ON THE SEPARATION OF NUTRITIONALLY DELETERIOUS AND INNOCUOUS FRACTIONS FROM THE ESTERS OF THERMALLY POLYMERIZED LINSEED OIL

A Thesis

by

Arthur Frederick Wells

Submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

McGill University

August 1952

ACKNOWLEDGMENTS

Thanks are extended to Prof. R.H. Common, who has directed these researches. His invaluable guidance and unfailing interest have provided encouragement and inspiration during the course of this work.

These researches form part of a larger research project conducted jointly by the Departments of Chemistry and Nutrition at Macdonald College. Members of the Department of Nutrition have conducted the feeding experiments and have assisted in the interpretation of results. Particular thanks are due to Miss Marion Berryhill, and to Miss Donna Crawford, to whom the burden of this work fell.

Thanks are due to the National Research Council for financial assistance. Besides direct research funds, a generous stipend was received by the author.

TABLE OF CONTENTS

Page

GENERAL INTRODUCTION

The shortage of edible fats and oils during the Second World War prompted research in this laboratory on the utilization of linseed oil for edible purposes. Linseed oil is unsuitable for the production of hydrogenated fats because of its marked tendency toward flavor reversion. This tendency appears to be due to its high content of linolenic acid.

A method was devised to remove a substantial part of the linolenic acid from the oil with the object of overcoming this flavor defect of hydrogenated linseed oil and producing a fat of reasonable flavor stability. This method consisted in thermally polymerizing the oil under selective conditions and solvent extracting the unpolymerized glycerides. The extract was then hydrogenated to give an edible shortening, while the residue was suitable for use in the paint industry.

Shortening produced by this method was bland in taste and of good flavor stability and baking quality. Unfortunately, when it was included in the diet of growing rats it caused a marked decrease in growth rate.

It has been shown that corn, peanut, rapeseed and soybean oils are also reduced in nutritive value by thermal polymerization, but results comparable with those obtained from linseed oil are attained only after a considerably longer heating period.

A continuing research program has been carried on in the Departments of Chemistry and Nutrition, Macdonald College, to determine the nature of the material responsible for the reduced nutritive value of heat polymerized oils. The present work is a continuation of that research.

REVIEW OF THE LITERATURE

Oxidation and Flavor Reversion

The autoxidation of unsaturated fatty acids and their glycerides has been studied from two opposite viewpoints. To the protective coating chemist, oxidation is an important and necessary reaction in the drying of oil films. To the chemist engaged in the manufacture or study of edible oil products, oxidation is an undesirable reaction to be reduced or abolished as far as possible. The course of the reaction and the products formed by oxidation are of interest to both; the protective coating chemist is interested in speeding up the reactions and, if possible, influencing them to obtain more stable products, while the edible oil chemist must reduce the reaction to the lowest possible rates.

Oxidation of unsaturated oils may be broken down into four stages: initiation, peroxide formation, peroxide decomposition, and polymerization. When an oil is exposed to air, a certain time elapses before there is an appreciable absorption of oxygen or formation of peroxides. The existence of such an induction period has been ascribed to the presence of natural inhibitiors, or antioxidants, in the oil. The induction period is followed by rapid absorption of oxygen and a rapid increase in peroxide value. This period of peroxide formation and accumulation is followed by a

period of peroxide decomposition, where peroxide value value falls although absorption of oxygen continues. The onset of the final phase, polymerization, is denoted by a rapid increase in viscosity.

It is now generally accepted that the first stable product formed during oxidation is a hydroperoxide. Farmer and Sutton (1943) oxidized methyl oleate at 35°C. under ultra-violet light. They isolated a monohydroperoxide oleate from the oxidized material by molecular distillation, and also by adsorption chromatography. The hydroperoxide contained one additional mole of oxygen and retained the double bond. They believed the hydroperoxide was attached to the carbon alpha to the double bond. Swift, Dollear and O'Connor (1946) also isolated the hydroperoxide by low temperature fractional crystallization from acetone.

The separation of an α-methylenic hydrogen atom requires about 80 kcal. of energy. It is difficult therefore to understand how a hydroperoxide could be formed. Farmer (1946) has suggested that the initial reaction is the addition of oxygen to one side of the double bond to form a bi-radical. The bi-radical then acts as a free radical which initiates a chain reaction. Thus a small amount of oxygen added across the double bond can initiate the formation of a large amount of hydroperoxide.

Khan, Brown and Deatherage (1951) studied the oxidation of methyl oleate, methyl stearolate and methyl 9, 10-dideu-

tero-oleate. They observed a more rapid oxidation of the acetylenic compound than of the olefinic compound. This is in contrast to the usual addition of ozone or halogens to a triple bond and suggested that the mechanism was not an addition to the double bond. On the other hand, the 9, 10-dideutero-oleate has a much longer induction period than the simple oleate, suggesting that olefinic hydrogen atoms and the double bond are involved in the initiation of oxidation. The authors believe that the initial attack is at the double bond and progresses to the -methylene group. Water formation occurs early in the process. At least some of the hydrogen of the water comes from the olefinic hydrogens. Most of the non-aqueous volatile cleavage products are peroxidic and are capable of oxidizing methyl oleate to the high melting form of dihydroxy stearic acid, whereas the commercially available peroxides tested gave only the low melting form.

Max and Deatherage (1951) studied the autoxidation of 8,8,11,11-tetradeutero cis-9-octadecene. They found that the tetradeutero compound oxidized at a much slower rate than octadecene itself. These results indicate that the sustaining reaction in autoxidation is the oxidative attack at the α-methylenic position. Only a small amount of deuterium was found in the water formed during oxidation; thus showing that the water does not come from the direct decomposition of the -hydroperoxide.

Zilch and his co-workers (Zilch and Dutton 1951, Fugge, Zilch, Cannon and Dutton, 1951) have attacked the problem of oxidation using countercurrent distribution methods to separate the products. They isolated unoxidized methyl oleate, a compounent consisting mainly of methyl oleate hydroperoxide, and a component consisting of peroxide scission products and other oxidized substances.

Infra-red absorption studies of oxidizing methyl oleate by Knight, Eddy and Swern (1951) indicate that cis-trans isomerization occurs concurrently with oxidation. Knight, Coleman and Swern (1951) analyzed oxidizing methyl oleate for peroxide oxygen, carbonyl oxygen, hydroxyl oxygen, oxirane oxygen, ester oxygen and carboxyl oxygen. Except when oxidation was carried out at temperature above 70°C., the sum of these values agreed closely with total oxygen by combustion. The discrepancy noted at higher temperatures was ascribed to formation of ether linkages.

The changes which occur in linoleic, linolenic and higher fatty acids during oxidation are even more complex than those occurring in oleic acid. Farmer, Koch and Sutton (1943) noted diene conjugation in oxidized linoleate and both diene and triene conjugation in oxidized linolenate. Oxidation of conjugated linoleic acid reduces the absorption at 2300 Å. Oxidation of conjugated triene results in reduced absorption at 2600-2800 Å and increased absorption at 2300 Å. (Holman, Lundberg & Burr, 1945)

The decomposition and polymerization reactions of peroxides are not well understood. The decomposition reactions of peroxides may involve several processes including dehydration, reduction, rearrangement and chain rupture. (Powers, 1949). The polymerization of oils induced by oxidation is now recognized as the process responsible for drying of oil films. However, present knowledge of the linkage between fatty chains is limited. Farmer, Bloomfield, Sundralingam & Sutton (1942) have proposed the view that the attack of a peroxide on a double bond results in an ether linked polymer. Powers, Overholt and Elm (1941) suggested that oxygen activates the double bonds resulting in carbon to carbon, vinyl-type polymerization. Bolland and Gee (1946) proposed that polymerization occurs by formation of peroxide linkages. Undoubtedly several mechanisms of oxidative polymerization may be acting at the same time.

The rate of oxidation of an oil may be increased by the addition of oxidation catalysts, or driers. The salts of manganese, cobalt and lead are used extensively for this purpose in the paint industry. Antioxidants are used in the edible oil industry to extend the induction period and thus delay the onset of rancidity. Antioxidants interfere with free radical chain propagation by reducing peroxides as soon as they are formed; thus a small amount of antioxidant will protect an oil for some time but is ineffective if added after oxidation has begun.

Some oils acquire objectionable flavors after undergoing oxidation to a degree less than that needed to produce rancidity. This flavor defect is called flavor reversion, although the taste of the reverted oil seldom resembles that of the original oil.

Flavor reversion is most pronounced in oils containing linolenic acid. Armstrong and McFarlane (1944) added ethyl linolenate to a non-reverting shortening and observed reversion which varied with the amount of linolenate added. They also noted that hydrogenated linseed oil reverted more rapidly than unhydrogenated oil. Lemon (1944) showed, by ultra-violet absorption studies, that hydrogenation of linseed oil produced dienoic acids which could not be conjugated by treatment with alkali. He assumed that the dienoic acid formed was a 9 : 15 isolinoleic acid, produced by the saturation of the middle double bond of linolenic acid. Reduction in the amount of this isolinoleic acid by further hydrogenation, decreased the tendency of the oil toward flavor reversion.

Flavor reversion in fish oils and in soybean oil may be due to non-glyceride components. Davies and Gill (1936) have shown that a nitrogenous non-glyceride component which is bound to the highly unsaturated acids is responsible for fishy flavors and odors of reverted fish oils. Mattil (1947) added the unsaponifiable material from hydrogenated soybean oil to cottonseed and peanut oils. The typical flavor and odor of reverted soybean oil soon developed. Golumbic, Schepartz and Daubert (1946)

prepared a synthetic soybean oil from purified acids. The flavors produced by heat and light treatments of this oil differed distinctly from those of soybean oil treated in the same manner. They were, however, inferior to cottonseed oil which had received the same treatment. Apparently both the linolenic acid and the non-glyceride constituents are involved in the flavor reversion of soybean oil.

Privett, Pringle and McFarlane (1945) developed a method to remove a substantial part of the linolenic acid from linseed oil. They believed that removal of linolenic acid should result in improved flavor characteristics of the residual oil. Their method consisted of thermal polymerization of the oil under conditions which were selective for the polymerization of linolenic acid. The resulting partially polymerized oil was then segregated with acetone. The acetone soluble fraction yielded an oil which could be hydrogenated to give a shortening with good flavor stability. The acetone insoluble fraction could be used as a paint and varnish oil.

Thermal Polymerization

Thermally polymerized unsaturated oils have been used for many years in the preparation of paints and varnishes. However, it is only within the last 25 years that there has been any concentrated effort to determine the structure of such oils and the mechanism of their formation. Within that

period much work has been done and valuable information has been gained, but the problem is not completely solved.

The observation by Scheiber in 1929, that the refrac- index of oils rose rapidly during the early stages of heating, led him to believe that the double bonds of un- saturated fatty acids are isomerized to conjugated positions before polymerizing. Kappelmeier (1933) suggested that a conjugated fatty acid could react with another unsaturated acid by a Diels-Alder addition to give a polymer. Scheiber (1936) then suggested a mechanism, whereby thermal polymer- ization of unconjugated oils consisted of two concurrent reactions: (1) Conjugation of the double bonds in the fatty acid residues, and (2) a 1 : 4, Diels-Alder addition of the conjugated structure with the double bonds of another fatty acid residue. A large amount of evidence has been published in support of this theory.

Bradley and Richardson (1940) found, by ultra-violet absorption studies, that the amount of conjugated diene increases at the beginning of the heating period and finally falls off again as it approaches the gel stage. Ahmad and Farmer (1940) polymerized 1, 4-pentadiene, which is a simpler compound than linoleic acid, but has the same arrangement of double bonds. Analysis of the dimers confirmed that a cyclo- hexene ring had been formed, and dehydrogenation and oxidation produced -phthalic acid, indicating that the mechanism must have been Diels-Alder addition.

The facts that conjugated oils polymerize much more rapidly than non-conjugated oils (Bradley and Johnston, 1940) and that the products of polymerization are the same for 9 : 12 linoleate as for 9 : 11 linoleate (Brod, France and Evans, 1939) lend further support to the belief that conjugation is the first step in polymerization. Radlove and Falkenberg (1948) have shown that previously conjugated linseed and soybean oils, bodied to the same viscosity as the non-conjugated oils, possess superior drying properties. These observations lend support, rather than opposition, to the Scheiber hypothesis, for complete dimerization of fatty acid radicals is never attained, even at the gel stage. However, Pashke, Jackson and Wheeler (1952) found differences in the refractive indices of dimers obtained from the polymerization of conjugated and unconjugated linoleate isomers, although they could detect no difference in the dimers by infra-red spectroscopy.

Polymerization of the simple alkyl esters of the unsaturated fatty acids has added to the knowledge of the polymerization reaction. Extensive studies by Bradley and Johnston (1940) on the thermal polymerization of methyl esters of the mixed fatty acids of several oils, led them to conclude that dienoic acids polymerize by Diels-Alder addition to form dimers which are monocyclic, while trienoic acids form dimers which are bicyclic, due to the closing of a second ring after the original Diels-Alder dimerization.

In spite of the evidence to support the Scheiber-Kappelmeier theory, it has not been universally accepted. Bernstein (1946, 1949) does not believe that conjugation is necessary for polymerization. He believes polymerization is a direct reaction between unsaturated bonds, influenced only by stearic considerations. Barker, Crawford and Hilditch(1951)[a] view the polymerization as occurring between the reactive methylene group of one fatty acid residue with a conjugated diene system by a modified 1,4-addition. Their view was based on the fact that in polymerized sunflower seed oil the iodine value of the "residual unsaturated acids", i.e. the unsaturated acids remaining after linoleic and conjugated diene acids had been accounted for, was greater than should be expected if Diels-Alder addition had occurred. They found that the mixed fatty acids of polymerized oils became increasingly soluble in acetone at -55°C. or below, with increased polymerization time. This, they felt, further substantiated their view. The isolation of monomeric isooleic esters from polymerized methyl linoleate(Paschke and Wheeler, 1949) may also lend some support to the theory that labile hydrogen atoms are involved in polymerization.

Paschke, Jackson and Wheeler (1952) have noted that thermal treatment of methyl linoleate results in cis-trans isomerization of the double bonds and that certain of these isomers are not converted to the conjugated form by the

usual 25 or 30 minute treatment with alkali, but require up to six hours for this change to reach completion. This might account for the high iodine values of the "residual unsaturated acids" noted by Barker, Crawford and Hilditch.

Adams and Powers (1944) found that iodine values decreased rapidly in the early stages of thermal polymerization with only a slight rise in the viscosity and mean molecular weight. This was ascribed to dimerization of fatty acid residues in the same glyceride, followed by transesterification to form dimeric and higher glycerides. Such dimerization of fatty acid residues in the same glyceride is commonly called "intrapolymerization". The term is not a satisfactory one, for the process does not entail molecular growth.

Several other workers have supported the view that the mechanism of polymerization does involve the formation of intramolecularly dimerized fatty acids in the early stages. Bradley and Pfann (1940) studies a sample of a very thoroughly heat-gelled drying oil. Analysis of the methyl esters derived from the solid phase of the gel, gave 75% of dimer and 25% of monomer. This is a higher proportion of dimers than could be attained if simple intermolecular dimerization of fatty acid chains had occurred.

Barker, Crawford and Hilditch (1951)[(a)] calculated the mean molecular weights of a series of heat bodied oils from the mean molecular weights of the corresponding esters. In

all cases the calculated mean molecular weight was higher than the corresponding observed mean molecular weight of the glyceride. They attributed the difference to the formation of intramolecular linkages. They also have shown that interesterification can take place at 300°C., without a catalyst. (1951)[b]. However, Joubert and Sutton (1952) polymerized pilchard oil and separated the unpolymerized glycerides by molecular distillation. Vacuum distillation of the methyl esters of the monomeric glycerides yielded only about 1.5% of dimeric esters. In this case the oil was brought to 273°C., held for 15 minutes and allowed to cool; the total time for which the temperature was in excess of 150°C. was 5-1/2 hours. Total dimeric fatty acid was about 11% to 12% of the whole oil.

Besides dimerization of fatty acid residues, several authors have noted the production of side-reaction products. These include hydrocarbons, aldehydes, free fatty acids, and low molecular esters. Pashke and Wheeler (1949) hydrogenated the monomers obtained from an almost completely polymerized sample of methyl linoleate. They obtained from this fraction 60% yield of a monomeric monoolefin, which would not hydrogenate to stearic acid. This amounts to about 12% of the whole ester.

The discovery that urea forms solid complexes with linear aliphatic compounds, but not with branched chain or cyclic compounds, suggests that urea complex formation

formation may provide a means of isolating these cyclic monomeric derivatives.

The Urea Addition Compounds of Linear Aliphatic Substances

An addition compound may be defined as a compound formed by the direct combination of two or more simpler substances. The existence of many addition compounds can be explained by union of the components by chemical bonds of various types. However, even in the absence of any apparent capacity for chemical combination, it is possible to obtain new crystalline substances by the union of certain pairs of such apparently unreactive compounds.

Powell (1948) has investigated a number of such addition compounds and has suggested they be named "clathrate" compounds. His work with quinol and the addition compounds of quinol with CO_2, HBr etc. led him to believe that the addition compound is formed by enclosing the smaller molecule inside a cavity formed by interlocking quinol molecules. The addition compounds of desoxycholic and apocholic acids with fatty acids, hydrocarbons etc. have been studied also and have been found to differ from the quinol compounds only by the fact that the size of the cavity may be varied to accomodate molecules of different sizes. The molecular ratios in which the components unite are, in all cases, small whole numbers.

The addition compounds of urea with linear aliphatic compounds resemble, to some extent, the clathrate compounds described by Powell. They differ from the compounds already discussed in that the molecular ratio of the components in a given compound is non-integral and that a definite relationship exists between the magnitude of the ratio and the chain length of the organic component.

The term "urea adduct" has become the generally accepted one to describe such an addition compound of urea. The existence of such compounds was discovered by M. F. Bengen in Germany about 1940. They were later studied intensively by W. Schlenk, whose very detailed account of these compounds was published in 1949. Zimmerschied, Dinerstein, Weitkamp and Marschner (1950) have substantiated and extended Schlenk's work and Redlich, Gable, Dunlop and Millar (1950) have also contributed to the physical chemistry of urea adduct formation.

The preparation of urea adducts is very simple. In the case of the lower aliphatic hydrocarbons it is sufficient to add the organic component to a saturated methanolic solution of urea. On shaking the adduct appears as a while crystalline precipitate.

In some cases finely powdered urea may be dissolved in the organic component by heating. On slowly cooling the solution the adduct may be crystallized as farily large

colorless needles. This method, however, is restricted to those organic components in which urea is soluble.

A third method is of limited applicability. In this case finely powdered urea is added directly to the cool organic component in a solvent such as benzene or ethylene chloride, i.e. a solvent which does not itself form a urea adduct. On shaking for a time the formation of the adduct takes place smoothly.

The yield of adduct increases with increasing concentration of urea and of the organic component in the solution. Under similar conditions of temperature and concentration, the higher homologues of a series give the greater yields. This fact gave an early indication of the increase of stability of the adducts with increasing chain length.

A striking feature of these compounds is that they all have the same crystalline form, being hexagonal needles. Urea itself crystallizes in tetragonal needles. The crystals of adducts whose organic component has a low vapor pressure are stable indefinitely in air. If the organic component has an appreciable vapor pressure, then decomposition sets in more or less rapidly on leaving the crystals in air, and they can only be preserved in an atmosphere saturated with the vapor of the organic component.

The adducts do not in general display definite melting points. Decomposition ordinarily sets in long before a fusion temperature is reached, and the organic component either volatilizes or sweats out in liquid form. The residual urea exhibits the normal tetragonal crystalline structure of urea and the normal urea melting point of 132.7°C.

The adducts are instantly decomposed by water. Thus if nonane-urea is thrown into water, the urea dissolves and the nonane separates as an oily layer on the surface of the solution.

While the formation of such compounds was first noted in relation to hydrocarbons, it was soon discovered that similar compounds are formed between urea and the following series:- aliphatic acids and their esters, alcohols, ethers, aldehydes, ketones, discarboxylic acids, unsaturated hydrocarbons, halogenides, amines, diamines, nitriles, dinitriles, thioalcohols, thioethers and other compounds.

A particularly remarkable outcome of the early survey work was the observation that adduct formation takes place only with difficulty, or not at all, in the case of branched chain compounds. Cyclic compounds, moreover, are not in general capable of forming addition compounds. Urea adduct formation, therefore, offers the possibility of separating linear aliphatic compounds from their branched-chain isomers

by a method based on differences in molecular shape instead of by more conventional methods depending on differences in chemical reactivity or molecular size.

A second remarkable observation was that, within each homologous series, a certain minimal chain length appears to be a prerequisite for adduct formation. It will be seen later how these observations may be brought into harmony with the results of crystallographic and calorimetric studies. Schlenk (1949) prepared the adducts of the normal paraffins from C_7 to C_{12} and also those of C_{16}, C_{20}, C_{24} and C_{28} compounds. On making careful analyses of the adducts prepared in various ways it was discovered that non-integral relations prevailed.

As already mentioned, a very slight degree of chain branching precludes adduct formation, as does also substitution by a phenyl group either laterally or terminally. It is true that this rule is not absolute in so far as very long chains can be induced to form adducts when substituted to a limited degree, i.e. the effect of the long chain seems to overcome the forbidding effect of a substituent, but in general the rule holds. E. V. Truter (1951) studied the formation of urea adducts with carboxylic esters. He reported chain lengths required to induce adduct formation of branched esters. A side chain methyl group on the same side of the molecule as the carbonyl oxygen requires a straight chain of

at least eight atoms. If substitution is on the opposite side, at least eleven atoms are required. A main chain phenyl group requires a straight chain of at least nine atoms if it is on the same side as carbonyl oxygen, whereas the slightly larger cyclohexyl group requires thirteen atoms.

Crystallographic studies of several urea adducts has shown that the urea molecules are arranged in a hexagonal lattice in which each unit cell contains six urea molecules.

The urea molecules are arranged in such a manner that there is a canal down the centre of the lattice. The dimensions of the canal are such that a stretched hydrocarbon chain will fit into it easily, whereas a phenyl group would require a very slight distortion and larger molecules would not fit at all. This type of molecular compound formation is somewhat analogous to the adsorp tion of n-paraffins in the crystal structure of chabazite while excluding isoparaffins as reported by Barrer (1945). However in the case of chabazite, the crystal lattice does not need to change to accommodate the adsorbed molecule as does the crystal lattice or urea.

Crystallography has also been of value in explaining the non-integral molecular combining ratios. The unit cell comprising six urea molecules is 11.1 Å long, so that one urea molecule corresponds to an interval of lattice length of 1.85 Å. Comparison of the length of lattice available with

the length of hydrocarbon chain in the adduct for a series of paraffins, shows that there is a uniform discrepancy of 2.4 Å, per molecule. The simplest explanation of this discrepancy is that 2.4 Å represents the interval between succeeding hydrocarbon molecules in the lattice. The space occupied by one molecule of hydrocarbon may then be represented by $((n \times 1.3) + 2.4)$ Å. This value divided by 1.85 Å will give the ratio of the number of urea molecules to hydrocarbon molecules in the adduct, and explains the non-integral nature of these ratios.

It is also possible to calculate the density of urea adducts from the density of tetragonal urea, the lattice dimensions of the tetragonal and hexagonal forms, and the density of the hydrocarbon. The calculated values are in excellent agreement with observed values.

The formation of urea adducts is an exothermic reaction. Schlenk has proposed that the energy transformation should be apportioned between three distinct, but simultaneous processes:

(a) The passage of the hydrocarbon molecules from the associated liquid state to the state of separated molecules.

> This process being physically analogous to vaporization, Schlenk assumed that the heat involved would be very nearly the same as the latent heat of vaporization.

(b) The urea molecules and they hydrocarbon molecules are brought into close association resulting in the liberation of heat.

(c) The urea passes from a tetragonal to a hexagonal lattice configuration. Since the tetragonal configuration is the more stable of the two, this transition almost certainly involves absorption of heat.

This partition of energy may be expressed by the equation: $Q_1 - Q_2 - Q_3 - L$

where Q_1 is the heat of formation of the adduct

Q_2 is the heat of association evolved by process (b)

Q_3 is the heat of lattice transition i.e. the heat absorbed by process (c)

and L is the latent heat of vaporization of the organic component, equal to the heat for process (a)

Q_2 may be expressed as cal per A of occupied lattice canal length. Q may be expressed as cal per A lattice length, as the whole lattice is transformed whether completely occupied or not. The equation then becomes:

$$Q_1 = (n \times 1.3)Q_2 - ((n \times 1.3) + 2.4)Q_3 - L$$

By selecting pairs of organic components of very different chain lengths, and hence different proportions of occupied and unoccupied lattice canal, it is possible to set up simultaneous equations from which values for Q_2 and Q_3 may be obtained. Agreement between the values of Q_2 and Q_3 calculated from different pairs of adducts is sufficiently

close to substantiate the basic validity of this interpretation.

The possible applications of urea adduct formation to oil chemistry are numerous. Schlenk and Holman (1950) have shown that if adduct formation is carried out fractionally, the saturated fatty acids form urea adducts more readily than the unsaturated fatty acids. Newey, Shokal, Meuller and Bradley (1950) utilized this property to segregate soybean methyl esters into a fraction of high iodine value and a fraction of low iodine value. The fraction having a high iodine value, when reconstituted to triglycerides, showed drying properties equal to those of linseed oil.

Holman and Schlenk (1950) have shown that urea adducts of unsaturated fatty acids are particularly resistant to autoxidation. This may offer a method of preserving such acids and their linear esters.

The experience of Linstead and Whalley (1950) who separated mixtures of esters, suggests that relatively small linear carboxylic acids might be separated from their branched isomers by converting the acids to esters of sufficient chain length to permit adduct formation.

Urea adduct formation has been used by Tiedt and Truter (1951) for the separation of linear and branched alcohols from wool fat. Secondary alcohols which formed adducts before, but not after, acetylation were demonstrated. J.R. Nunn (1952) has isolated sterculic acid, a fatty acid containing a

cyclopropane ring, from the kernel oil of Sterculia foetida, by means of urea adduct formation.

The simplicity of the technique and the unique nature of the separations afforded, i.e. separation by shape rather than by size or chemical reactivity, promise that urea adduct formation may become of increasing importance as an analytical and preparative aid in oil chemistry.

An analogous series of thiourea adducts has been reported by Redlich, Gable, Beason and Millar (1950) and has been studied in detail by Schlenk (1951). However, thiourea will form adducts only with larger more compact molecules and will probably not find such wide application to oil chemistry as the urea adducts.

The Toxicity of Heat Polymerized Oils

Mention has already been made of a method developed by Privett et al. (1945) to reduce the tendency of linseed oil toward flavor reversion. However, the inclusion of linseed oil prepared by this method in the diet of growing rats resulted in a marked reduction in growth rate. Animals receiving polymerized oils, excreted dark, sticky feces and, when the level of oil in the diet was high, showed oily matted coats (Crampton, Farmer and Berryhill, 1951). Lassen, Bacon and Dunn (1949) also noted abnormal feces from adult rats fed polymerized sardine oil.

Linseed, rapeseed, corn, peanut and soybean oils were reduced in nutritive value by thermal polymerization. The extent of the damage appears to depend on the unsaturation of the oil and on the duration of thermal treatment. Highly unsaturated oils, such as linseed, required much less time than more saturated oils for the same thermal damage to take place. (Crampton, Farmer, and Berryhill, 1951).

High levels of polymerized oils in the diet resulted in greater growth depression than low levels. Feeding the oil by dropper, apart from the non-lipid portion of the diet, did not result in any improvement in the growth rate. It would appear that polymerized oil exerts its growth depression by a direct toxic action, rather than by destruction of some essential component, either in the oil itself or in the non-lipid portion of the diet.

The damage to the nutritive value of linseed oil by thermal polymerization does not appear to be associated with either peroxidation or destruction of Vitamin E. (Crampton, Common, Farmer, Berryhill and Wiseblatt, 1951)[a].

Farmer, Crampton and Siddall (1951) have shown that inclusion of polymerized linseed oil in the diet of pregnant female rats had an adverse effect on the number of rats born and on the survival time of those born. Vitamin E destruction was apparently not the cause for this effect, for administration of Vitamin E did not improve the results.

It appears unlikely that the unsaponifiable material in linseed oil is responsible for the appearance of toxicity in the heated oil. Crude and alkali-refined linseed oils yield polymerized fractions of identical toxicity. Ergosterol and lecithin, added to commercial shortening and heated at 275°C. for 18 hours, did not render the samples any poorer than the pure shortening control (Gass,1947).

Wiseblatt (1950) attacked the problem by converting the heat polymerized oil to the corresponding mixed ethyl esters. Distillation of these esters gave a distillable, monomeric fraction and an undistillable, polymeric fraction. These fractions were included in rat diets which were baked before feeding. Results of these feeding trials showed that, while the monomeric fraction was no better than the unfractionated mixed ester, the polymeric fraction was much worse. Six out of eight rats receiving the polymeric fraction lived less than one week. (Crampton, Common, Farmer, Berryhill and Wiseblatt, 1951)[b]. However, rats in this group ate only about 2 gm. of feed per day. While it has been established that feed intake at this level will prevent death from starvation, reduced digestibility of the polymerized esters might lower the digestible caloric intake to a point where death from starvation ensued.

The fact that the monomeric esters were reduced in nutritive value, suggests that this fraction may contain products of side-reactions which are nutritionally deleterious.

The possibility that cyclic monomeric esters may be present in the distillable ester fraction, is suggested by the work of Pashke and Wheeler (1949). While nothing is known of the effects of feeding such material, several cyclic fatty acids, both natural and synthetic, have been shown to be toxic to animals.

The Toxicity of Branched-Chain and Cyclic Fatty Acids

The study of branched-chain and cyclic fatty acids has been chiefly centered on their relationship to the acid-fast bacteria causing tuberculosis and leprosy. These bacteria are characterized by the possession of a fatty or waxy envelope. Cyclic fatty acids have been shown to be the agents responsible for the beneficial effect of chaulmoogra oil on leprosy, while branched-chain fatty acids have been isolated from several species of acid-fast bacteria.

In connection with the extensive study of the fats and waxes of the tubercule bacilli by Anderson and his group, Anderson and Chargaff (1929) separated from the acetone soluble fat of these bacilli, two fatty acids which they called tuberculostearic and phthioic acids. Anderson (1929) reported that these acids, particularly phthioic acid, were active biologically and that when injected subcutaneously produced tubercule-like lesions at the site of the injection. Tuberculostearic acid was later identified by

Spielman (1934) as 10-methyl stearic acid. The structure of phthioic acid is still somewhat in question, but it appears to be a branched-chain acid, probably a tri-substituted acetic acid. (Robinson, 1940).

It was believed by some that the biological reactions produced by injection of phthioic acid was due to impurities derived from bacterial residues. However, Robinson (1940) injected two synthetic branched-chain fatty acids and observed a reaction in both cases. Intraperitoneal injection of $\alpha\alpha$-dimethyl n-decylacetic acid produced peritonitis and leukemia in rabbits, while methyl di-n-octylacetic acid produced the cell reactions typical of phthioic acid.

Buu-Hoï and Ratsimamanga (1943) claim similar results from the injection of $\alpha\alpha$-dimethyl stearic acid into guinea pigs. Their treatment consisted of intraperitoneal injection of 50 mg. of the acid once a week for one month. They noted a marked decrease in haemoglobin and an increase in the ratio of monocyte/lymphocyte. Haemoglobin decreased 50% by the end of three weeks. M/L x 100 went up to 40 to 90 from the 4th to the 20th hour after injection, whereas controls receiving only the inert vehicle remained at about 10 to 20.

Autopsy of the treated guinea pigs revealed adhesions between the intestinal loops themselves and between the loops and the posterior wall of the abdominal cavity. They

also noted granulations similar to the lesions of tuberculosis on the liver and diaphragm. Microscopic examination of these lesions showed: (a) Giant Langerhans type cells; (b) epithelial cells grouped in masses, (c) great numbers of monocytes, some showing mitosis, and (d) necrotic foci.

The linear form of the acid, arachidic acid, was tested but did not produce any of these effects.

Paraf et al.(1945) gave four intraperitoneal injections of 25 mg. each to guinea pigs over a two-week period, of an unsaturated branched-chain acid, $\alpha\alpha$-dimethyl ω-tridecylenic acid. These injections resulted in cachexia ending in death. Subcutaneous injections of like doses caused shock and loss in weight but did not kill. The lethal effect of intraperitoneal injections (but not the loss in weight) was inhibited by concurrent injection of large doses of nicotinamide.

Cagnant et al. (1950) studied the physiological action of the ester of the same acid. They found no toxic symptoms when they injected doses of 0.6 cc. subcutaneously into healthy mice or guinea pigs. The ester appeared to have a bacteriotrophic effect when injected into guinea pigs infected with septic tuberculosis, but did not produce patent lesions.

Polgar (1948) injected 3:13:19 trimethyl tricosanoic acid into experimental animals and obtained effects comparable to those obtained with phthioic acid.

It would appear from these reports that high molecular weight branched-chain fatty acids are capable of producing tubercule-like lesions. Medium molecular weight branched-chain fatty acids may produce a modified physiological action. Esters and amides are inactive. The production of the physiological reaction appears to depend on the presence of a free carboxyl group and it has been suggested that these acids act either through association of carboxyl groups, to inhibit certain enzyme systems, or by reason of the peculiar surface phenomena displayed by these acids.

While the beneficial effects of chaulmoogra oil have been known for a long time, the structures of the principal fatty acids of chaulmoogra oil, chaulmoogric and hydnocarpic acids, were not fully elucidated until 1925. (Shriner and Adams, 1925). These acids have been proven to be responsible for the curative properties of chaulmoogra and allied oils.

The oral toxicity to dogs of chaulmoogric and cyclopentenyl acetic acids was demonstrated by Bernhard and Muller (1938). They attempted to study the metabolism of these acids but were unable to isolate any of the metabolic end products from the urine.

Buu-Ho'i and Ratsimamanga (1941) also reported that chaulmoogric and hydnocarpic acids were toxic and ascribed the toxic properties to the cyclopentene ring.

Sartory et al.(1950) have studies the toxicity of chaulmoogric and hydnocarpic acids as well as a number of related synthetic acids. They administered subcutaneous and intra-peritoneal injections at dosages of 0.1 and 0.6 cc. in the case of rats and 0.6 and 1.0 cc. in the case of guinea pigs. The following symptoms were noted in all cases: (a) Very severe convulsions, (b) respiratory spasms, (c) a decreased pulse rate, (d) elevated arterial pressure, (e) vaso constrictions which arrested peristaltic movements of the digestive tract, (f) death, (0.6 cc. injected into rats caused death in three to six hours), and (g) in cases of delayed death, methaemoglobin formation.

From the series of acids and esters used, the authors believe that the presence of a cyclic structure bearing on isolated double bond is necessary for toxicity. Whether the cyclic structure is 5-membered or 6-membered apparently makes no difference, but either exhaustive hydrogenation or substitution of an aromatic group for the monoethenoid ring supresses the toxicity. This is in contrast to the bacteriocidal action of these acids, which action is not diminished by hydrogenation. The similar activity of acids and esters in this series excludes the mechanism involving the inhibi- of ensyme systems, as has been proposed for the branched-chain fatty acids.

METHODS

Thermal Polymerization

The apparatus used is illustrated in Figure 1. It consists of a two litre, three necked flask fitted with 29/42 standard taper side necks and a 45/50 standard taper centre neck. One side neck is fitted with a 29/42 inner joint carrying a sealed-in inlet tube, which reaches to the bottom of the flask, and a side arm which serves as an outlet for volatile material. The other side neck is fitted with a 29/42 inner joint drawn down to fit snugly around a mercury thermoregulator. (Central Scientific Co., Merc to Wire). The centre neck is fitted with a 45/50 inner joint drawn down to fit a 360°C. thermometer. The thermometer and thermoregulator are sealed in with short lengths of rubber tubing.

The flask is heated by a hemispherical mantle, the top portion of the flask being insulated with a layer of glass wool. Temperature control is maintained by the thermoregulator which is attached to a mercury relay. (Central Scientific Co., Merc to Merc). The electrical connection from relay to mantle is made through a Powerstat variable transformer set at 80 volts to prevent overheating the mantle. This arrangement gives temperature control to about $\pm$ 1.5°C., of the desired temperature.

Figure 1. Apparatus For Thermal Polymerization

At the beginning of the heating period a slow flow of CO_2 was bubbled through the oil. When the temperature reached 180°C., the flow was made rapid enough to keep the oil surging vigorously. Timing was begun when the oil reached 275°C. This usually required about 1½ hours. At the end of the heating period, the heating mantle was shut off and removed to facilitate rapid cooling of the oil. The full flow of CO_2 was continued until the oil temperature reached 150°C., and thereafter a slow flow was continued until room temperature was reached. This method of polymerization produced oils with low acid values and good color and blandness.

Alcoholysis of Oils

Oils were prepared for alcoholysis by a preliminary extraction with anhydrous alcohol to remove free fatty acids, using approximately 30 ml.alcohol per 100 gm.oil, alcoholysis was carried out as follows: 30 gm. of anhydrous ethanol, or 22 gm. of anhydrous methanol, each containing 0.5 gm. NaOH, was used for each 100 gm. of oil. The oil and the alcoholic solution of NaOH were heated separately to 55°C. in the steam bath and then mixed together at this temperature. They were held at 55 °C.. for two hours and then allowed to cool for several hours or overnight. The dark red, glycerol rich layer which settled out was drawn off in a separatory funnel and discarded. The esters were

washed with large quantities of hot distilled water until all soaps and excess NaOH were removed. They were then dried under reduced pressure.

The anhydrous alcohols used in this preparation were prepared by the magnesium method of Lund and Bjerrum (1931).

Vacuum Distillation of Esters

The esters were distilled in a simple, all-glass apparatus. The distilling flask was filled with clean glass wool to prevent foaming and bumping. It was heated by a wax bath. (Fisher Bath Wax).

No suitable manometer was available for accurate measurement of pressure, so distillation temperature limits were established on esters of alkali-refined linseed oil. These limits were observed in subsequent distillations. It was found that the distillable esters came over between 150° and 180°C. When the vapor temperature had passed the maximum, the bath temperature was taken up to 240°C. If no further rise in vapor temperature occurred, the bath was removed and the residue allowed to cool without breaking the vacuum.

For quantitative determination of percentage distillable esters, it was found convenient to use a small bulb blown on a piece of Pyrex glass tubing (7 to 9 mn. diameter) furnished with a short side arm and bulb to act as receiver. The bulbs were of a size to hold conveniently a charge of

0.5 to 0.7 gm. of ester. After distillation the bulb and side arm were broken apart and weighed. The residue and distillate were then removed by ether and the weights of distillate and residue obtained by difference. It was found that this simple technique gave consistent quantitative results and that there was little or no loss of material.

Urea Adduct Formation

Urea combines with linear methyl or ethyl esters of C_{18} fatty acids in the weight ratio of approximately 3 gm. of urea to 1 gm. of ester. In order to ensure complete precipitation of linear esters, a weight of urea equal to four times the estimated weight of monomeric esters present was used for the formation of urea adducts.

As dimeric esters are soluble only to a limited extent in methanol, commercial absolute ethanol was used as a solvent. One ml. of solvent was used for each gm. of urea used. This is not a sufficient quantity of ethanol to dissolve all the urea but it was found that adduct formation proceeded smoothly in the pasty mixture so formed.

Where large quantities of urea adduct were required, it was found that 500 gm. batches of the ester could be processed conveniently. A detailed description of such a preparation follows:

Five hundred gm. of ester was dissolved in 2 l. of absolute ethanol. To this was added, slowly with stirring,

2 kgm. of urea. The mixture was held at about 50°C. for one-half hour and then allowed to cool slowly to room temperature overnight. The next morning the precipitated adduct was removed on a Büchner funnel and washed on the funnel with several portions of absolute ethanol saturated with urea.

The adduct was thrown into a large volume of warm water, in which the urea dissolved. The esters so freed separated from the mixture and the aqueous layer was drawn off in a separatory funnel and discarded. It was found that the addition of a small amount of sodium chloride aided in the separation of the two phases and reduced the loss of ester due to emulsification. The ester was then washed with warm water and dried under reduced pressure.

The filtrate plus alcoholic washings, containing the esters which would not form adducts, was diluted with a large volume of water. This served to throw these esters out of solution. The aqueous phase was drawn off and the esters were washed and dried under reduced pressure.

While the method outlined entailed some loss due to emulsification, the esters were not subjected to temperatures higher than about 50°C. at any time. The addition and removal of solvent, which might add objectionable flavors and odors, was not necessary. This is important where the fractions are intended for use in animal feeding trials.

Where quantitative measurements of yields were desired, the ester was recovered from the aqueous mixture formed when the adduct was decomposed, or when the non-adduct forming esters were diluted with water, by extraction with ether. The ether was then removed under an atmosphere of CO_2 and the yields of adduct forming and non-adduct forming esters determined by weighing.

Mean Molecular Weights

Mean molecular weights were determined by cryoscopy in purified cyclohexane. The concentration of solute was kept below 5% for all determinations. The solutions were cooled by an ice-water bath and stirred with a stainless steel loop stirrer. A Beckmann thermometer enabled freezing points to be estimated to within $\pm 0.002°C$. Observed molecular weights were corrected for free fatty acid content by the method of Bernstein (1948).

Cyclohexane was purified for cryoscopy as follows: A quantity of cyclohexane was mechanically shaken with about 10% of its volume of 20% fuming sulfuric acid for twelve hours. The acid layer was drawn off and the cyclohexane was shaken for another twelve hours with a similar portion of fuming sulfuric acid. This was again drawn off and excess acid in the cyclohexane was removed by the addition of granular barium hydroxide. The cyclohexane was then decanted and distilled through a Stedman column.

The cryoscopic constant of the cyclohexane was determined using resublimed naphthalene and found to be 20.19. This value compares favorably with the value, 20.2 given by Glasstone (1946).

Iodine Values

In previous work in this laboratory the "Rapid Wijs" method of Hoffmann and Green (1939) has been used. Recently Benham and Klee (1950) have proposed a method which consists essentially of the use of the Rosemund-Kühnhen reagent with the addition of a mercuric acetate catalyst. The authors claim satisfactory results for oils which are difficult to halogenate by other methods. (Klee and Behham, 1950). Both of these methods have been used in the present work. The method used is indicated where iodine values are given.

Fatty Acid Composition

The fatty acid composition of oils or esters was determined by spectroscopy as recommended by the Spectroscopy Committee, American Oil Chemists' Society (1949).

Figures 2 and 3 show the constant temperature bath used for the isomerization procedure. It consists of two large cylindrical cans fitted one inside the other, with the intervening space filled with diatomite to provide insulation. The cover has openings for three reaction tubes, a heater, a stirrer, a thermometer and a thermoregulator. A rod type

heater is used and the mercury thermoregulator (Central Scientific Co., Merc to Wire) is connected to a mercury relay (Central Scientific Co., Merc to Merc) which is mounted on a panel above the bath. The panel is also fitted with switches for the heater and the stirrer which is also mounted on it. This arrangement has been found to provide temperatures which are constant to within $\pm 0.1^{\circ}C$.

An oxygen absorption train constructed according to the directions of Meites and Meites (1948) is mounted on the back of the bath. The oxygen absorption train consists of three flasks; the first two flasks each contain 200 ml. of 0.1 M vanadyl sulfate, 200 gm. amalgamated zinc and a little free sulfuric acid, the third flask contains dry glass wool. From this flask the purified nitrogen is led through a a drying tube containing calcium chloride and thence to a manifold. Separate tubes lead the nitrogen from the manifold to each of the reaction tubes. The reaction tubes are fitted with gas distribution heads similar to those described by the Spectroscopy Committee (1949).

Acid Values

A sample of 5 to 10 gm. of the oil was weighed in a tared 250 ml. flask and dissolved in 50 ml. of a mixture of ethanol and toluene (1 : 1 by volume) which had been neutralized to phenolphthalein just before use. One ml. of 1% phenolphthalein solution was added and the solution was titrated immediately

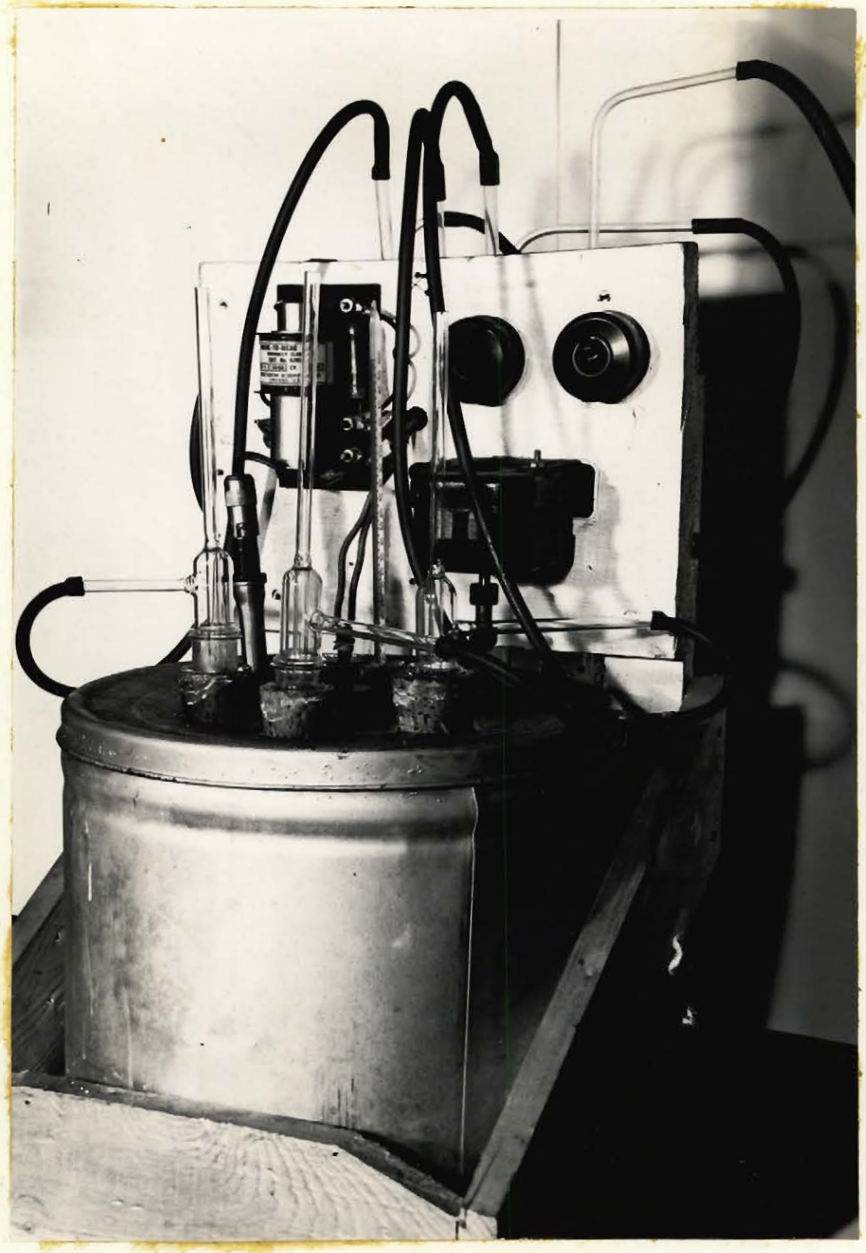

Figure 2. Constant Temperature Bath
Front View

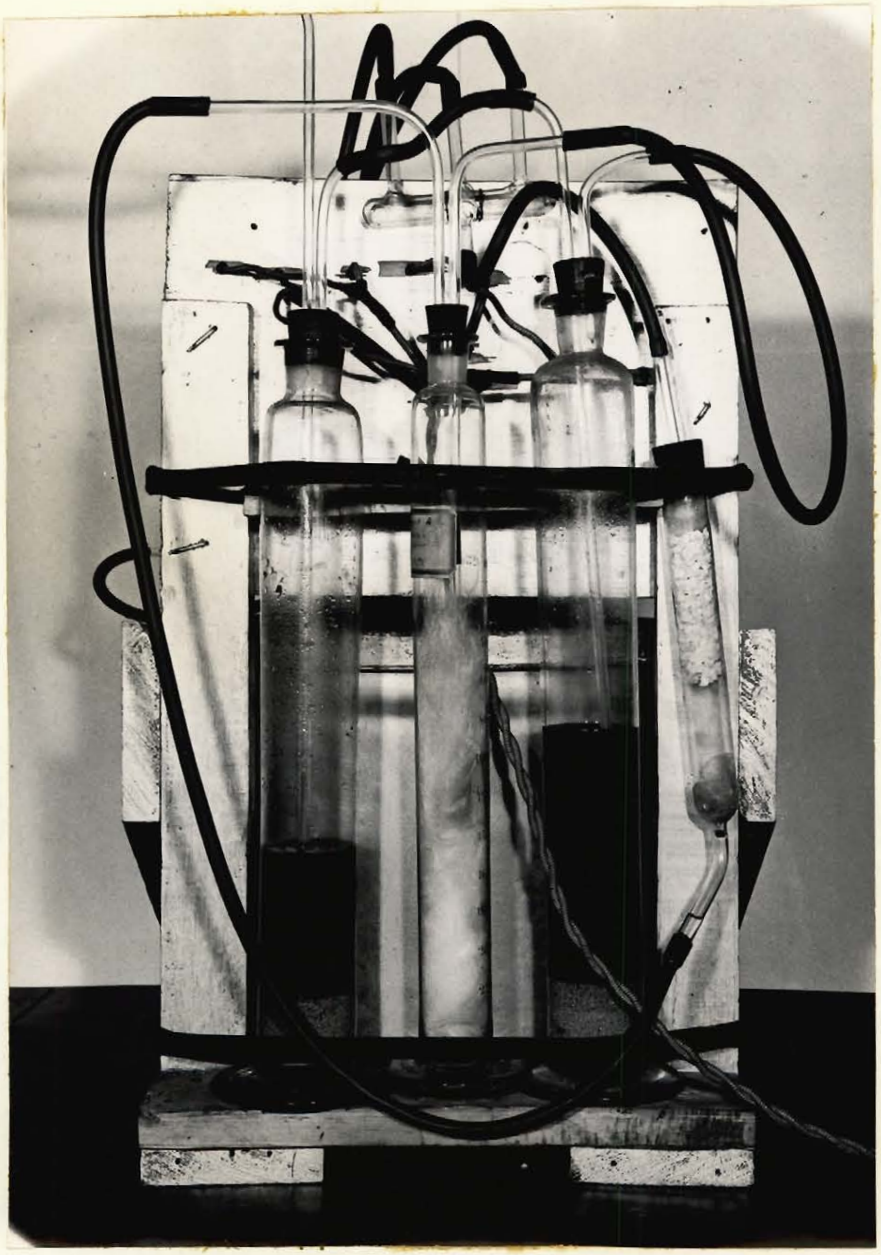

Figure 3. Constant Temperature Bath
Back View

with 0.05 N KOH solution using vigorous agitation. Acid values were calculated as per cent oleic acid.

Peroxide Values

Peroxide values were determined by the method of Skellon and Wills (1948). Where it was desired to determine the extent of oxidation of oil fractions after they were mixed in the diet, the modification of this procedure described by Crampton, Common, Farmer, Berryhill and Wiseblatt (1951)[2] was used. Peroxide values are reported as mg. of peroxide oxygen per kgm. of oil.

Hydroxyl Values

Hydroxyl values were determined by the method of Ogg, Porter and Willits (1945). The method is comparatively simple and the results were found to be reproducible.

C-Linked Methyl Groups

The determination of carbon linked methyl groups was performed according to the directions of Barthel and La Forge (1944). This method depends on the principle that in the oxidation of aliphatic compounds by chromic acid of the strength used, all carbons are oxidized to carbon dioxide with the exception of those representing terminal methyl groups. These latter are oxidized to acetic acid which can be recovered and determined.

Refractive Indices

Refractive indices were determined at 25°C. with a Zeiss immersion refractometer and auxiliary prism. A sodium vapor lamp was used for illumination.

Feeding Experiments

All feeding trails were conducted by members of the Department of Nutrition, Macdonald College. Albino rats were used as the experimental animals. Feeding experiments were begun when the rats were 28 to 35 days old and were extended over the following twenty-eight days. The animals were given feed and water ad libitum. Feed consumptions and liveweight gains were recorded.

The basal diets were as follows:

White flour	44.0	or	54.0
Oil fraction	20.0	or	10.0
Milk powder	19.0		
Casein	11.5		
Yeast	3.0		
Bone meal	2.0		
Salt	0.5		

EXPERIMENTAL

Section 1

Preparation of Termally Polymerized Linseed and Soybean Oils of Known Dimeric Acid Content

Introduction

Wiseblatt (1950) has shown that the increase of refractive index of linseed oil during thermal polymerization bears a linear relation to dimeric acid residues during polymerization. He suggested that this property might be utilized for the preparation of thermally polymerized oils of known di eric acid content.

It was considered desirable to investigate this relationship with soybean oil, and to obtain data on these batches of linseed and soybean oil for future use.

Procedure and Results

Batches of alkali-refined oil were heated at 275°C for 6.5 hours and 12 hours in the case of linseed oil, and for 12 hours and 24 hours in the case of soybean oil. The methyl esters were prepared by alcoholysis and the percentage of dimeric acids was estimated from the undistillable residue. The results for the two oils are given in Table 1. These results are plotted in Figures 4 and 5. The reflective indices corresponding to 9% and 18% of dimeric acids were then read from the graphs. Samples were polymerized to these refractive indices and the oils were then analyzed for dimeric acid content as before. The results are shown in Table 11.

Table I

Refractive Indices and Dimeric Acid Contents of Polymerized Linseed and Soybean Oils

Oil	Time of Heating (hours)	Refractive Index (25°C)	Per cent Dimeric Acids
Linseed	0	1.4790	0
	6.5	1.4829	16.9
	12	1.4861	26.7
Soybean	0	1.4732	0
	12	1.4748	10.4
	24	1.4766	20.2

Table II

Realization of Dimeric Acid Contents of Polymerized Linseed and Soybean Oils from Refractive Index Measurements

Oil	Per cent Dimeric Acid Desired	Refractive Index (25°C.) Corresponding to Desired Dimeric Acid Content	Per cent Dimeric Acid Realized
Linseed	9	1.4809	9.5
	18	1.4832	18.0
Soybean	9	1.4746	8.9
	18	1.4762	17.8

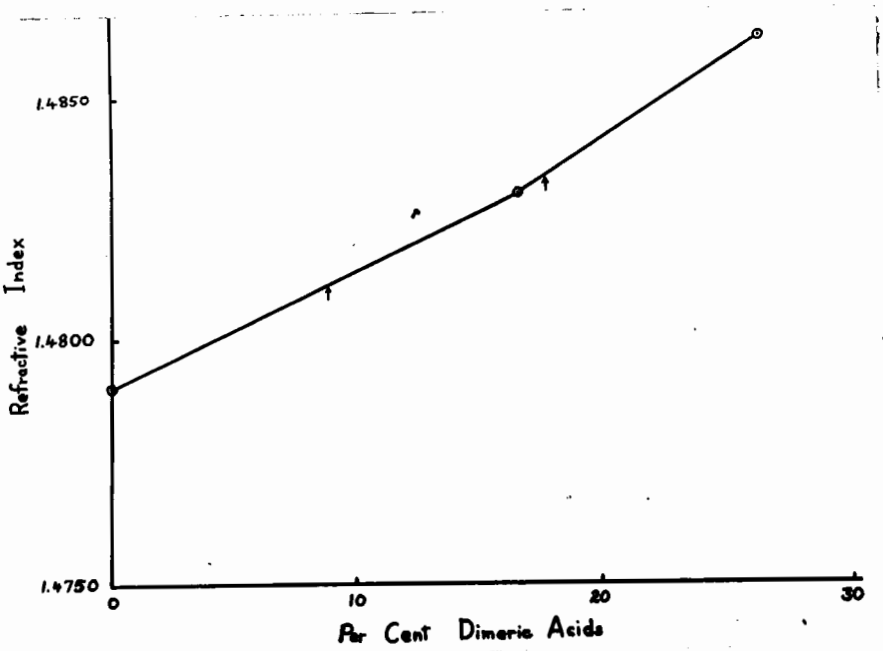

Figure 4

Relationship between Refractive Index
and Per cent Dimeric Acids
Linseed Oil

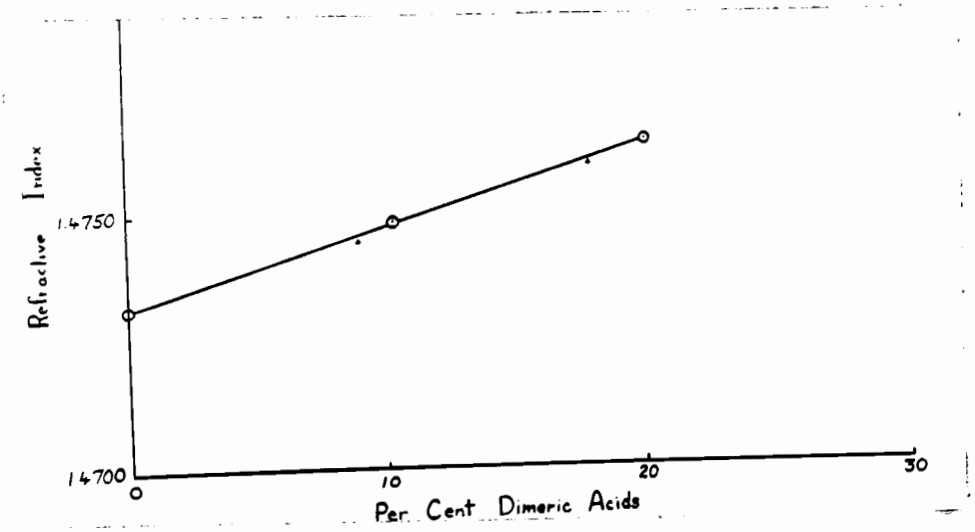

Figure 5

Relationship between Refractive Index
and Per cent Dimeric Acids
Soybean Oil

Discussion

Figures 4 and 5 show that the three points give a line which is almost straight in each cose. It is evident therefore, that the predominant reaction affecting the increase of refractive index, is dimerization of fatty acid residues. Table II shows that polymerized oils of a desired dimeric fatty acid content can be obtained with reasonable precision by polymerizing to the appropriate refractive index.

Section II

The Relationship between Dimeric Acid Content and Nutritive Value in Thermally Polymerized Linseed and Soybean Oils

Introduction

Previous work by Wiseblatt (1950) has shown that dimeric esters, isolated from the mixed ethyl esters of thermally polymerized linseed oil, were toxic when included in rat diets which were baked before being fed. If the dimeric acid residues in heated oils are responsible for their toxicity, the toxicity of a heated oil should be directly proportional to the amount of dimeric acid it contains. The lower order of toxicity of soybean oil might be explained as being due to its slower rate of polymerization.

It was believed that comparison of the nutritive value of linseed and soybean oils, polymerized so as to contain the same amount of dimeric acids, might provide some evidence of the toxicity or innocuity of dimeric acids. Valuable information might also be provided by comparison of the nutritibe value of diets containing oils polymerized for different periods of time, but with the level of oil in the diet adjusted so that both diets contain the same level of dimeric acids.

Alkali-refined linseed and soybean oils were thermally polymerized to contain 9% and 18% of dimeric fatty acid

residues by the method outlined in Section I. These levels were chosen because 18% is approximately the level of dimeric acid residues found in the acetone-soluble seggregate of linseed oil which has been thermally polymerized for 12 hours at 275°C. (Wiseblatt 1950).

These four polymerized oils were incorporated into rat diets at two levels, 10% and 20%. These diets were fed to eight groups of albino rats for a 28 day test period. Results of this feeding trial are summarized in Table III.

Discussion

Table III shows that the nutritive values of diets containing polymerized linseed oil vary with the amount of dimeric acids contained in those diets. A diet which included 20% of linseed oil, heated to contain 9% of dimeric acids, gave weight gains which were identical to those produced by a diet which included 10% of linseed oil, heated to contain 18% of dimeric acids. Diets which contained smaller amounts of dimeric acids gave better gains; diets which contained more dimeric acids gave poorer weight gains.

However, where the diets contained polymerized soybean oil instead of polymerized linseed oil, no such simple relationship existed. A diet including 20% of soybean oil, polymerized to contain 9% of dimeric acids, was just as

nutritious as a similar diet containing only 10% of this oil. The nutritive values of these diets were high, and it would seem that polymerization of soybean oil to this degree had not impaired its nutritive value.

Diets including 10% of soybean oil, polymerized to contain 18% of dimeric acids, were reduced in nutritive value, and diets containing this oil at the 20% level were still less nutritious. All diets containing soybean oil were superior to the corresponding diets containing linseed oil.

Although the results obtained with linseed oil in this experiment might support the view that dimeric fatty acids are responsible for the toxicity of polymerized oils, acceptance of this view requires its restriction to linseed oil and the proposal of a different toxic agent in polymerized soybean oil.

A simpler explanation is that toxic material accumulated in the linseed oil during heating in proportion to the accumulation of dimeric acids but that the proportion of toxic material formed for a similar accumulation of dimeric acids during heating of soybean must have been very much smaller. In fact, the results suggest that toxic material may not have formed during the heating of the soybean oil until polymerization had proceeded to a considerable extent. The apparent existence

of such an induction period in the formation of toxic material from soybean oil suggests that this material may be formed as the result of two or more consecutive chemical reactions.

TABLE III

The Nutritive Value of Diets Containing Known Amounts of Dimeric Acids Derived from Linseed and Soybean Oils

Oil	Per cent Dimers in Oil	Per cent Oil in Diet	Average Daily Gain (gm.)	Average Daily Feed Intake (gm.)	Gain per 1000 Gross Calories (gm)
Linseed	9	10	3.7	10.2	74
		20	3.2	8.9	67
	18	10	3.2	9.7	67
		20	2.1	7.1	55
Soybean	9	10	4.2	10.9	79
		20	4.1	9.6	79
	18	10	3.8	10.8	72
		20	3.4	9.1	69

Section III

A Comparison of the Nutritive Values of Fractions Obtained by Vacuum Distillation and by Urea Adduct Formation from the Ethy l Esters of Thermally Polymerized Linseed Oil

Introduction

Previous work in this laboratory (Wiseblatt, 1950) has shown that undistillable ethyl esters of thermally polymerized linseed oil are toxic to rats when included in diets which were baked before being fed. However, the distillable fraction was less nutritious than the ethyl esters of whole unheated linseed oil. Subsequent work has shown that baking seriously reduces the nutritive value of diets containing ethyl esters. (Wiseblatt, 1950).

A possible hypothesis to explain the reduced nutritive value of the distillable ethyl esters was that they contained cyclized monomeric material. Evidence for the presence of cyclic monomers in thermally polymerized methyl linoleate has been presented by Paschke and Wheeler (1949). Since urea will form crystalline adducts with linear aliphatic compounds, but not with cyclic or branched chain compounds, urea adduct formation should remove linear aliphatic material from the ethyl esters of thermally polymerized oil, leaving behind cyclic monomeric material as well as dimeric esters.

It was considered desirable, therefore, to re-assess the nutritive value, in unbaked diets, of monomeric fractions,

obtained by vacuum distillation of the ethyl esters of heated linseed oil, and to compare the results with those obtained with monomeric fractions separated by urea adduct formation.

Procedure and Results

Alkali-refined linseed oil was polymerized for 12 hours at 275°C. The resulting polymerized oil was converted to the mixed ethyl esters by alcoholysis. A portion of these esters was distilled under reduced pressure to give a distillable, monomeric fraction and an undistillable dimeric fraction. Another portion of the mixed esters was treated with urea to give an adduct-forming fraction, consisting of linear ethyl esters, and a non-adduct-forming fraction, consisting of dimeric ethyl esters and other compounds which would not form urea adducts. The yields of these fractions are given in Table IV. These yields of themselves provided grounds for suspecting the presence of non-linear monomeric material in the esters of the heat polymerized linseed oil.

Table IV

Yields of Various Fractions
Obtained from the Ethyl Esters
of Thermally Polymerized Linseed Oil

Ester Fraction	Yield (%)
Distillable esters	65
Undistillable esters	35
Adduct-forming esters	50
Non-adduct-forming esters	50

These fractions were incorporated into rat diets at a level of 10%. Two control diets were also prepared. One of these diets contained 10% of unheated linseed oil and the other contained 10% of the mixed ethyl esters of unheated oil.

Two groups of rats were assigned to each diet. The diet for one group was stored at room temperature while the diet for the other group was refrigerated. Peroxide values were determined on the oil fractions extracted from the diets at the ends of the second, third and fourth week of the trial. The results of this feeding experiment are shown in Table V while Table VI shows the results of the peroxide value determinations.

A large number of animals died during the course of this experiment, from non-dietary causes. Post-mortem examination of all animals surviving the test showed no evidence of ill-health.

It should be remarked here that a preliminary investigation was carried out to determine if cyclic fatty acids were likely to form urea adducts. The free fatty acids of chaulmoogra oil were prepared by saponification and acidification of the resulting soaps. It was found that only about 15% of these fatty acids would form urea adducts. Since chaulmoogra oil contains 80% or more of cyclic fatty acids, it is evident that cyclic fatty acids were not precipitated. This conclusion received further support from the subsequent publication of similar findings by Martinez Moreno et al (1951).

Table V

The Nutritive Values of Diets Containing Various Fractions of the Ethyl Esters of Linseed Oil

Oil	Dry Matter Digestibility of Diet (%)	Storage Conditions	No. of Animals Surviving Test	Gain per 100 gm. of Digested Feed
1. Unheated Linseed Oil	94	Room temp.	5	45
		Refrig.	2	46
2. Ethyl Esters of Unheated Oil	93	Room temp.	3	15
		Refrig.	2	42
3. Esters of Polymerized Linseed oil	91	Room temp.	5	35
		Refrig.	3	34
4. Adduct Forming Fraction of 3.	93	Room temp.	4	26
		Refrig.	6	35
5. Non-Adduct Forming Fraction of 3.	88	Room temp.	4	27
		Refrig.	4	28
6. Distillable Fraction of 3.	93	Room temp.	6	33
		Refrig.	6	31
7. Undistillable Fraction of 3.	86	Room temp.	3	40
		Refrig.	4	37

Table VI

Peroxide Values of Oils Extracted from Diets Containing Various Fractions of the Ethyl Esters of Heated Linseed Oil

Oil	Storage Conditions	Peroxide Value at the End of: (mg. peroxide oxygen per kg. fat)		
		2nd week	3rd week	4th week
1. Unheated Linseed Oil	Room temp.	185	282	309
	Refrig.	162	189	216
2. Ethyl Esters of Unheated Oil	Room temp.	12,320	17,612	14,208
	Refrig.	505	732	812
3. Esters of Polymerized Linseed oil	Room temp.	348	384	412
	Refrig.	185	225	365
4. Adduct Forming Fraction of 3.	Room temp.	15,720	11,776	11,520
	Refrig.	2,193	4,096	8,735
5. Non-Adduct Forming Fraction of 3.	Room temp.	664	1,387	1,759
	Refrig.	658	753	840
6. Distillable Fraction of 3	Room temp.	146	655	824
	Refrig.	91	266	326
7. Undistillable Fraction of 3.	Room temp.	262	435	537
	Refrig.	137	384	416

Discussion

Table IV shows that the yield of distillable material was 15% greater than the yield of adduct forming material. This difference must have been due to some material in the mixed esters which is volatile at reduced pressures but will not form urea adducts, e.g. branched chain acids, hydroxy acids or cyclic acids. It has been shown that the cyclic fatty acids of chaulmoogra oil will not form urea adducts. It seemed possible that cyclization of fatty acid residues might have occurred during the thermal treatment. The presence of the esters of such cyclic fatty acids would account for the difference in yields noted above. While it is true that hydroxy acids would also be unlikely to form adducts, the evidence available, and which is discussed later, suggests that hydroxy acids cannot account for more than a small proportion of the non-adduct-forming material.

No marked difference in the nutritive value between the refrigerated and unrefrigerated diets was observed except for those diets containing linear monomeric esters as the only fat source, i.e. the esters of unheated linseed oil and the urea adduct forming esters of heated oil. In those two cases, the nutritive value of the diet stored at room temperature was much lower than that of the refrigerated diet. Table VI shows that the peroxide values of oil extracted from these diets were much higher when the diet was stored at room temperature than when

it was refrigerated. It appears likely that peroxidation of the oil component was responsible for the reduction in nutritive value of some of these diets, especially those in which peroxidation of the oil component was extensive.

While storage of the diet under refrigeration was effective in protecting the ethyl esters of unheated oil from peroxidation, this was not the case with the adduct forming esters of polymerized oil. It has been shown previously that peroxidation does not explain the production of toxicity in heated oils. It is therefore suggested that the low nutritive value of the urea adduct forming fraction of the esters of heated oil was the consequence, not of the presence of toxic constituents of polymerized oil, but of the presence of harmful peroxides.

Comparison of the nutritive values of the refrigerated diets shows that the adduct forming esters were better nutritionally than the non adduct forming esters and that the undistillable esters were better than the distillable esters. The only material which is common to both the non adduct-forming esters and the distillable esters is monomeric ester, which would not form urea adducts. It is possible that this material may contain cyclic esters.

It would appear that the principal effect of dimeric ethyl esters is to reduce the digestibility of the diet. Diets free from dimeric esters (Diets 1, 2, 4 and 6) were equally

well digested, while diets containing dimeric esters (Diets 3, 5 and 7) were less well digested. The reduction in diet digestibility was proportional to the amount of dimeric esters in the diet. Although the reduction in the digestibility of the diet was small, it must be kept in mind that these reductions were effected by only 10% of dimeric acids in the case of Diet 7 and even less in the case of Diets 3 and 5. These latter diets contained approximately 3.5% and 7% of dimeric esters respectively.

Mention has already been made of the high peroxide values attained in the ethyl esters of unheated oil and in the adduct forming esters of heated oil. The ethyl esters of heated oil did not peroxidize to as great an extent as the ethyl esters of unheated oil. This fact suggests that heat polymerization either protects the natural antioxidants in the oil or forms new antioxidants. However, the effects of other diet constituents on the course of the oxidation reaction is not known. These effects may be considerable and could lead to erroneous conclusions being drawn from peroxide values determined in this manner.

Section IV

The Oxidative Stability of Various Fractions of the Ethyl Esters of Heated Linseed Oil

Introduction

The observations on the peroxide values of the various fractions used in the feeding trial reported in Section III were of sufficient interest to prompt a special examination of the susceptibility of such fractions to oxidation. This was the more desirable because the peroxidation of the fractions as present in the diets was complicated by the possible effects of other dietary components.

Procedure and Results

Methyl esters were prepared from both heated and unheated linseed oil by alcoholysis. The esters from the heated oil were segregated (a) into distillable and undistillable fractions by vacuum distillation and (b) into adduct forming and non adduct forming fractions by treatment with urea. The distillable fraction was further segregated by urea adduct formation. Table VII shows the yields of the various fractions obtained by these segregation techniques.

These fractions were examined for susceptibility to peroxidation. A simple stability test was carried out by maintaining samples of the various fractions in an air oven at 98°C and making peroxide value determinations after suitable intervals of time. These data are presented in Table VIII and are shown graphically in Figures 6, 7 and 8.

Table VIII

Peroxide Values of Various Fractions of the Methyl Esters of Heated Linseed Oil

Oil Fraction	Peroxide Values after heating time shown (mg. peroxide oxygen per kg. oil)					
	0	1	3	6	9	14.5
Unheated Oil	218	256	338	444	548	802
Heated Oil	0	46	75	110	159	208
Esters of Unheated Oil	263	890	1,226	1,822	2,244	3,178
Esters of Heated Oil	191	274	482	742	1,052	1,746
Adduct-forming Esters	568	682	962	1,594	2,148	2,952
Non-adduct-Forming Esters	206	204	274	338	440	720
Undistillable Esters	8	20	36	71	111	175
Distillable Esters	24	100	386	652	1,120	1,772
Adduct-Forming Distillate	105	250	570	1,058	1,494	2,456
Non-Adduct Forming Distillate	56	84	134	300	500	894

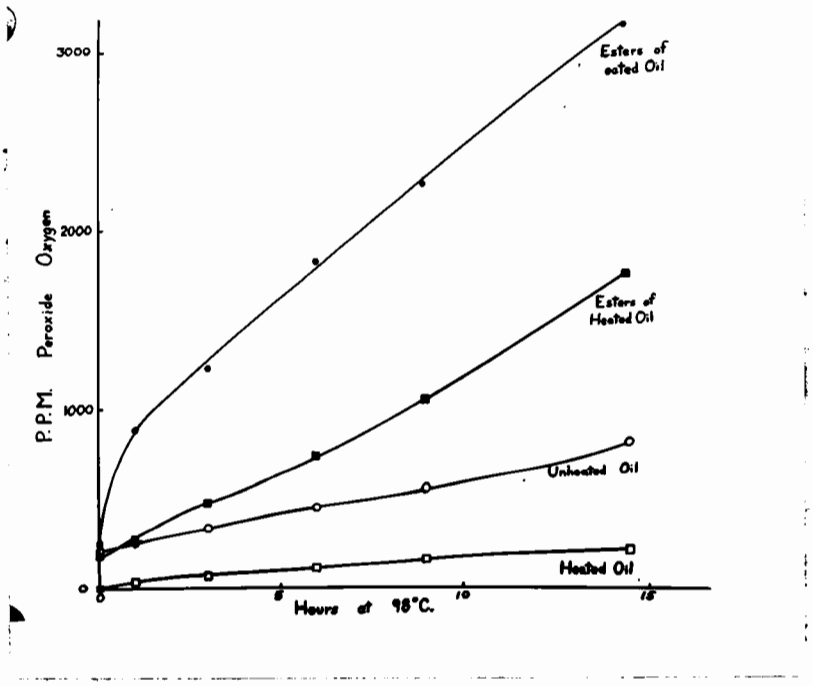

Figure 6.

The Oxidative Stability of Heated and Unheated Linseed Oils and Their Esters

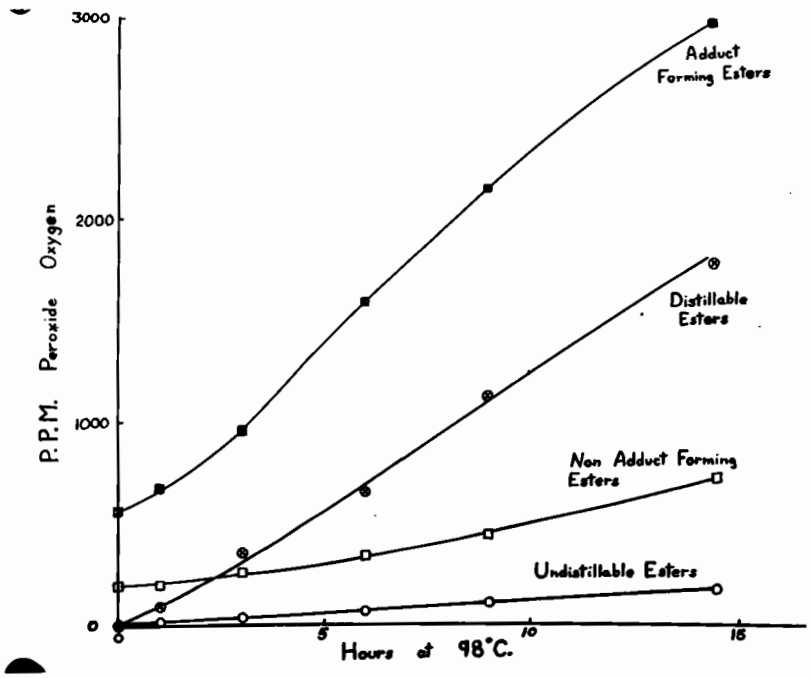

Figure 7

The Oxidative Stability of Fractions of the Esters of Heated Linseed Oil

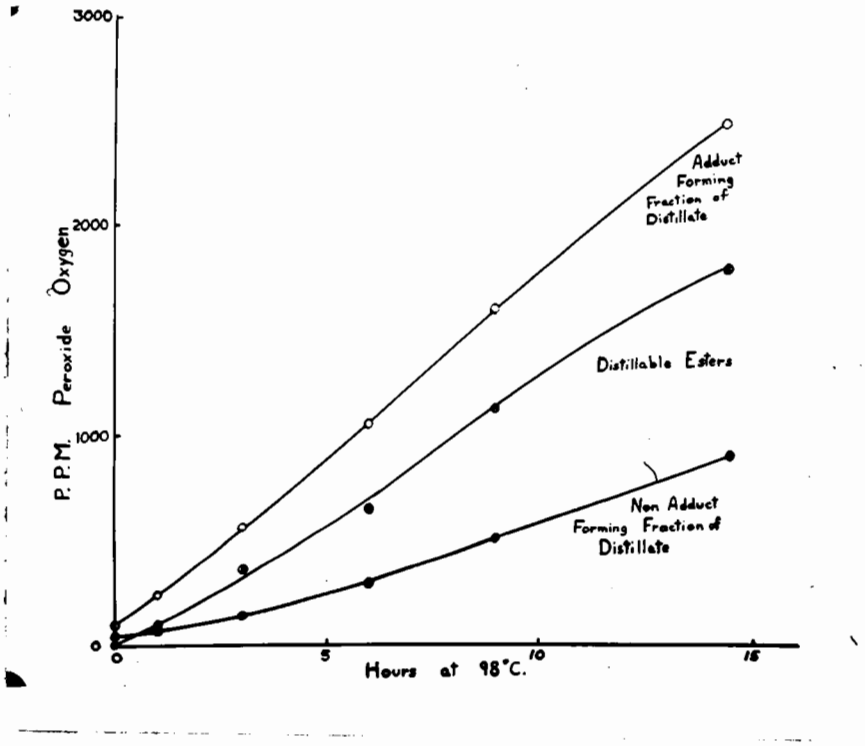

Figure 8

The Oxidative Stability of Fractions of the Distillable Esters of Heated Linseed Oil

Table VII

Yields of Various Fractions from the Methyl Esters of Heated Linseed Oil

Source	Fraction	Yield (%)	Yield as % of original mixed ester
Mixed Ester	Distillable	72	72
	Undistillable	28	28
Mixed Ester	Adduct forming	58	58
	Non adduct forming	42	42
Distillable Esters	Adduct forming	83	60
	Non adduct forming	17	12

Discussion

Figure 6 shows that the esters of unheated oil peroxidized more rapidly than the unheated oil itself. This observation accords with the known susceptibility of the simple alkyl esters of fatty acids to peroxidation. While the esters of heated oil peroxidized more rapidly than the heated oil itself, these esters peroxidized distinctly less rapidly than the esters of unheated oil.

Figure 7 shows that the adduct forming fraction of heated linseed oil peroxidized very rapidly. The rate of peroxidation of this fraction was approximately the same as that of the unheated esters. These observations suggest that the esters of heated oil may have contained material which slowed the rate of peroxidation and which did not form urea adducts.

The data for the peroxidation of fractions separated by vacuum distillation shows that distillation destroyed peroxides present in the esters of heated oil. The distillable fraction peroxidized at a slower rate than the fraction separated as a urea adduct. The undistillable fraction peroxidized at a somewhat lower rate than the corresponding fraction which did not form an adduct. Thus both fractions separated by distillation were slightly more resistant to peroxidation than the corresponding fractions separated by adduct formation. It seemed possible either (a) that heating during distillation destroyed peroxides already present, to such a degree that peroxidation was not resumed at the rate realized prior to distillation, or (b) that material produced during distillation tended to render the ester more resistant to peroxidation than they were originally.

Figure 8 shows the results of stability tests on the fractions, obtained by urea segregation of the distillable esters. The adduct forming fraction of the distillable esters was less resistant to peroxidation than the whole distillable fraction. In fact, it peroxidized at approximately the same rate as the adduct-forming fraction of the whole esters. The non adduct-forming fraction was considerably more resistant to peroxidation than the whole distillable fraction.

It seems evident that distillation of the esters of whole heated linseed oil yielded a monomeric fraction which contained

material not present in the monomeric fraction separated by urea adduct formation, this material being of such a nature that it enhanced resistance to peroxidation.

The difference in yields of monomeric material by urea segregation and by distillation, which were noted in Section III, have been confirmed in this experiment. This experiment has shown also that monomeric material which did not form urea adducts could be separated by urea segregation of the distillable esters. There is not sufficient evidence to conclude that this material represents monomeric esters of cyclic fatty acids, but the available data would be consistent with this view. Urea segregation of the distillable esters gave yields of adduct forming material which were comparable to the yields obtained directly from the whole mixed esters.

Section V

Segregation of Toxic and Non-toxic Fractions from the Esters of Heat Polymerized Linseed Oil

Introduction

Results of the experiment reported in Section III were rather unsatisfactory due to excessive peroxidation of the oil fractions. The death of several animals from non-dietary causes during the course of that experiment also reduced the value of the results obtained. However, the experiment provided some indication that the toxic material resulting from heat polymerization might reside in a fraction which was distillable but which would not form urea adducts.

Repetition of that experiment, with provision for adequate protection of the oil fractions from peroxidation, was considered desirable.

Procedure and Results

Alkali refined linseed oil was heat polymerized for 12 hours at 275°C. Ethyl esters were prepared from this oil by alcoholysis. The following fractions of the ethyl esters were then prepared:

1. Linear monomers from whole esters:- that portion of the ethyl esters of heated linseed oil which formed urea adducts with ease.
2. "Cyclized" monomers and dimers from whole esters:- that portion of the esters of heated oil which did not form urea adducts.

3. Linear and "cyclized" monomers from distillate:- that portion of the esters of heated linseed oil which was distillable at reduced pressure.
4. Linear monomers from distillate:- that portion of the distillable esters which formed urea adducts.
5. "Cyclized" monomers from distillate:- that portion of the distillable esters which did not form urea adducts.
6. Dimers by distillation:- The residue after removal of the monomeric esters by distillation.

It is to be understood that the designations of the fractions given here do not imply that the natures of the fractions are definitely established. Whether the non-adduct forming monomeric material from Fractions 2, 3 and 5 is cyclic or branched is not established. However, since the existence of such material has been suggested by Paschke and Wheeler (1949), the term "cyclized monomer" will be used here for convenience.

Special precautions were taken to prevent autoxidation of the oil fractions in the diets. All oil fractions were protected immediately after preparation by the addition of 0.1% of Tenox II (A commercial preparation of the Eastman Tennessee Corp., containing 20% butylated hydroxyanisole, 4% citric acid, 6% propyl gallate and 70% propylene glycol).

In addition to the above protection, the fractions which segregated with urea were protected during that operation by the addition of 0.025% of Tenox II before the beginning of the urea

segregation. The adduct forming material was protected during washing by the addition of 0.025% of Tenox II immediately after the adduct was decomposed.

The mixed diets were stored in the refrigerator. Peroxide values were determined on the oil extracted from the mixed feeds at the end of the second and fourth weeks of the trial. The values secured are reported in Table IX.

Table IX

Peroxide Values of Oils Extracted from the Mixed Feeds at Two Weeks and Four Weeks after Preparation. Feeds Stored in Refrigerator

Oil fraction in the diet	Peroxide Value (mg. peroxide oxygen per kg. oil)	
	After 2 weeks	After 4 weeks
Fraction 1	44	98
Fraction 2	38	88
Fraction 3	42	90
Fraction 4	46	102
Fraction 5	38	92
Fraction 6	33	75

The nutritional tests on the six oil fractions were performed in two replicates, each comprising observations on 60 animals. Half of the animals received diets containing 10% oil; while the remainder received diets containing 20% oil. The results of these tests are shown in Tables X and XI.

Table X

The Effect of Feeding Fractions of Polymerized Linseed Oil at 10% Level in Rat Diets

Fraction Fed	No. of Animals Surviving Test	Average Gain (gm)	Average Feed Intake (gm)	Mean Gain Adjusted to equal Digestible Cal. Intake	Digestibility of dry matter %
1. Linear monomers of whole esters	10	107	300	72	95
2. "cyclic" monomers and dimers of whole esters	10	39	205	58	87
3. Linear and "cyclic" monomers of distillate	10	66	228	66	94
4. Linear monomers of distillate	10	108	299	75	95
5. "cyclic" monomers of distillate	0	-	-	-	-
6. Dimers	10	77	276	64	87

Table XI

The Effect of Feeding Fractions of Polymerized Linseed Oil at 20% Level in Rat Diets

Fraction Fed	No. of Animals Surviving Test	Average Gain (gm)	Average Feed Intake (gm)	Mean Gain Adjusted to equal Digestible Cal. Intake	Digestibility of dry matter %
1. Linear monomers of whole esters	10	101	266	72	93
2. "Cyclic" monomers and dimers of whole esters	2	-32	141	4	81
3. Linear and "cyclic" monomers of distillate	9	4	118	52	94
4. Linear monomers of distillate	10	57	193	63	94
5. "Cyclic" monomers of distillate	0	-	-	-	-
6. Dimers	7	13	168	71	55

Animals receiving diets containing Fraction 5, i.e. "cyclized" monomer, consumed only small amounts of feed. The possibility that the death of some or all of the rats in this group might have been caused by low feed intake, was examined by the addition of a supplementary group of five rats to the second replicate of the experiment. These rats were allowed to consume the basal oil-free diet ad libitum but were dozed daily by dropper with a quantity of "cyclized" monomer equal to 10% of their measured voluntary intake of the fat free mixture.

The intake of the basal diet under these conditions was low, but it was adequate for maintenance and in one case permitted some gain. Nonetheless, all these rats died within 16 days, thus demonstrating that the mortality in Group 5 could not be explained on the grounds of the low food intake.

Some chemical characteristics of these oil fractions were determined and are shown in Table XII.

Discussion

It may be seen from Table IX that peroxidation of the diets was relatively slight. There was no appreciable difference between the fractions in this respect. The addition of a suitable antioxidant and storage of the diets in the refrigerator appear to provide adequate protection. It may be reasonably concluded that the results of the feeding trials reported in this Section were due to the effects of constituents other than products of peroxidation.

Table XII

Yields and Characteristics of Fractions of Ethyl Esters of Heated Linseed Oil Used in the Feeding Trials

Oil Fraction	Yield as % of total Esters of Heated Oil	Iodine Value	Refractive Index (25°C)	Mean Molecular Weight	Hydroxyl Value (% OH)
1. Linear Monomers of whole Esters	46	118.2	1.45345	293	0.23
2. "Cyclic" monomers and dimers of whole esters	54	162.7	1.47561	472	0.46
3. Linear and "Cyclic" monomers of distillate	60	130.1	1.45684	294	0.22
4. Linear monomers of distillate	49	124.8	1.45494	293	0.14
5. "Cyclic" monomers of distillate	11	170.7	1.46986	300	0.34
6. Dimers	40	159.9	1.48017	550	0.64

The most striking feature of the results of these feeding trials was related to mortality. It is clear that the presence of "cyclized" monomers in the diet was associated with toxicity. Rats consuming the diets containing Fraction 5, which contained the highest concentration of this material, did not survive the full 28 day test period. A comparison of the results of Diet 2, containing a mixture of approximately three parts of dimeric esters to one part of "cyclized" monomer, with the results of Diet 6, shows clearly the much more harmful nature of the monomeric material. The deaths in Group 6 were associated with partial starvation and excessive diarrhoea, whereas the animals of Group 5 displayed neither diarrhoea, nor digestive disturbances, nor overt clinical symptoms suggestive of the cause of death.

The animals receiving fractions consisting mainly of straight chain monomers, i.e. Groups 1 and 4, all survived the test and remained in good health. Growth of the rats in these groups was comparable to that obtained in previous tests with esters of unheated oil. It should be pointed out that this was the first occasion on which a fraction prepared from heated oil gave such favorable growth response in the experiments conducted at Macdonald on this subject.

The gains of the rats receiving the various fractions reflect the toxic effects of the "cyclized" monomeric material and the adverse effects of the dimeric esters. The dimeric ester fraction was associated with low digestibility, and this was

doubtless the cause of the diarrhetic condition of the rats receiving this material.

The relative efficiencies of the diets per unit digestible calories eaten show that the straight chain monomeric esters and the dimeric esters were equally well "metabolized", whereas the presence of "cyclized" monomeric material was reflected at once by sharp reduction in the utilization of digestible calories.

Fractions 1 and 4 should both consist mainly of the straight chain adduct forming material. However, it will be noted from Table XII that Fraction 1 had a lower iodine number and a lower refractive index than Fraction 4. Moreover, the yield of Fraction 1 represented 46% of the total esters, whereas the yield of Fraction 4 was 49%. These differences in yield and characteristics of presumptive straight chain monomeric material according to the route by which it has been prepared, suggest that in the case of Fraction 4 the adduct-forming fraction has probably carried with it by entrainment a small proportion of material incapable of itself of forming an adduct, i.e. "cyclized" monomer.

On referring to Table XI it will be noted that Fraction 1 was also superior to Fraction 4 in nutritive value. This circumstance reinforces the view that Fraction 4 contained a small proportion of the non-adduct forming material ("cyclized" monomer), and that the "cyclized" monomeric ester is either of itself the deleterious component or that the deleterious material accompanies this ester fraction.

SECTION VI

Investigation of the Nature of the Non-Adduct-Forming Fraction of the Distillable Ethyl Esters of Heated Linseed Oil

Introduction

The discovery that the non-adduct-forming fraction of the distillable ethyl esters of heated linseed oil was toxic to rats prompted further investigation of the nature of this fraction. It is evident that the material did not possess a linear structure because it would not form a urea adduct. However, the material might have either a branched or cyclic structure.

Procedure and Results

A sample of the non-adduct forming fraction of the distillable ethyl esters of heated linseed oil, prepared for the feeding trial reported in Section V was studied.

Since the iodine value of this fraction, 170.7 (Table XII), indicated that the material was mainly dienoic, an investigation was made of the ultra-violet absorption spectra of this material. Figure 9 shows the absorption spectra obtained before and after alkali-isomerization for 25 minutes at 180°C and after six hours isomerization at 180°C. The six hour isomerization time was used on account of the possibility of the presence in this fraction of cis-transisomers of ethyl linoleate. Paschke, Jackson and Wheeler (1952) have found that trans, trans-linoleate requires six hours to reach a maximum isomerization. However, in this case the presence of a small amount of trienoic fatty acid may

invalidate calculations from this absorption spectrum.

The fatty acid composition of this fraction was calculated from the ultra-violet absorption data obtained from the 25 minute isomerization time, by the method recommended by the Spectroscopy Committee, American Oil Chemists' Association (1949). These values are set out in Table XIII. Although these values are reported as "linoleic" and "linolenic" acids, it must be kept in mind that the actual acids present may not be linoleic and linolenic acids, but may include isomers of these acids which give diene and triene conjugation on alkali-isomerization.

Table XIII

Fatty Acid Composition of the Non-Adduct Forming Fraction of the Distillable Ethyl Esters of Heated Linseed Oil

% Conjugated Diene	2.9
% Conjugated Triene	0.0
% "Linoleic" acid	4.7
% "Linolenic" acid	2.1
Total	9.7

In order to determine the number of carboxyl groups per molecule, the saponification equivalent was determined for comparison with the mean molecular weight.

Saponification equivalent 330
Mean Molecular weight (Table XII)...300

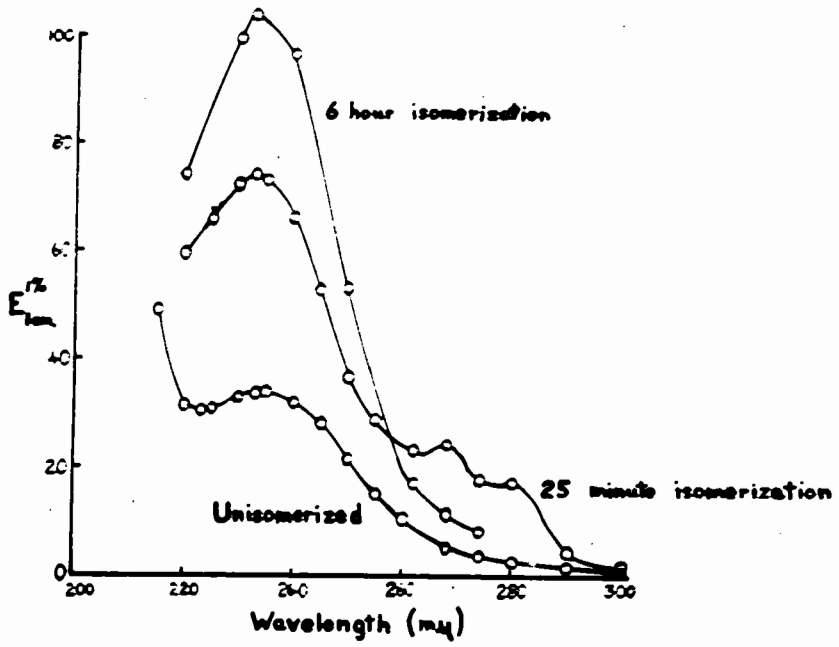

Figure 9

Absorption Spectra of the Non-Adduct Forming Fraction of the Distillable Ethyl Esters of Heated Linseed Oil

In an effort to determine if this fraction consisted of branched chain or cyclic esters, carbon-linked methyl groups were determined and compared with those obtained from the adduct-forming fraction of the same oil. This comparison is shown in Table XIV.

If branched chain fatty acids were present, yields of acetic acid should have been greater from this fraction than from the adduct-forming fraction. If cyclic acids were present, two possibilities arose; (1) That the ring was terminal. In this case yields of acetic acid should be lower for this fraction than for the urea adduct forming fraction. (2) That the ring was not terminal. In this case the yields from both fractions should have been the same.

Table XIV

Terminal Methyl Numbers of Adduct Forming and Non-Adduct Forming Fractions of the Distillable Ethyl Esters of Heated Linseed Oil

Sample	Weight of Sample (mg.)	Yield of Acetic Acid (millequivalents)	Terminal Methyl No.
Blank	0.0	0.012	
Non-Adduct Forming Distillate	21.1	0.131	1.70
Adduct Forming Distillate	23.6	0.148	1.74

Discussion

The first notable result of this experiment was that, although the non-adduct-forming fraction of the distillable ethyl esters of heated linseed oil had an iodine value of 170.7, less than 10% of the fraction could be accounted for as "linoleic", "linolenic" or conjugated diene acids. Even if "linoleic" acid was calculated from the absorption spectrum of the fraction after six hours isomerization, this amount was increased only slightly. It appears that this fraction contained a large amount of a dienoic fatty acid in which the double bonds could not be conjugated even by a six hour isomerization.

The small percentage of OH in this fraction, noted in Section V (Table XII), may habe been due to monoglycerides. Monoglycerides, resulting from incomplete conversion of the oil to the ester, would be concentrated in this fraction. On this basis, the fraction contained approximately 3.5% of monoglyceride. Such monoglycerides could conceivably carry some of the "linoleic", "linolenic" and conjugated diene acids noted above.

When results of the determination of the saponification equivalent were compared with the mean molecular weight (Table XII), it was found that the saponification equivalent was about 10% higher. This may indicate that the fraction contains a small amount of hydrocarbons or other compounds which do not bear a carboxyl group.

The results of the determination of carbon linked methyl groups presented in Table XIV suggests that the material consisted mainly of esters containing a non-terminal ring.

It is evident that the non-adduct-forming fraction of the distillable ethyl esters of heated linseed oil was not homogeneous. It apparently contained small amounts of "linoleic", "linolenic" and conjugated diene acids, as well as small amounts of hydroxyl-bearing compounds, possibly monoglycerides, and small amounts of hydrocarbons or other compounds which did not bear a carboxyl group. However, the weight of the evidence available points to monomeric esters containing a non-terminal cyclic structure as the most probable major constituent of the non-adduct-forming fraction of the distillable esters.

Section VII

Some Observations on the Mechanism and Kinetics of the Thermal Polymerization of Soybean Oil

Introduction

Continuation of the study of the toxicity of heated vegetable oils necessitates its extension to oils other than linseed oil. As a preliminary to nutritional studies on soybean oil, it was considered desirable to make a brief study of its thermal polymerization.

Procedure and Results

Raw soybean oil was alkali-refined with 3% of 20° Baume NaOH. The oil was then bleached with 2% of activated bleaching clay (Super Filtrol) for 15 minutes at 100°C. The resulting oil was a light straw color and contained 0.04% of free fatty acids (calculated as oleic).

Five hundred gram lots of the alkali refined oil were heat polymerized at 275°C for 3,9,15, 21 and 30 hours. These polymerized oils were converted to the mixed methyl esters by alcoholysis. Portions of the mixed esters were vacuum distilled and the fatty acid compositions of the distillates were determined.

Tables XV, XVI and XVII show the chemical characteristics of the oils, the mixed esters and the distillable esters respectively

Table XV

Chemical Characteristics of Heated Soybean Oils

Hours Heated	Mean Molecular Weight	Iodine Value	Refractive Index (25°C)	Acid Value (%f.f.a.)
0	867	144.8	1.4733	0.04
3	863	130.7	1.4737	0.66
9	912	124.4	1.4744	0.43
15	1000	119.2	1.4754	0.56
21	1103	110.1	1.4767	0.75
30	1302	100.3	1.4786	0.69

Table XVI

Chemical Characteristics of Mixed Methyl Esters of Heated Soybean Oil

Hours Heated	Mean Molecular Weight	Iodine Value	Refractive Index (25°C)	Acid Value (% f.f.a)
0	293	144.5	1.4560	0.06
3	298	131.6	1.4564	0.09
9	305	124.3	1.4572	0.11
15	315	118.2	1.4583	0.08
21	332	112.5	1.4593	0.10
30	364	102.2	1.4608	0.05

Table XVII

Chemical Characteristics of the distillable Methyl Esters of Heated Soybean Oil

Hours Heated	Mean Molecular Weight	Iodine Value	Refractive Index	F.F.A. (%)
0	293	144.5	1.4560	0.08
3	295	132.3	1.4559	0.10
9	290	129.8	1.4558	0.12
15	294	123.0	1.4556	0.10
21	293	117.0	1.4554	0.12
30	291	108.6	1.4548	0.08

NOTE:

Iodine values reported in Tables XV, XVI and XVII were determined by the method of Hoffman and Green (1939).

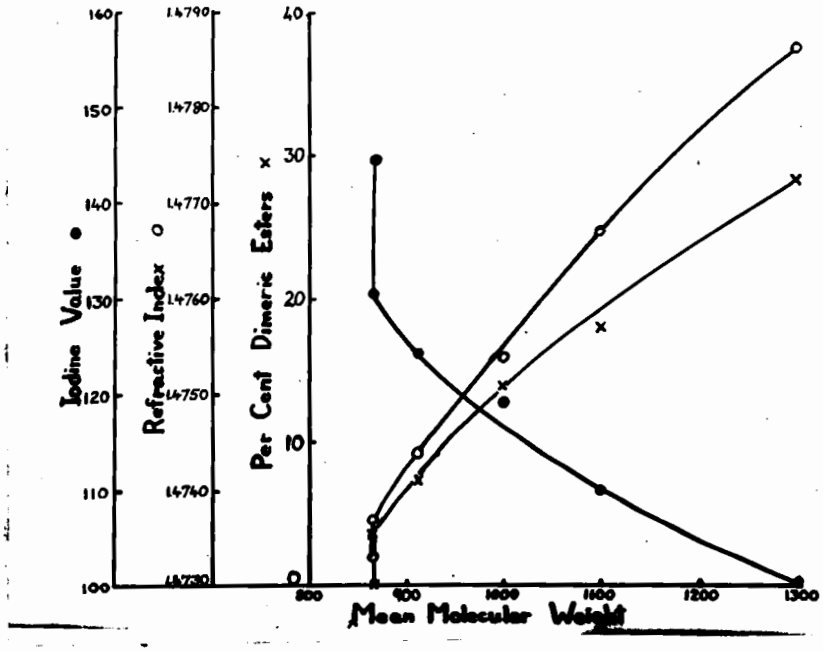

Figure 10

Relationship of Chemical Characteristics to Mean Molecular Weights for a series of Thermally Polymerized Soybean Oils

Figure 10 shows the relationship between some of the chemical characteristics of the heat polymerized oils and their mean molecular weights.

Table XVIII shows the fatty acid compositions of the distillable methyl esters of this series of heat polymerized soybean oils. In Table XIX these values are presented as percentages of the total methyl esters. Figure 11 shows the changes in fatty acid composition of the total methyl esters. Figure 12 shows the proportion of the original linolenic and linolenic acids remaining as polymerization proceeds. Iodine values rather than bodying times have been used for abscissae for these figures because of the difficulty of estimating the effects of heat-up and cool-down times.

It should be emphasized again that, while fatty acid compositions are reported in terms of linolenic and linoleic acids, the acids actually present may not have been linolenic and linoleic acids but may have included isomers of those acids which gave diene and triene conjugation on alkali-isomerization.

Table XVIII

Fatty Acid Composition of the Distillable Methyl Esters of Heated Soybean Oils

Hours Heated	0	3	9	15	21	30
% Conj. Diene	0.0	0.9	5.6	4.9	5.8	5.4
% Linolenic	8.0	7.1	5.4	3.2	2.4	1.2
% Linoleic	56.4	52.8	34.9	30.8	24.6	17.9
% Arachidonic	1.1	0.4	0.2	trace	trace	0.0
% Oleic	19.0	22.9	37.5	43.8	49.3	45.6
% Saturated	11.1	11.5	12.0	12.9	13.5	15.5

Table XIX

Fatty Acid Composition of Total Methyl Esters

% Conj. Diene	0.0	0.9	5.2	4.2	4.8	3.9
% Linolenic	8.0	6.8	5.0	2.8	2.0	0.9
% Linoleic	56.4	50.8	32.2	26.5	20.2	12.8
% Arachidonic	1.1	0.4	0.2	trace	trace	0.0
% Oleic	19.0	22.0	34.6	37.7	40.4	32.7
% Saturated	11.1	11.1	11.1	11.1	11.1	11.1
% Dimers	0.0	3.7	7.8	14.0	18.0	28.3

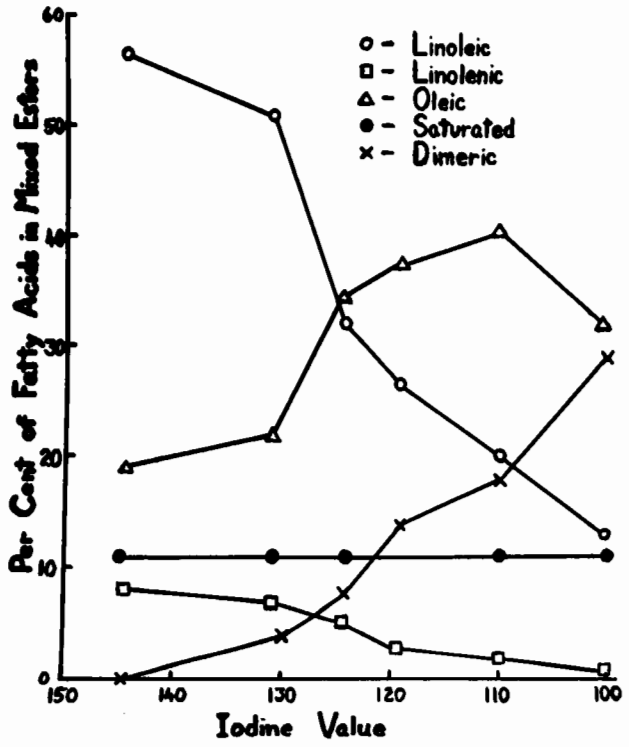

Figure 11

Changes in Fatty Acid Composition
During Thermal Polymerization of
Soybean Oil

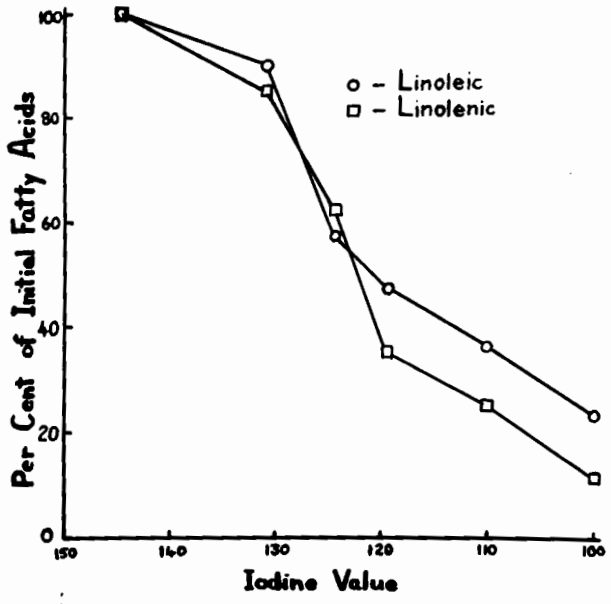

Figure 12

Disappearance of Linoleic and Linolenic Acids during Thermal Polymerization of Soybean Oil

The values for oleic acid given in Tables XVIII and XIX were calculated by assuming that the content of saturated acids in the oil did not change appreciably during thermal polymerization. This method of calculation was adopted on account of the known unreliability of the results of iodine value determinations on polymerized oils. Another factor which argued for the adoption of this method of calculating oleic acid was the possibility that the bodied oils might contain dienoic acids which could not be alkali-isomerized to conjugated diene by the usual treatment.

Figure 13 shows the relationship between dimeric acid content and mean molecular weight for this series of soybean oils. This figure also includes a theoretical relationship between dimeric acid content and mean molecular weight. The theoretical relationship is based on the assumption that intramolecular dimerization of fatty acid residues does not occur.

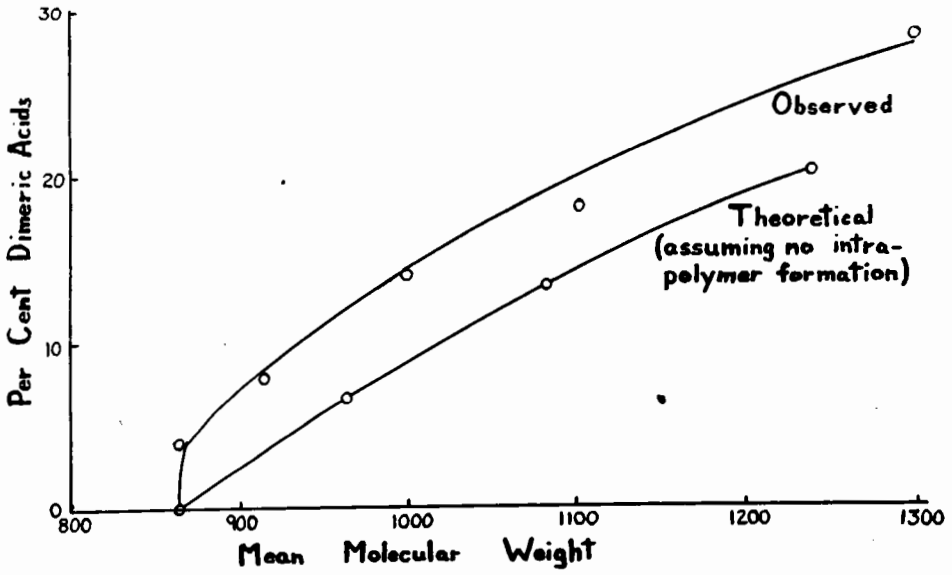

Figure 13

Relationship between Mean Molecular Weight
and Dimeric Acid Content in
Heated Soybean Oil

Iodine values reported thus far in this section have been determined by the Rapid Wijs method of Hoffman and Green (1939). Iodine values were also determined on the polymerized oils by the method of Benham and Klee (1950). It was found that constant iodine values were obtained at the end of one hour by this method if chloroform was used as the solvent. Table XX shows these values. The relationship between iodine values determined by this method and the dimeric acid content of the oils is shown in Figure 14.

Discussion

Figures 11 and 12 show that the reactions taking place during thermal polymerization apparently proceed in three steps. During the first phase of the reaction the content of linolenic and linoleic acids decreased only slightly, while the content of oleic remained at nearly the same level. It will be noted from Table XIX that during this same period only a very small amount of conjugated diene was formed.

Table XX

Iodine Values of Thermally Polymerized Soybean Oils

Hours Heated	% Dimeric Acids	Iodine Value
0	0	128.0
3	3.7	126.2
9	7.8	124.2
15	14.0	120.9
21	18.0	119.5
30	28.3	115.5

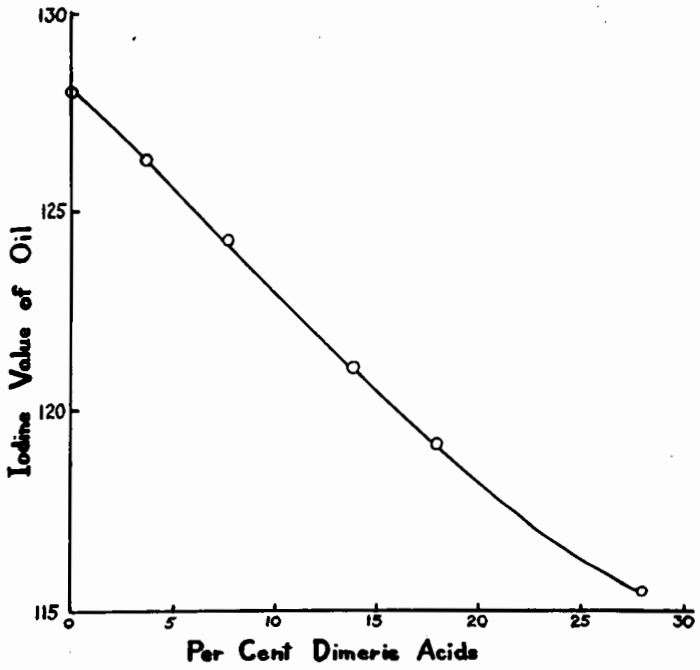

Figure 14

Relationship between Benham and Klee Iodine Value and Dimeric Acid Content in Heated Soybean Oil

During the second phase of the reaction the content of linolenic and linoleic acids decreased rapidly, while the content of oleic acid increased rapidly. The content of conjugated diene also increased during this period.

During the final phase the rate of disappearance of linolenic and linoleic acids was somewhat slower. The oleic acid content remained at a high level, dropping only slightly in the final sample. The remarkable similarity of the rates of disappearance of linolenic and linoleic acids is also shown in Figure 12.

Interpretation of these data is very difficult. However, the slow rate of disappearance of linolenic and linoleic acids during the initial phase, emphasizes the role of conjugation as an initial step in these reactions.

The theoretical relationship between mean molecular weight and dimeric acid content as shown in Figure 13 was derived from the following consideration:

Let one consider a hypothetical system containing ten triglyceride molecules, each of molecular weight X, the total weight of such a system would be 10X and the mean molecular weight would be X. If union now occurs between two fatty acid residues in different triglyceride molecules, the number of molecules in the system becomes 9. The total weight of the system is still 10X, so the mean molecular weight becomes 10X/9.

The number of fatty acid residues in such a system would be 30. After a single dimerization reaction, two of these fatty acid residues would form a dimeric acid and thus the weight percentage of dimeric acids in the system would be $\frac{2}{30} \times 100$ or 6.7%.

Following this line of reasoning, and assuming the absence of intramolecular dimerization reactions, the relation between mean molecular weight and dimeric acid content may be calculated for a series of oils of increasing mean molecular weight. It makes no difference whether the succeeding stages involve union between monomeric glyceride and a polymeric glyceride, or between two polymeric glycerides, since in each case the number of molecular entities is decreased by one for each union of a pair of fatty acid residues.

The equation describing this implied relation is:

$$Y = 66.7 - 66.7 \frac{M}{X}$$

Where Y = per cent of dimeric acids

X = mean molecular of heated glycerides

and M = mean molecular weight of glycerides before heating.

It may be noted in Figure 13 that the curve representing the relation of observed dimeric acid contents to mean molecular weight falls above the curve representing the theoretical relation. The divergence of these two curves suggests

that intramolecularly dimerized material was present in these oils.

Intramolecular dimerization could conceivably take place as follows:

(a) Two fatty acid residues in the same triglyceride might unite. A system containing dimeric acid residues produced in this way would show no increase in mean molecular weight.

(b) Union might take place between fatty acid residues which have not yet polymerized and which are located in a dimeric glyceride. This type of intramolecular dimerization would give double the dimeric acid content expected from the mean molecular weight on the assumption of no intrapolymer formation.

(c) Union might occur between fatty acid residues in a higher polymeric glyceride. In this case the percentage of dimeric acids would be less than twice that to be expected on the assumption of no intrapolymer formation, but would still be greater than this hypothetical value.

While the results shown in Figure 13 do not show definitely which type of intramolecular dimerization occurred, the initial rise in dimeric acid content without any increase in mean molecular weight suggests that intramolecular dimerization occurred, at this early stage, between fatty acid residues in the same monomeric glyceride molecule.

Examination of similar data for linseed oil reported by Wiseblatt (1950) showed the same divergence of the observed and theoretical curves. Moreover, Wiseblatt's data for the acetone soluble fractions of a series of thermally polymerized linseed oils, showed that the observed contents of dimeric acids were more than double those calculated from the mean molecular weights by the given equation. It appears therefore, that intramolecular dimerization occurs within single triglyceride molecules in the case of polymerized linseed oil and it is possible that the same type of reaction occurs in the case of polymerized soybean oil.

Mention should be made of the apparent linearity of the relationship between dimeric acid content and Benham and Klee idoine values as shown in Figure 14. It will be noted that the iodine value declined about 0.43 units for each unit decline in the figure for per cent dimeric acids. This decline in iodine value corresponds to the loss of one double bond for each dimeric acid formed. A Diels-Alder condensation of fatty acid residues would result in the loss of two double bonds for each dimeric acid formed. Although these results may have been simply an analytical coincidence, they appear to provide some support for the mechanism of thermal polymerization suggested by Barker, Crawford and Hilditch (1951).[a] The mechanism proposed by these authors has already been discussed in the Review of Literature.

GENERAL DISCUSSION

It has been shown that, in thermally polymerized linseed and soybean oils, the increase in refractive index bears a linear relationship to the increase in dimeric acid content. This relationship has been utilized for the preparation of polymerized oils of known dimeric acid content. However, it was necessary to establish this relationship for each batch of oil before proceeding with the preparation of these polymerized oils. It is possible, that examination of this relationship in a large number of samples might provide data from which a general equation could be derived for each oil, relating the refractive index corresponding to a given dimeric acid content with the initial refractive index of the oil. In other words, the slope of the line relating these two characteristics may bear some ascertainable relationship to the initial refractive index.

Urea segregation techniques have provided a means of separating a nutritionally satisfactory fraction from the ethyl esters of thermally polymerized linseed oil. The fact that this fraction was as nutritious as the ethyl esters of unheated linseed oil, suggests that any linear isomers formed during the heat treatment, either by cis-trans isomerization or by a change in the position of double bonds, did not

reduce the nutritive value of the fraction.

The most toxic fraction separated during these experiments appeared to consist mainly of cyclic monomeric esters in which the ring is not terminal. Research is now being conducted by the Departments of Chemistry and Nutrition, Macdonald College, to determine the biological effects of this fraction when it is injected into experimental animals. The results of these experiments will be compared with the results obtained by previous workers, who had injected known branched chain and cyclic fatty acids into experimental animals.

Monomeric cyclic esters appeared to form the major part of the non-adduct-forming fraction of the distillable ethyl esters of heated linseed oil. The iodine value of this fraction suggests that the cyclic esters were dienoic. Presumably these cyclic, dienoic esters were formed from linolenic acid residues. It is not known if cyclic fatty acid residues are formed during heating, in oils which contain only linoleic and more saturated fatty acids. If cyclic fatty acid residues are, in fact, the toxic agent in polymerized linseed oil, it still remains to be seen if cyclized linoleic acid, which would presumably be monoethenoid, is toxic. Investigations of the nutritional value and chemical composition of thermally polymerized sunflower seed oil, which contains little or no linolenic acid, may provide valuable information concerning the formation and possible toxicity of cyclized linoleic acid.

SUMMARY

1. Linseed and soybean oils are reduced in nutritive value by thermal polymerization.

2. This reduction in nutritive value is related to the unsaturation of the oil and to the length of the thermal treatment. However, the content of dimeric acids bears a different relation to the reduction in nutritive value in linseed oil than it does in soybean oil.

3. Dimeric ethyl esters of thermally polymerized linseed oil decrease the digestibility of rat diets in which they are included. However, these esters do not cause any reduction in the utilization of digestible calories.

4. The fraction of the ethyl esters of thermally polymerized linseed oil which forms adducts with urea appears to be as nutritious as the ethyl esters of unheated linseed oil.

5. A fraction which is extremely toxic to rats has been separated from the ethyl esters of thermally polymerized linseed oil, by a combination of distillation and urea segregation techniques.

6. This toxic fraction appears to be composed mainly of cyclic monomeric esters in which the ring is not terminal. These esters appear to be dienoic but the double bonds are separated so that they cannot be conjugated by alkali-isomerization.

7. A study has been made of the oxidative stability of several fractions of the ethyl esters of polymerized linseed oil. The non-adduct-forming fraction of the distillable esters appears to contain material which increases the oxidative stability of that fraction.

8. The course of thermal polymerization of soybean oil has been studied. Data are presented which appear to lend some support to the view that thermal polymerization proceeds by a mechanism which involves the formation of a single carbon-to-carbon linkage between fatty acid residues.

9. Evidence is presented also for the formation of intra-polymers during the course of thermal polymerization of soybean oil.

REFERENCES

1. Adams, H. E. and P. O. Powers, 1944.
   Mechanism of heat bodying linseed oil
   Ind. Eng. Chem 36: 1124-7.

2. Ahmed, A. and E. H. Farmer, 1940.
   Mechanism of polymerization Part V Dimerization of unconjugated pentadiene
   J. Chem. Soc. 1940: 1176-8

3. Anderson, R. J. 1929
   Chemical investigation of biologically active lipids of tubercule bacilli.
   Proc.Nat. Acad. Sci. 15: 628-33.
   (C.A. 23: 5502, (1929)

4. Anderson, R. J. and E. Chargaff, 1929
   The chemistry of the lipoids of the tubercule bacilli IV Concerning tuberculestearic acid and phthioic acid.
   J. Biol Chem 85: 77-88.

5. Armstrong, J. G. and W. D. McFarlane, 1944.
   Flavour reversion in linseed shortening
   Oil and Soap 21: 322-7

6. Barker, C. R.V. Crawford and T. P. Hilditch 1951(a)
   Some chemical changes associated with the thermal polymerization of drying oils
   J. Oil and Color Chemists Assoc. 34: 215-31

7. Barker, C. R.V. Crawford and T.P. Hilditch, 1951(b)
   Thermal interchange of acyl groups in triglycerides
   J. Chem. Soc. 1951: 1194-1200

8. Barrer, R.M. 1945.
   Separation of mixtures using zeolites as molecular sieves, Part 1. three classes of molecular-sieve zeolite.
   J. Soc. Chem. Ind 64: 130-1

9. Barthel, W.F. and F. B. LaForge, 1944
   Determination of C-linked methyl groups
   Ind. Eng. Chem. Anal Ed 16: 434-5

10. Bengen, M.F. 1940.
    German Patent Appl. O.Z. 12438 (1940)
    Technical oil mission Reel 6, p. 263

11. Banham, G.H. and L. Klee, 1950
An improved method for the determination of iodine numbers.
J. Am. Oil Chem. Soc. 27: 127-9

12. Bernhard, K. amd L. Muller, 1938
Behavior of hydrocyclic compounds in the animal body. IV Metabolism of cyclopentenyl and cyclopentyl fatty acids.
Z. PhysiolChem 256: 85-9.
(Cited from C.A. 33: 1377, (1939)

13. Bernstein I.M. 1946.
Heat polymerization of noncomjugated vegetable oils
J. Polymer Sci. 1: 495-528

14. Bernstein I.M. 1948
Polymer fractionation of heat polymerized non-conjugated vegetable oils.
J. Phys. & Colloid Chem. 52: 613-61

15. Bernstein, I.M. 1949.
Thermal polymerization of linseed and soybean oils
J Oil and Color Chemists Assoc. 32: 447-60

16. Bolland, J.L. and G. Gee, 1946
Kinetic studies in the chemistry of rubber and related materials III thermochemistry and mechanisms of olefin oxidation
trans Farady Soc. 42: 244-52

17. Bradley, T. F. and W. B. Johnston, 1940
Drying oils and resins. Reactions involving the carbon-to-carbon unsaturation during the thermal treatment of some esters of unsaturated C18 fatty acids.
Ind. Eng. Chem. 32: 802-9 (1940)

18. Bradley, T. F. and H. F. Pfann, 1940.
Drying oils and resins. Constitution of a drying oil gel
Ind. Eng. Chem 32: 694-7

19. Bradley, T. F. and D. Richardson, 1940.
Drying oils and resins. Ultraviolet absorption study of esters of the acids of drying oils.
Ind. Eng. Chem. 32: 963-9

20. Buu-Ho'i and A.R. Ratsimamanga, 1941.
Toxicity of chaulmoogra oil and its derivatives; role of the double bond.
Bull Soc Chim Biol 23: 459-64.
(Cited from C.A. 37:180, (1943)

21. Buu-Ho'i and A.R. Ratsimamanga, 1943.
Sur la production des lesions type tuberculeux par des acides gras synthetiques --αα-disubstitutes.
Compt Rend Soc Biol 137: 189-90

22. Brod, V.S., W.G. France and W.L. Evans, 1939.
Thermal polymerization of ethyl eleostearate and 9, 11- and 9, 12-ethyl linoleate.
Ind. Eng. Chem. 31: 114-8

23. Cagnant, P., A. Sartory and J. Meyer, 1950.
Contribution a l'étudie des propriétés biologiques des acides gras ramifiés. II Sur les propriétés physiologique de l'acideα-dimethyl-tridecylenique et de quelques autres acidesαα- disubstitutés.
Ann Inst Pas teur 78: 382-91

24. Crampton, E.W., F.A. Farmer and F.M. Berryhill, 1951
The effect of heat treatment on the nutritional value of some vegetable oils.
J. Nutrition 43: 431-40

25. Crampton, E.W., R.H. Common, F.A. Farmer, F.M. Berryhill and L. Wiseblatt, 1951(a).
Studies to determine the nature of the damage to the nutritive value of some vegetable oils from heat polymerization. I The relation of autoxidation to decreas e in the nutritional value of heated linseed oil.
J. Nutrition 43: 533-9

26. Crampton, E.W., R.H. Common, F.A. Farmer, F.M. Berryhill and L. Wiseblatt, 1951(b).
Studies to determine the nature of the damage to the nutritive value of some vegetable oils from heat treatment. II Investigation of the nutritiousness of the products of thermal polymerization of linseed oil.
J. Nutrition 44: 177-89

27. Davies, W.L., and E. Gill, 1936.
Investigations on fishy flavor.
J. Soc. Chem. Ind. 55: 144-6T

28. Farmer, E.H., 1946.
Peroxidation in relation to olefinic structure.
Trans. Faraday Soc. 42: 228-36

29. Farmer, E.H., G.F. Bloomfield, A. Sundralingam and D.A. Sutton, 1942.
The course and mechanism of autoxidation reactions in olefinic and polyolefinic substance, including rubber.
Trans. Faraday Soc. 38: 348-56

30. Farmer, E.H., H.P. Koch and D.A. Sutton, 1943.
The course of autoxidation reactions in polyisoprenes and related compounds. Part VII Rearrangement of double bonds during autoxidation.
J. Chem. Soc. 1943: 541-7

31. Farmer, E.H., and D.A. Sutton, 1943.
The course of autoxidation reactions in polyisoprenes and allied compounds. Part IV The isolation and constitution of photochemically formed methyl oleate peroxide.
J. Chem. Soc. 1943: 119-22

32. Farmer, F.A., E.W. Crompton and M.I. Siddall, 1951.
The effect of heated linseed oil on reproduction and lactation in the rat.
Science 113: 408-10

33. Fugger, J., K.T. Zilch, J.A. Cannon and H.J. Dutton, 1951.
Analysis of fat acid oxidation products by countercurrent distribution methods. II Methyl oleate.
J. Am. Chem. Soc. 73: 2861-4

34. Golumbic, C., A.I. Schepartz and B.F. Danbert, 1946.
Flavor reversion in soybean oil. III The preparation and flavor characteristics of a simulated soybean oil.
Oil and Soap 23: 380-1

35. Gass, J.H., 1947.
Studies on the processing of vegetable oils.
M. Sc. Thesis, McGill University

36. Glasstone, S., 1946.
Textbook of physical chemistry. Second Edition, 1946.
D. Van Nostrand Company, New York

37. Hoffman, H.D. and C.E. Green, 1939.
A rapid method for the determination of iodine number.
Oil and Soap 16: 236-8

38. Holman, R.T., W.O. Lundberg and G.O. Burr, 1945.
Spectrophotometric studies of the oxidation of fats.
II The oxidation of dienoic fatty acids.
III Ultra violet absorption spectra of oxidized octadecatrienoic acids.
J. Am. Chem. Soc. 67: 1386-90, 1390-4

39. Holman, R.T., and H. Schlenk, 1950.
Separation and stabilization of fatty acids by urea complexes.
J. Am. Chem. Soc. 72: 5001-4

40. Joubert, F.J., and D.A. Sutton, 1952.
The chemistry of polymerized oils. I Characteristics of some pilchard stand oil fractions.
J. Am. Oil Chem. Soc. 29: 287-91

41. Kappelmeier, C.P.A., 1933.
Chemical processes of standoil formation.
Farben-Ztg. 38: 1018-20, 1077-9
(C.A. 27: 4425, 1933)

42. Khan, N.A., J.B. Brown and F.E. Deatherage, 1951.
Autoxidation of methyl oleate, methyl stearolate, and methyl 9, 10-dideutero-oleate.
J. Am. Oil Chem. Soc. 28: 105-9

43. Klee, L., and G.H. Benham, 1950.
The determination of the true iodine numbers of oils containing conjugated double-bond systems.
J. Am. Oil Chem. Soc. 27: 130-3

44. Knight, H.B., J.E. Coleman and D. Severn, 1951.
Reactions of fatty materials with oxygen. IX Analytical study of the autoxidation of methyl oleate.
J. Am. Oil Chem. Soc. 28: 498-501

45. Knight, H.B., C.R. Eddy and D. Severn, 1951.
Reactions of fatty materials with oxygen. VIII Cistrans isomerization during autoxidation of methyl oleate.
J. Am. Oil Chem. Soc. 28: 188-92

46. Lassen, S., E.K. Bacon and H.J. Dunn, 1949.
The digestibility of polymerized oils.
Arch. Biochem. 23: 1-7

47. Lemon, W.H., 1944.
Flavor reversion in hydrogenated linseed oil.
I The production of an isomer of linoleic from linolenic acid.
Can. J. Research 22F: 191-8

48. Linstead, R.P., and M. Whalley, 1950.
The formation of crystalline complexes between urea and esters, and their application to the separation of mixtures of esters.
J. Chem. Soc. 1950: 2987-9

49. Lund and Bjerrum, 1931.
Ber. 64: 210
(Cited from L.F. Fieser. Experiments in Organic Chemistry. Second Edition, 1941. D.C. Heath and Company, New York)

50. Martinez Moreno, J.M., A. Vazquez Roncero and J. Fernandez Jimenez, 1951.
Application of crystalline addition products of urea and organic substances to the chemistry of fats. I Separation of mixtures of triglycerides and fatty acids. Preliminary laboratory note.
Anales real soc espan fis y quim, 47B: 229-34
(C.A. 45: 9281 (1951)

51. Mattil, K.F., 1947.
Flavor stability of soybean oil. I The role of the non-saponifiable fraction.
J. Am. Oil Chem. Soc. 24: 243-6

52. Max, R.A., and F.E. Deatherage, 1951.
The synthesis and autoxidation of 8, 8, 11, 11-tetradeuterocis-9-octadecene.
J. Am. Oil Chem. Soc. 28: 110-14

53. Meites, L., and T. Meites, 1948.
Removal of exygen from gas streams.
Anal. Chem. 20: 984

54. Newey, H.A., E.C. Shokal, A.C. Mueller and T.F. Bradley, 1950.
Drying Oils and Resins. Segregation of fatty acids and their derivatives by extractive crystallization with urea.
Ind. Eng. Chem. 42: 2538-41

55. Nunn, J.R., 1952.
The structure of sterculic acid.
J. Chem. Soc. 1952: 313-8

56. Ogg, C.L., W.L. Porter and C.O. Willits, 1945.
Determining the hydroxyl content of certain organic compounds.
Ind. Eng. Chem. Anal. Ed. 17: 394-7

57. Paraf, J., J. Deshordes, Buu-Hoi, A.R. Ratsimamanga and P. Cagniant, 1945.
Physiological properties of a new -disubstituted unsaturated acid.
Compt Rend Soc Biol 139: 863-5.
(Cited from C.A. 40: 6654, (1946)

58. Paschke, R.F., and D.H. Wheeler, 1949.
Thermal polymerization of unsaturated fatty esters. Normal methyl linoleate.
J. Am. Oil Chem. Soc. 26: 278-83

59. Paschke, R.F., J.E. Jackson and D.H. Wheeler, 1952.
Thermal polymerization of drying oils. Isomers of methyl linoleate.
Ind. Eng. Chem. 44: 1113-8

60. Polgar, N., 1948.
Component acids of the lipids of human tubercule bacilli.
Biochem J. 42: 206-11

61. Powell, H.M., 1948.
The structure of molecular compounds. Part IV. Clathrate compounds.
J. Chem. Soc. 1948: 61-73

62. Powers, P.O., 1949.
Mechanism of the oxidation of drying oils.
Ind. Eng. Chem. 41: 304-9

63. Powers, P.O., J.L. Overholt and A.C. Elm, 1941.
Formation and deterioration of paint films. Polymerization of the vinyl type in the drying of oils.
Ind. Eng. Chem. 33: 1257-63

64. Privett, O.S., R.B. Pringle and W.D. McFarlane, 1945.
The elimination of flavor reversion in linseed shortening by heat polymerization and solvent segregation of the oil.
Oil and Soap 22: 287-9

65. Radlove, S.B., and L.B. Falkenberg, 1948.
Comparative evaluation of polymers of heat-bodied conjugated and non-conjugated linseed and soybean oils.
J. Am. Oil Chem. Soc. 25: 1-3

66. Redlich, O., C.M. Gable, L.R. Beason and R.W. Miller, 1950.
Addition compounds of thiourea.
J. Am. Chem. Soc. 72: 4161-2(1950)

67. Redlich, O., C.M. Gable, A.F. Dunlop and R.W. Miller, 1950.
Addition compounds of urea and organic substances.
J. Am. Chem. Soc. 72: 4153-60

68. Robinson, Sir R., 1940.
Some aspects of the chemotherapy of tuberculosis.
J. Chem. Soc. 1940: 505-9

69. Sartory, A., J. Meyer and P. Cagnant, 1950.
Contribution a l'etudie des proprietes biologiques des acides gras ramifies. I Sur les proprietes toxiques des quelques acides ramifies non satures.
Ann. Inst. Pasteur 78: 93-6

70. Scheiber, J. 1929.
Reactions in the formation of stand oils.
Farbe u. Lack. 1929: 585-7.
(C.A. 24: 978, 1930)

71. Scheiber, J. 1936.
Standoil preparation.
Fette u. Seifen 43: 103-5.
(C.A. 30: 7880, 1936)

72. Schlenk, W.J. 1949.
Die harnstoff addition der aliphatisch verbindungen.
Annalen 565: 204-40

73. Schlenk, W., Jr. 1951.
Thiourea addition of organic compounds.
Annalen 573: 142-62.
(C.A. 46: 4998, 1952)

74. Schlenk,H., and R.T. Holman, 1950.
The urea complexes of unsaturated fatty acids.
Science 112: 19-20

75. Shriner, R.L. and R. Adams, 1925.
Structure of chaulmoogric and hydnocarpic acids.
J. Am. Chem. Soc. 47: 2727-39

76. Skellon, J.H., and E.D. Wills, 1948.
Iodimetric methods of estimating peroxidic oxygen.
Analyst 73: 78-85

77. Spectroscopy Committee, American Oil Chemists' Society, 1949.
Report of the Spectroscopy Committee.
J. Am. Oil Chem. Soc. 26: 399-404

78. Spielman, M.A., 1934.
The chemistry of the lipids of tubercule bacilli.
XXXIX The constitution of fuberculostearic acid.
J. Biol.Chem.106: 87-96

79. Swift, C.E., F.G. Dollear and R.T. O'Connor, 1946.
The oxidation of methyl oleate. I The preparation, properties and reactions of methyl hydroperoxido oleate.
Oil and Soap 23: 355-9

80. Tiedt, J., and E.V. Truter, 1951.
Some normal alcohols occurring in wool wax.
Chem. and Ind. 1951: 911-12

81. Truter, E.V., 1951.
Urea complexes of some branched-chain and cyclic esters.
J. Chem. Soc. 1951: 2416-9

82. Wiseblatt, L., 1950.
On the nature of the nutritionally deleterious constituents of heated vegetable oils.
M. Sc. Thesis, McGill University

83. Zilch, K.T., and H.J. Dutton, 1951.
Analysis of fat acid oxidation product by countercurrent distribution methods. Model compounds.
Anal. Chem. 23: 775-8

84. Zimmerschied, W.J., R.A. Dinerstein, A.W. Weitkamp and R.F. Marschner, 1950.
Crystalline adducts of urea with linear alphatic compounds.
Ind. Eng. Chem. 42: 1300-6