adjacent molecules, effectively "zipping" them together (Ward, 1969).

Thus, the microcrystalline structure coupled with the hydrogen bonding between the adjacent molecules may account for a readily accessible surface of Avicel to give a better orientation to the lipid molecules and thus provide for greater oxygen access to the lipids.

Figures 4, 5 and 6 show the amount of oxygen absorbed by the lipid sources when on a promine-D surface. Here it

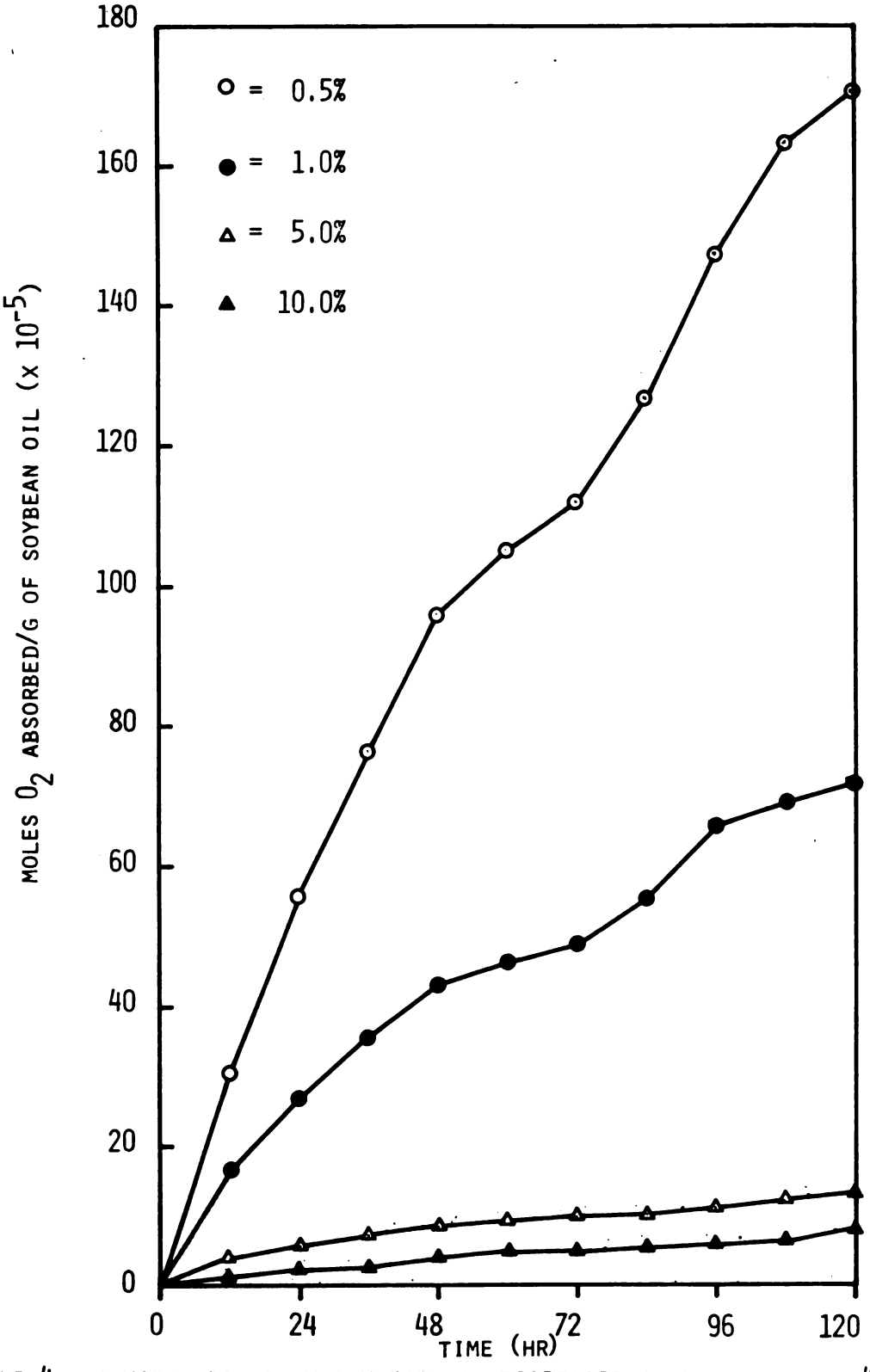


FIGURE 4. OXYGEN UPTAKE BY SOYBEAN OIL DISPERSED ON D-PROMINE AT $45^{o}C$.

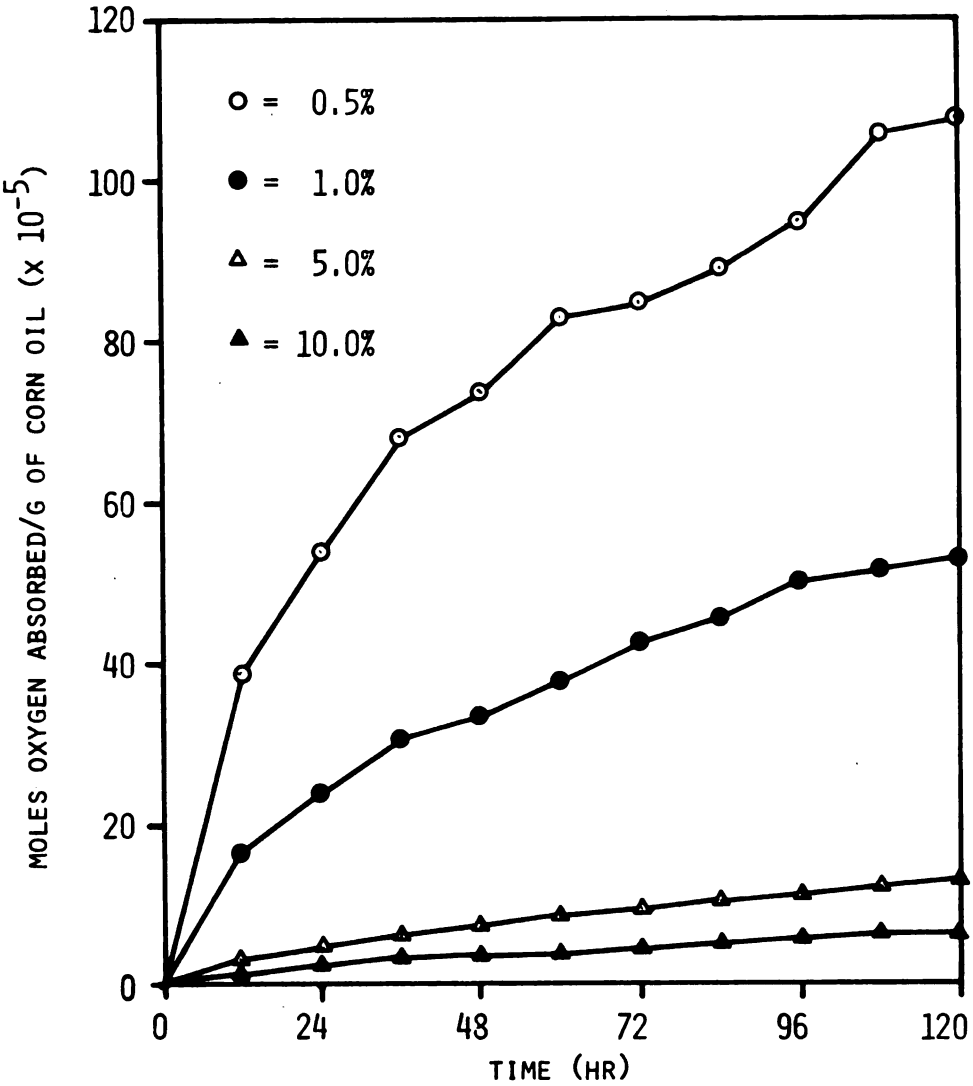


FIGURE 5. OXYGEN UPTAKE BY CORN OIL DISPERSED ON D-PROMINE AT $45^{\circ}C$.

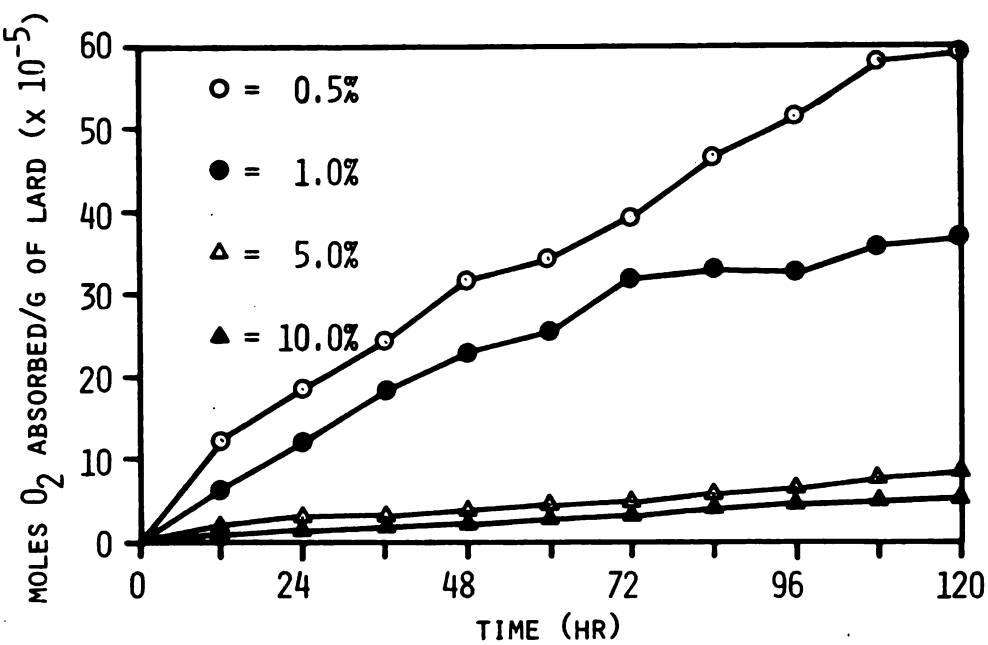


FIGURE 6. OXYGEN UPTAKE BY LARD DISPERSED ON D-PROMINE AT 45°C.

is seen that the amount of oxygen absorbed was lower than on Avicel, but a similar trend exists. That is, the oxygen absorbed increased with an increase in unsaturation of the lipids used and higher oxidation effects occurred in the systems having the lower lipids to promine-D ratios. Promine-D, a soy protein isolate, is a general purpose, functional sodium proteinate designed for use in processed food systems. Its protective effect against lipid oxidation may be due to the orientation of lipids on the promine-D surface. A free radical chain terminating mechanism could arise from the presence of protein (Karel et al., 1975). Togashi et al. (1961), working with model systems, studied the autoxidation of cotton-seed oil in films on glass, or alternatively on gelatin with a smooth dry surface. The extent of oxidation on the gelatin surface was almost one-half that on the glass. The protective effect of proteins may be a result of the convolutions in protein which reduces the surface area of lipid molecules.

It was reported by Bishov et al. (1961) that substances possessing a charge exert a protective effect against lipid oxidation. Their study showed that lysine and arginine salts of safflower fatty acids were extremely stable against lipid oxidation. Koch et al. (1971) pointed out that basic amino acids were the only class of compound to confer stabilization against autoxidation of linoleic acid. Promine-D used in this study is high in lysine, which might have been a reason for its protective effect.

Thus, in essence, the protective effect of promine-D on lipid oxidation could be because of one of several reasons. It could be as a result of convolutions in the protein surface, charge effect, or the presence of certain amino acids like lysine. It may also be due to the interaction of lipid free radical with the protein surface which take them out of the normal pathway.

Figures 7, 8 and 9 illustrate the amount of oxygen absorbed by the lipids when on a Na-caseinate surface. Here, the oxidation effect in terms of oxygen absorbed was minimal compared to that on Avicel or promine-D. The induction period was as great as 36 hours before any measurable oxygen absorption was recorded.

The casein components are colloidally distributed as polydisperse stable micellar aggregates in association with calcium and phosphate, and lesser amounts of magnesium and citrates. The total complex, of undefined structure, is generally referred to as the calcium caseinate-calcium phosphate complex (Tumerman and Webb, 1965). The organic phosphorous in casein, recognized as a critical site for calcium binding and interpolymer complexing, could play a role in lowering the degree of oxidation effect when lipids are dispersed on Na-caseinate. Watts (1950) reported the possible synergistic effect of polyphosphates on oxidative rancidity. The presence of -SH groups which are themselves labile to oxygen may also be responsible for the antioxygenic property of the casein. Aside from this, the charge group

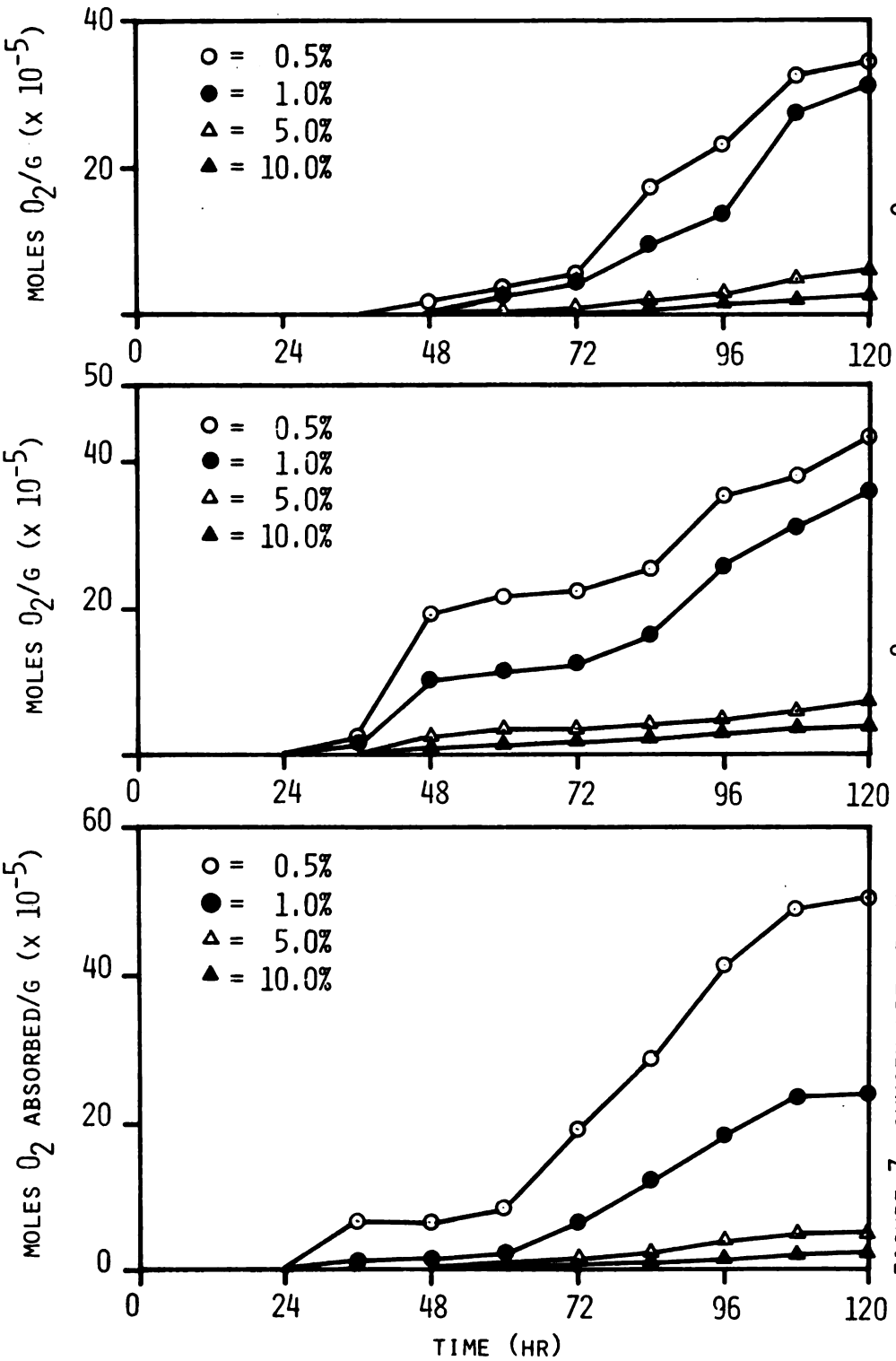


FIGURE 9. OXYGEN UPTAKE BY LARD DISPERSED ON CASEIN AT 45°C.

FIGURE 8. OXYGEN UPTAKE BY CORN OIL DISPERSED ON CASEIN AT 45°C.

FIGURE 7. OXYGEN UPTAKE BY SOYBEAN OIL DISPERSED ON CASEIN AT 45°C.

stabilization of casein particle may have provided the protective effect to lipid molecules dispersed on the casein surface. Casein exists in more or less uncoiled, random structures rather than in tightly coiled specific structures (Jenness and Patton, 1959). This structural difference of casein may also have provided the protective effect by reducing the surface area of the exposed lipid molecules.

Thus, the presence of oxygen labile -SH groups, charge stabilization of casein particles and its uncoiled random structure may be responsible for the stability of lipids exposed on the casein surface. Besides, the orientation of lipid molecules in casein film may also have accounted for the protective effect.

It was noted that the lower ratios of lipid to Avicel or to proteins gave a greater oxidation effect. This may be because of greater dispersion of the available lipid molecule or greater surface to volume ratio in the case of lower ratios of lipids.

It is also important to mention that in the case of the higher ratios of lipids (5.0, 10.0%) to Avicel or to proteins there was only an insignificant change in the oxidation throughout the oxygen uptake studies. It may be that the thickness of the fat layer protected against lipid oxidation by offering resistance to flow of oxygen across and/or through the reaction-matrix. Higher ratios of lipids may act as a viscous drag on neighboring lipid molecules reducing the flow of oxygen through them.

Diene Conjugation Studies

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. This region is between about 200-400 nm. The absorption of isolated double bonds, nonconjugated and saturated materials within the spectral range of 200-400 nm is very weak, while the conjugated double bonds give rise to intense, well-defined absorption maxima. This is the basis for the ultraviolet absorption method for determining oxidative effects. Swern (1961) reported that when oils containing linoleate or more highly unsaturated systems are autoxidized, the diene conjugation as measured by absorption of ultraviolet radiation at 233 nm increased parallel with oxygen uptake and peroxide formation in the early stages of oxidation.

Angelo et al. (1975) compared the peroxide value determinations and spectrophotometric assay of conjugated diene hydroperoxides while studying shelf life stability of peanut butter during short- and long-term storage. They found good correlation between the two methods over 4 and 12 week storage periods. Conjugated diene hydroperoxide method is simpler than the peroxide value determinations. It requires smaller samples and is quicker. Besides, it does not require additional reagent and is not dependent upon a chemical reaction or color development.

In this study, absorbance due to diene conjugation was

determined along with the oxygen uptake study to more fully characterize the oxidation of soybean oil, corn oil and lard when they are dispersed on Avicel and protein surfaces.

Table 2 gives the absorbance due to diene conjugation when soybean oil, corn oil, and lard are dispersed in the ratios of 0.5, 1.0, 5.0 and 10.0 percent on Avicel. As can be seen, the absorbance due to diene conjugation was higher in the soybean oil dispersed on Avicel, followed by lesser absorbance in corn oil and still less in lard.

The lower ratios of lipids to Avicel exhibited higher absorbances due to diene conjugation indicating greater oxidative effects. Also, the absorbance due to diene conjugation increased with time indicating the progress of oxidation with the time.

In the systems with higher lipid to Avicel ratios, the changes in the absorbance due to diene conjugation were not very significant suggesting little oxidation. It did, however, tend to follow the pattern of oxygen uptake. The absorbance due to diene conjugation of 5.0 and 10.0 percent corn oil on Avicel are almost equal to that of the same proportions of soybean oil on Avicel. When lard was dispersed on Avicel, the absorbances due to diene conjugation were quite low thus indicating a lower degree of oxidation. Here again, the lower ratios of lard to Avicel gave higher absorbances confirming that lower ratios of dispersion of lipid accounted for greater oxidative effect.

Table 3 shows the absorbance due to diene conjugation in

Table 2. Absorbance Due to Diene Conjugation in Soybean Oil, Corn Oil, and Lard on Avicel at 45°C.

Sample System	% Lipids	Diene Conjugation (233 nm)[a]		
		48 Hr	96 Hr	120 Hr
Avicel + Soybean Oil	0.5	.346	.456	.649
	1.0	.220	.358	.401
	5.0	.063	.086	.134
	10.0	.028	.073	.097
Avicel + Corn Oil	0.5	.242	.325	.336
	1.0	.138	.258	.294
	5.0	.052	.088	.123
	10.0	.041	.092	.118
Avicel + Lard	0.5	.154	.197	.203
	1.0	.084	.168	.194
	5.0	.056	.060	.084
	10.0	.051	.047	.061

[a]Average of two samples.

soybean oil, corn oil and lard when on promine-D at 45°C. The same trend follows as in the use of Avicel. That is, diene conjugation was higher when soybean oil was used followed by corn oil, which in turn was followed by lard. Also, the lower lipid ratios accounted for higher absorbances due to diene conjugation as they did for higher oxygen uptake (Figures 4, 5 and 6). The higher lipid to promine-D ratios showed insignificant increases in absorbances due to diene conjugation suggesting little oxidation effect.

The increases in the diene conjugation for each lipid when on a promine-D surface is in agreement with oxygen uptake measurements. Also, the diene conjugation of each lipid was less when dispersed on promine-D than when each lipid was on Avicel, confirming that the oxidation effect was slower on promine-D surface in comparison to Avicel.

Table 4 gives the absorbance due to diene conjugation in soybean oil, corn oil, and lard when on Na-caseinate surface. Here again, the very same trend followed as with Avicel and promine-D. That is, the lower ratios of lipids to Na-caseinate exhibited higher absorbances due to diene conjugation.

The absorbances due to diene conjugation in corn oil are almost equal to that of the soybean oil. The peroxide value of corn oil determined at the end of the study was higher than that of soybean oil (Table 5), which might be responsible for equal changes in absorbance due to diene conjugation in soybean oil and corn oil. Lard showed

Table 3. Absorbance Due to Diene Conjugation in Soybean Oil, Corn Oil, and Lard on Promine-D at 45°C.

Sample System	% Lipids	Diene Conjugation (233 nm)[a]		
		48 Hr	96 Hr	120 Hr
D-Promine + Soybean Oil	0.5	.300	.400	.425
	1.0	.171	.259	.346
	5.0	.055	.075	.080
	10.0	.040	.059	.060
D-Promine + Corn Oil	0.5	.106	.234	.335
	1.0	.085	.198	.205
	5.0	.007	.008	.012
	10.0	.002	.049	.007
D-Promine + Lard	0.5	.004	.066	.082
	1.0	.004	.009	.015
	5.0	.005	.008	.009
	10.0	.004	.009	.010

[a]Average of two samples.

Table 4. Absorbance Due to Diene Conjugation in Soybean Oil, Corn Oil, and Lard on Na-Caseinate at 45°C.

Sample System	% Lipids	Diene Conjugation (233 nm)[a]		
		48 Hr	96 Hr	120 Hr
Casein + Soybean Oil	0.5	.046	.094	.126
	1.0	.040	.092	.098
	5.0	.006	.008	.009
	10.0	.003	.005	.009
Casein + Corn Oil	0.5	.050	.096	.130
	1.0	.052	.010	.124
	5.0	.008	.010	.018
	10.0	.004	.009	.014
Casein + Lard	0.5	.020	.050	.076
	1.0	.013	.018	.022
	5.0	.005	.007	.015
	10.0	.003	.003	.006

[a]Average of two samples.

Table 5. Product Characteristics.[a]

(a) % Moisture	
Promine-D	5.3
Casein	1.6
Avicel	5.2
(b) Peroxide Value (t meq/1000 g oil)	
Soybean oil	6.096
Corn oil	10.150
Lard	16.256

[a]Determination made at the end of the study.

minimum increases in absorbance due to diene conjugation.

The oxidation effect in terms of oxygen uptake was found to be minimum when lipids were dispersed in Na-caseinate surface (Figures 5, 6 and 7), and so did increases in absorption due to diene conjugation, indicating the possible protective effect to lipids dispersed on Na-caseinate.

Corliss (1968) suggested that although ultraviolet measurements of conjugated dienes do not indicate the precise stage of lipid oxidation, these can be used on combination with quantitative determinations to aid in understanding the autoxidation of unsaturated lipids. And this study very well proves the importance of such a combined study of oxygen uptake and spectrophotometric study of conjugated diene hydroperoxide.

Protein Solubility

An attempt was made in this study to determine losses in protein solubility following oxidation.

Light (1974) reported that the solubility of proteins varies with ionic strength, pH, temperature, and polarity of the solvent.

Here, however, when we refer to protein solubility, our concern is what happens to the chemical property of the protein after it interacts with oxidized lipid. To what extent does it become modified? Oxidized lipids react with proteins to form lipo-protein complexes in which lipids

are bound to the proteins in part by physical forces, in part by covalency.

Pokorny and Janicek (1975) reported that secondary reactions of lipo-protein complexes lead to brown color compounds of limited solubility. Tappel and Roubal (1966 a,b) suggested that protein insolubilization can result from protein-protein interaction initiated by lipid-free radicals.

In this study, the solubility of proteins extracted from the lipid-protein complex was measured by the Biuret method at 540 nm. The Biuret reaction requires at least a sequence of two or more peptide bonds to develop the characteristic pink-violet or purple colored complex in the presence of copper in alkaline solution.

Because of little loss of the solubility of promine-D and Na-caseinate as measured by the characteristic color reaction, the values were not quantitated in terms of percent loss in solubility. The absorbance values do explain that at the levels of oxidation achieved, there was no appreciable impairment of the solubility of promine-D and Na-caseinate.

Table 6 shows changes in the solubility of promine-D at the end of 48, 96 and 120 hours of oxygen uptake studies as measured by the Biuret method at 540 nm. As can be seen, the absorbance at 540 nm of promine-D extracted from the lipid-protein complex was slightly lower than that from the untreated promine-D, suggesting some change in the solubility of the original promine-D. But there is little correlation

Table 6. Effect on Promine-D Solubility during Lipid Oxidation.

Sample System	% Lipids	Absorbance (540 nm)[a,b,c]		
		48 Hr	96 Hr	120 Hr
D-Promine + Soybean Oil	0.5	.385	.375	.410
	1.0	.395	.380	.375
	5.0	.395	.395	.385
	10.0	.370	.400	.420
D-Promine + Corn Oil	0.5	.375	.380	.410
	1.0	.395	.360	.410
	5.0	.380	.395	.420
	10.0	.385	.395	.430
D-Promine + Lard	0.5	.345	.350	.335
	1.0	.350	.355	.330
	5.0	.358	.365	.340
	10.0	.394	.394	.355

[a]Average of two samples.

[b]Untreated D-promine absorbance at 540 nm = 0.42.

[c]Biuret method.

Table 7. Effect on Casein Solubility during Lipid Oxidation.

Sample System	% Lipids	Absorbance (540 nm)[a,b,c]		
		48 Hr	96 Hr	120 Hr
Casein + Soybean Oil	0.5	.295	.280	.290
	1.0	.350	.410	.430
	5.0	.355	.275	.400
	10.0	.360	.355	.365
Casein + Corn Oil	0.5	.360	.346	.365
	1.0	.395	.390	.390
	5.0	.430	.430	.440
	10.0	.395	.390	.400
Casein + Lard	0.5	.394	.375	.380
	1.0	.400	.398	.400
	5.0	.400	.400	.410
	10.0	.425	.410	.420

[a]Average of two samples.

[b]Untreated (control) casein - absorbance at 540 nm = .45.

[c]Biuret method.

between loss of protein solubility and lipid oxidation at the levels of oxidation achieved.

Table 7 shows the changes in the solubility of Na-caseinate following oxygen uptake studies. The data do not indicate that at the levels of oxidation achieved there is a loss of the solubility of casein. The absorbance values do not follow any pattern, in any combinations of lipids to Na-caseinate. However, the absorbance of untreated casein is slightly higher than the absorbance values of Na-caseinate extracted from the lipid-casein matrix, indicating some change in the solubility of the original casein.

Thus, at the levels of oxidation achieved, there is little change in the solubility of promine-D and Na-caseinate. Longer oxidation periods and more sensitive tests may give more conclusive evidence about the effect of oxidized lipids on solubility of the proteins used in this study.

SUMMARY

This investigation was undertaken to develop more information about protein lipid-interactions and their possible role in lipid oxidation in foods.

The results of this study, as demonstrated by oxygen uptake, diene conjugation, and protein solubility studies, indicated the possible protective effect protein surfaces had as compared with microcrystalline cellulose on lipid oxidation. The findings of this study were that:

a. oxidation increased with an increase in unsaturation of lipids;
b. there was a good correlation between oxygen uptake and diene value;
c. oxidation occurred more readily at lower ratios of lipid to proteins;
d. lipid oxidation occurred more readily on an Avicel surface than on protein surfaces;
e. oxidation of lipids progressed less rapidly on casein than on promine-D; and
f. little correlation was obtained between loss of protein solubility and lipid oxidation at the levels of oxidation achieved.

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