THE EFFECT OF HEAT POLYMERIZATION ON THE COMPOSITION AND NUTRITIVE VALUE OF MENHADEN OIL

A Thesis

by

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

McGill University

April 1957

ACKNOWLEDGEMENTS

The author wishes to express his thanks to Dr. R.H.Common for his supervision and direction during the course of this work. Thanks are also due to Professor Alfred Taurins, McGill University, for determining the infrared spectrograms and also for advising on their interpretation.

The author also wishes to thank Canada Packers Limited, Montreal, and Dr. H.J.Lips, National Research Council, Ottawa, for donating oil samples used in this study.

The assistance of Mr. G.O.Henneberry in the construction of an all glass flash distillation apparatus, is gratefully acknowledged.

Members of the Department of Animal Nutrition have conducted the feeding trial experiment and have supplied the author with detailed reports on the results of these trials. Special thanks are due to Dr. F.A.Farmer and Mrs. E.Say who did the majority of this work.

Thanks are extended to the Canadian Committee on Edible Fats and Oils, National Research Council of Canada, for financial assistance received during the course of this investigation.

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GENERAL INTRODUCTION

Research in this laboratory on the utilization of linseed oil for edible purposes, was originally stimulated by the shortage of edible oils and fats during the second world war. This research was concerned with overcoming the unpleasant odour and taste associated with linseed oil subsequent to hydrogenation. The tendency to flavour reversion has been attributed to the isolinoleic acid produced by hydrogenation from linolenic acid present in large quantities in linseed oil.

A method of removing a portion of the linolenic acid was developed. It was suggested that thermal polymerization would involve primarily the linolenic radicals. The polymeric triglycerides thus formed could then be separated by virtue of their insolubility in acetone. It was presumed that the residual soluble fraction should then be relatively lower in linolenic acid radicals, and to that extent less liable to flavour reversion. In the event, the hydrogenated acetone-soluble fraction displayed good stability as regards flavour reversion, and produced a shortening suitable in taste and baking properties. Studies on the nutritive value of this shortening however, showed that it was toxic when incorporated in the diet of young rats.

Thermally polymerized peanut, corn, rapeseed and soybean oils all displayed lowered nutritive values.

Further work in this laboratory has effected a separation of the monomeric portion of the ethyl esters of the polymerized oil into two fractions, viz., a very injurious fraction which fails to form adducts with urea, and a fraction, capable of forming adducts with urea, and comparable in nutritive value to the unheated esters.

The yield of the nutritionally injurious non-adduct forming monomeric material could be related to the degree of saturation exhibited by the component fatty acids of the oil itself.

The present work has been concerned, firstly, with a further study of the components of this injurious fraction, and secondly with a study on the nutritional value of similar ester fractions from thermally polymerized menhaden oil.

PART I

REVIEW OF THE LITERATURE

Thermal Polymerization

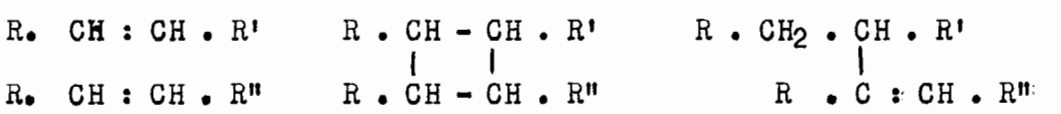
Thermal treatment of unsaturated triglyceride oils results in the formation of covalent links between fatty acid chains. Saponification of the thermally polymerized triglyceride mixture yields monomeric, dimeric and smaller amounts of trimeric (and higher) acids.

Early theories on thermal polymerization proposed the Diels-Alder reaction as the mode of union.

Scheiber (1929) noticed that, whereas refractive index values increase steadily with time of polymerization of linseed and other common drying oils, in the case of tung oil refractive index fell during the early stages of thermal polymerization. Scheiber correctly interpreted this difference as due to the fact that eleostearic acid is conjugated, whereas the acids of linseed oil are not. He explained his observations on the theory that conjugation must necessarily occur before polymerization. In the case of tung oil, conjugation was already very extensive, hence loss of conjugated bond systems and consequent decrease of refractive index occured during thermal polymerization. Kappelmeier (1933) advanced the idea of a 1, 4 - Diels-Alder type addition of a dienophile to a conjugated diene, as being a possible mechanism for the thermal polymerization reaction. These two proposals were combined into the Scheiber-Kappelmeier theory of thermal polymerization (Scheiber, 1936), which involves two steps: (1) thermal conjugation of unsaturated fatty acyl groups to conjugated dienes, and (2) the addition of an unsaturated fatty acid residue (dienophile) to the conjugated diene

by a Diels-Alder type addition to produce a dimer containing a cyclohexene ring. Conjugation of unsaturation was therefore considered an essential stage in the polymerization (Bradley and Richardson, 1940). The apparent induction period in thermal polymerization during which unsaturation was consumed with little increase in viscosity, was explained as the time required for conjugation and intra-glyceride reaction. Bradley and Richardson, accordingly, regarded intra-glyceride reactions as a necessary preliminary to thermal polymerization, which was considered to proceed by acyl group interchange. Another hypothesis, due to Sunderland (1945), proposed that a hydrogen separation reaction is responsible for linkage. A double bond in one molecule opens to receive at one end a hydrogen atom from a methylene group in another molecule and becomes joined at the other end to the methylene group site. Thus the linkage formed is a single carbon-carbon bond.

Brocklesby (1941) proposed a scheme in which two direct carbon-to-carbon bonds are formed between two ethenoid groups in different residues. This mechanism would cause the formation of a four carbon ring, which then rearranges to produce a non-cyclic, branched dimer.



This reaction was thought to accur only intermolecularly because of the shift in the fatty acyl chains which would rupture any intra-molecular compound. This theory was advanced before the mobility of hydrocarbon chains was fully realized.

The Diels-Alder mechanism for thermal polymerization of poly-unsaturated fatty esters has received support from the findings of

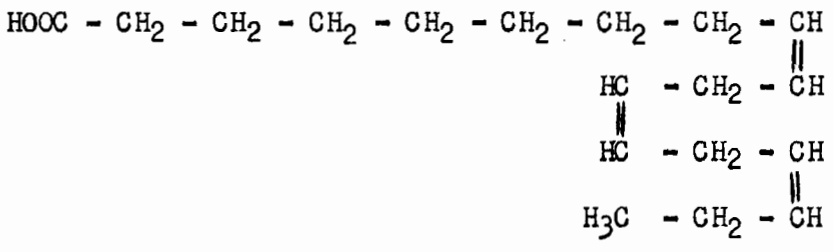
recent workers (Boelhouwer, Tien and Waterman, 1953; and Paschke and Wheeler, 1955a). It is now known that, although six membered rings are formed in heated oils, intra-glyceride reactions are quantitatively unimportant in the early stages of convential thermal polymerization (Sims, 1956). In addition, the contribution of conjugation and geometric isomerization to reaction rate and mechanism has been assessed.

Influence of Amount and Type of Unsaturation.

The common types of fatty acids encountered in a drying oil are: (1) stearic acid, octadecanoic acid; (2) oleic acid, Δ-9 octadecanoic acid; (3) linolenic acid, Δ-9, 12, 15 octadecatrienoic acid. Fish oils contain a wide variety of saturated and unsaturated fatty acids, with chain lengths ranging from twelve to twenty-six carbon atoms. The high degree of unsaturation exhibited by fish oil fatty acids is also extremely variable, and is known to exceed five double bonds per molecule (Bailey, 1951a).

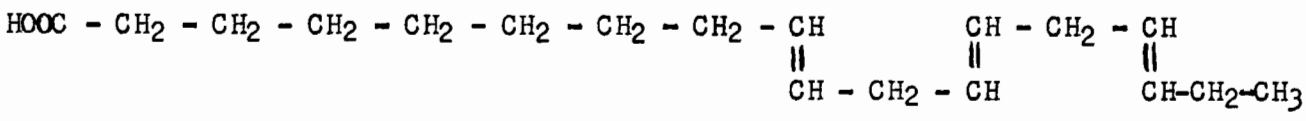
The following structures illustrate the effect of geometric configuration in unsaturated fatty acids. Double bonds have a planar configuration, and geometric isomerization about the plane of the double bond changes the conformation of the fatty acid in space.

Geometric Isomerization in Octadecatrienoic Fatty Acids:



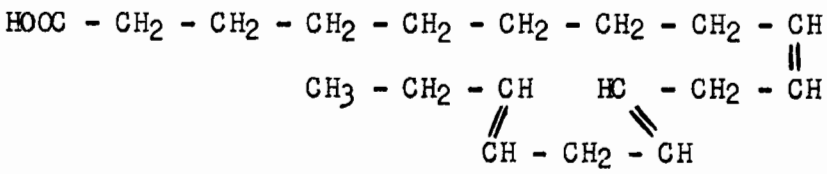
<u>cis</u>, <u>cis</u>, <u>cis</u> Δ-9, 12, 15 octadecatrienoic acid.

N - Linolenic Acid



trans, trans, trans Δ-9, 12, 15 octadecatrienoic acid.

α - Eleostearic Acid.



cis, trans, trans Δ-9, 12, 15 octadecatrienoic acid.

β - Eleostearic Acid.

Work by Paschke, Jackson and Wheeler (1952) and Paschke and Wheeler (1955a) on the polymerization of methyl esters of pure isomeric fatty acids clarified many polymerization problems. With various isomers of methyl linoleate, the reaction was shown to be pseudo-unimolecular. Normal (non-conjugated) linoleate was considered to isomerize and react with both conjugated and non-conjugated material. Trans-linoleate was shown to polymerize more rapidly although its rate of conjugation was essentially the same as that of the parent cis compound. Since polymerization rates of cis-cis, cis-trans and trans-trans non-conjugated linoleates were quite similar, thermal cis-trans isomerization was not considered to control the rate of reaction. Moreover, Gourley (1951) working with linseed oil, has shown that assumption of the trans configuration is a more rapid reaction than thermal polymerization.

With conjugated linoleate isomers, however, geometric configuration was more important; trans-trans conjugated linoleate polymerized much

faster than alkali-conjugated linoleate. Moreover the reaction followed first order kinetics more closely than second order kinetics. Geometric isomerization was, therefore, considered to be the cause.

Gast, Bell and Teeter (1956) recently studied the mechanism of a Diels-Alder-type reaction between diethylazodicarboxylate and trans, trans Δ-9, 11 octadecadienoic acid in various solvent systems and with acidic catalysts. It was found that the reaction followed second order or pseudo second order kinetics. Probably the reaction was more complex than the overall reaction kinetics indicated.

Thermal polymerization studies of normal methyl linoleate and α- and β- eleostearate (Paschke et al.1955a) indicated that the kinetics of linoleate polymerization were consistant with the conjugation theory of non-conjugated polyenes. Monomeric eleostearates were shown to disappear in a second order manner with the β isomer polymerizing faster. Side reactions, producing cyclic and less reactive monomers were observed to the extent of 5 - 15 per cent.

The products formed during thermal polymerization include hydrocarbons, acrolein, aldehydes, free fatty acids, low molecular weight esters and many other products. Some of these products originate from pyrolytic decomposition, others are formed as by-products of the main polymerization (Bailey,1951c). Although there is some tendency for oils to increase in acidity during heat bodying, it is quite possible to produce a high-viscosity product from alkali-refined linseed oil with an acid value not greater than 3 or 4 (Wells and Common, 1953). Monomeric iso-oleates that could not be hydrogenated to stearate have been found in heat-bodied oils (Paschke and Wheeler, 1949.)

The NAFD fractions isolated from sunflower seed, soybean and linseed oils (Pritchard, 1954) also represents less well known products of thermal polymerization.

Structural analysis of the linseed oil NAFD fraction has been extended by the wider application of spectroscopy and by ozonolytic degradation methods (Macdonald, 1957).

One aspect of this investigation that had not been studied until recently was whether the isomerization reaction took place more readily than polymerization. That is whether the movement of double bonds to the conjugated position could be the rate-controlling step in the postulated reaction scheme. Research by Rushman and Simpson (1955), has shown that with methyl linoleate the conjugation reaction is much slower than polymerization and is thus not rate-controlling. Polymerization was shown to proceed by second order kinetics that could be explained in terms of hydrogen abstraction and not by a Diels-Alder mechanism.

Work with triglyceride oils rather than methyl esters, has produced like information. Using viscosity to measure the progress of thermal polymerization, reaction rates were measured and activation energies for the polymerization of linseed, safflower and tung oils were calculated (Sims, 1955a). The energy required for the polymerization of naturally conjugated oils was shown to be almost 50% less than that of the initially non-conjugated oils. Differences in response to catalysts and inhibitors also suggested a difference in mechanism for the two types of oil. More recent investigations (Sims, 1956), where extent of reaction was followed by consumption

of monomeric acids and by disappearance of unsaturation, showed that the kinetic orders for the polymerization of these two types of oils were different. Activation energies obtained in this experiment again suggested marked differences in reaction mechanism.

Influence of Temperature.

Thermal polymerization of linseed, safflower and tung oils has been studied in Canada (Sims, 1955a). Using rate constants obtained from viscosity measurements, apparent overall energies were obtained for thermal polymerization of linseed, safflower and native and isomerized tung oils. The non-conjugated oils had activation energies of about 35 K. cal. per mole, whereas tung oil - isomerized or native - required an activation energy of 23 K. cal. per mole. Although isomerized tung oil polymerizes more rapidly than the native oil they have similar activation energy requirements. The faster rate of polymerization of isomerized tung oil can, therefore, be attributed to a larger probability factor. At temperatures greater than 260°C. thermal polymerization appeared to become complicated by side reactions.

Intra-Glyceride Reactions

Intra-glyceride reactions in thermal polymerization have been investigated in a number of ways. Early attempts to show the existence of cyclohexene rings in heated oil by aromatization of the ring and oxidation of the side chain gave inconclusive results. Wells and Common (1953) have suggested that the failure of NAFD to form adducts with urea may be due to the presence of a non-terminal ring structure.

The presence of cyclic monomers among the products of the heat treatment of glycerides and fatty esters has often been suggested (Bradley and Johnston, 1940), but the evidence for them has been indirect.

Recently, Clingman, Rivett and Sutton (1954) used bromine substitution followed by dehydrohalogenation and oxidation to show the presence of six-membered rings in dimeric fatty acids obtained from heated linseed and safflower oils and methyl linoleate and eleostearate. Subsequently Paschke and Wheeler (1955b) used a similar technique to show the occurrence of six-membered rings in monomeric heated methyl eleostearate. Macdonald (1956) provided evidence for cyclic monomers in the NAFD of heated linseed oil. This evidence was obtained by methods involving aromatization via allylic bromination and dehydrohalogenation, followed by oxidation to a simple aromatic acid, phthalic acid. Because of low yields in some of the reaction steps in this method, it is not possible to state with precision the relative amounts of ring formation that occur when fatty acids of different degrees and types of unsaturation are heated. However, it can be said that normally conjugated fatty acids cyclize more readily than do fatty acids with initially non-conjugated unsaturation.

Boelhouwer, Geerands and Waterman (1955) have used a method of ring analysis based on specific refractivity and molecular weight to estimate the number of rings per molecule in the monomeric and polymeric fractions of heated oil. At a polymerization temperature of 300°C. they found that the monomeric and polymeric fractions from linseed oil contained two rings per triglyceride molecule. Applying a similar method of ring analysis, these workers have also studied methyl linoleate, methyl linolenate, tung oil and methyl eleostearate.

Molecular distillation has been used to estimate the amount of intra-glyceride reaction. Van Loon (1953) used this method to study the influence of temperature on cyclization reactions of tung oil. He showed that between 170° and 300°C. cyclization was a minor reaction, whereas at temperatures greater than 300°C. the conversion of eleostearic acid to its cyclic isomer became extensive. Reasoning that isomerization of α- to β-eleostearic acid would proceed rapidly at high temperatures, Van Loon (1953) compared the composition of isomerized tung oil polymer with that obtained from native tung oil. Oil isomerized with iodine and heated at 170°C. contained even less distillable acyl groups than native oil heated at 280°C.

The effect of reaction temperature, extent of reaction and type of unsaturation on intra-glyceride reactions has been studied by Sims (1955c). The effects of reaction temperatures and geometric configuration on the ratio of polymeric acyl groups to polymeric glycerides found in heated tung oils at various extents of reaction have been elucidated. Tung oil isomerized to the β configuration gave the greatest amount of intra-glyceride reaction. High temperatures gave essentially the same effect as prior isomerization after the reaction had proceeded beyond a 50 per cent increase in molecular weight.

The intra-glyceride reactions of initially non-conjugated oils suggest that, with oils of this type, intra- glyceride reactions play a much less important part in the changes brought about by heat. It appears that, in the early stages of normal high temperature polymerization, these reactions are insignificant. At low reaction temperatures, however, higher polymeric acyl group contents were

measured, indicating incomplete randomization of the acyl groups at low temperatures during small extents of reaction. The general conclusion, from information obtained by molecular distillation (Paschke and Wheeler, 1954), dilution polymerization (Sims, 1954) and a combination of molecular distillation and ring analysis (Boelhouwer and Waterman, 1952), is that, contrary to earlier hypotheses, intra-glyceride reactions play a minor role in the early stages of conventional, high-temperature thermal polymerizations.

The Viscosity of Bodied Oils

The viscosity of polymerized oils, which has been used as an empirical measure of extent of bodying, has been further investigated. Ordinary fatty oils exhibit the flow behaviour of true Newtonian liquids (Bailey, 1951b). However, Weltmann (1948) has shown that at very high rates of shear, they may behave as thixotropic plastics, presumably because of the tendency of micelles or other aggregates to become orientated under high shearing stresses. Under such conditions polymerized oils decrease quite markedly in viscosity. In polymerized linseed oils, Tollemaar and Bolthof (1946) have shown that the decrease is greater the greater the variation in molecular weight of the aggregates in the oil. i.e., is at a maximum when a non-polymerized oil is mixed with a highly polymerized oil. Polymerized oils become less thioxtropic as their temperature increases. Relations between viscosity and polymer content have been established for safflower, linseed, and tung oils (Sims, 1955d). Conjugated and non-conjugated oils give different functions and those of the two non-conjugated oils resemble each other.

The dependence of activation energy of viscous flow on the molecular weight and concentration has been studied by Sims (1956). Using low rates of shear, at which the flow was normal Newtonian, the activation energy of viscous flow was found to be a function of both molecular weight and concentration.

Molecular Weight Distribution in Heated Oils

Using the Bernstein fractionation technique (Bernstein, 1948), Walker, MacKay and Taylor (1953) were able to show the presence of large amounts of material with an average molecular weight of 150,000 in heated linseed oil. This technique has also been used to compare the molecular weight distribution of polymer in heated linseed and tung oils (Sims, 1955c). The results of this investigation suggest that in tung oil the monomer disappearance is more rapid than in linseed oil and that the dimers so formed tend to go to tetramers quickly.

Recently, Hoeve and Sutton (1955) have presented satisfactory information on the polymer distribution in heated linseed oil. From the experimentally determined proportions of monomeric, dimeric and trimeric acyl groups, the extent of reaction and molecular weight distribution were calculated on the assumption that the acyl groups are completely and randomly esterified with glycerol. Their calculations were based on Flory's* theory of polycondensation reactions Further work by Hoeve and Sutton (1956) using slowly polymerizing sunflower seed and linseed oil, substantiated the validity

* (1946)

of the assumption that acyl groups are randomly and completely esterified with glycerol.

Whether or not Flory's theory can be applied to rapidly polymerizing oils or marine oils, depends largely upon the fatty acid distribution over the component glycerol molecules. In the case of marine oil polymerization, it is improbable that an initial state of random fatty acid distribution exists, since preliminary kinetic studies of menhaden oil polymerization in this laboratory, indicate that Flory's theory cannot be applied to the initial two-hour polymerization period.

ADDUCTION COMPOUNDS OF UREA AND LINEAR ALIPHATIC SUBSTANCES

A number of instances is known where two compounds, each capable of a separate existence and having no obvious means of chemical union, may unite to form a new crystalline substance, usually described as a crystalline molecular compound. The ratios of the component molecules in each substance may be constant or variable, simple or complex. The urea adduction compounds of straight chain aliphatic molecules discovered by Bengen in 1940, belong to the general group of crystalline molecular compounds.

In these urea adduction compounds the molecular ratio of the components is non-integral. The compounds obey the law of constant composition in that the molecular ratio of urea to organic component is constant for that particular organic compound, but the molecular ratio is related to the chain length of the organic compound.

Bengen's (1940) discoveries included the observation that normal straight chain hydrocarbons form urea adducts, but that cyclic, branched-chain and highly unsaturated molecules do not. This rule is not rigid, in so far as some cyclic and branched-chain structures will form adducts if the attached hydrocarbon chain is of sufficient length (Truter, 1951).

Since the original German patent came to light, Schlenk (1949) and Zimmerschied, Dinnerstein, Weitkamp and Marschner (1950) have reported in detail on the formation of urea complexes in relation

to the shape of the organic molecules, their composition, crystal structure and energy of formation. These results have been confirmed and extended by Redlich, Gable, Dunlop and Miller (1950) and by Knight, Whitnauer, Coleman, Noble and Swern (1952).

Urea adduct formation, therefore, offers the possibility of separating linear aliphatic compounds from their branched-chain isomers by a method based on molecular shape, instead of more conventional methods depending on chemical activity and molecular size.

Crystallographic studies of urea adducts have shown that urea molecules are arranged in a hexagonal lattice with each unit cell containing six urea molecules. The internal diameter of the canal down the centre of the lattice is such that a stretched hydrocarbon chain will fit into it easily. The unit cell comprising six urea molecules is 11.1 A., corresponding to a lattice length interval of 1.85 A. Comparison of lattice length with length of hydrocarbon chain for a series of paraffins shows that there is a uniform discrepancy of + 2.4 A. per molecule. This discrepancy represents the interval between succeeding hydrocarbon molecules in the lattice. It is possible, therefore, to give the ratio of the number of urea molecules to hydrocarbon molecules in the adduct and to explain the non-integral nature of these ratios.

Nicolaides, Laves and Niggli (1956), using X-ray diffraction patterns of a single urea adduct crystal, have developed a method of locating substituents on the hydrocarbon chain of molecules that form adducts with urea. They were also able to determine the

chain length of the adducting moiety.

The possible applications of urea adduct formation to oil and fat chemistry are numerous. Schlenk and Holman (1950a) found that fractions with markedly different iodine values could be separated by varying the ratio of urea to fatty acid. By simply using a single urea complex separation at room temperature, Parker and Swern (1957) were able to prepare concentrates of linolenic acid from linseed oil. Mehta and Sharma (1956), working with heat-bodied linseed oil, were able to isolate a cyclized product by a combination of acetone extraction and urea adduct formation.

Urea adduct fractionation can be applied to the separation of gemmetric isomers (Schlenk and Holman,1950b). Normal cis, cis, Δ-9, 12 linoleate is now prepared routinely as the non-urea adducting fraction of fresh sunflower seed methyl esters (Paschke, 1956). Urea will also form addition complexes with poly-alcohol monoesters (Moreno, Cruz and Janer del Valle, 1956). In this connection, it is of interest to note that methanolysis of monoglycerides will occur during urea adduct formation (Aylwood and Wood, 1955). The disturbance of chemical equilibria by inclusion type compound formation is known to occur often. Aylwood and Wood (1955) have reported the existence of two types of crystalline adducts: (1) large hexagonal needles typical of the majority of inclusion type compounds, and (2) smaller hexagonal biprisms appearing as hexagonal plates and containing saturated long chain monoglycerides.

Since the fractionation of fatty acid mixtures by urea adduct formation depends on both the extent of unsaturation and on geometric isomerization, this method is now applied extensively to marine oil

research. Domartt, Miyauchi and Summerwell (1955) were able to separate a mixture of menhaden oil fatty acids into fractions of almost any desired iodine value. Fractions with iodine values ranging to above 300, a degree of unsaturation corresponding to 3.4 double bonds per molecule, were isolated.

Thiourea inclusion compounds are less stable than their urea analogues, and hence possess a low heat of formation, lower free energy and a decrease in molal ratio of thiourea to reactant as compared with urea to reactant. Schlenk (1956) demonstrated that desoxycholic acid conforms with urea and thiourea in its properties as a reagent for the fractionation of fatty acid mixtures. Quantitative recovery of the adducting fatty acid moiety was impractical.

In this laboratory the urea fractionation technique has been successfully applied to the separation of the ethyl esters of thermally polymerized vegetable and marine oils into nutritionally innocuous and nutrionally deleterious fractions.

PHYSIOLOGICAL AND NUTRITIONAL EFFECTS OF HEATED OILS

Heat-treatment of unsaturated oils for edible purposes was developed in Germany and Occupied Norway during the war 1939-1945. A deodorization-polymerization process was devised in order to produce from fish oils a substitute for vegetable oils for fish canning. The process is described in British Intelligence Objectives Sub-committee report No. 1477 (1947). It involves alkali refining and bleaching followed by deodorization at 280° - 300°C. at 10-15 mm for 8 to 12 hours in a vessel of acid proof steel. The process was said to lower the nutritional value of oil. A similar process was applied to herring oil in the United States (Lasser, Bacon and Dunn, 1949). It is obvious that the heat treatment used must have led to extensive polymerization.

The initial stages of high temperature hydrogenation would involve various isomerizations, and some of these changes are likely to be similar to those that precede polymerization in heated oils. The formation of fatty acid isomers during hydrogenation has been studied by Feuge, Cousins, Fore, Du Pré and O'Connor (1953). They showed that the amount of diene conjugation increases with increase in temperature, catalyst concentration, and hydrogen dispersion. The amount of non-conjugated trans isomer increased with catalyst concentration and hydrogen. dispersion. Recently it has been shown that during the catalyzed thermal polymerization of methyl linoleate, hydrogen transfer between linoleate esters occurs and this produces fractions of varying degrees of unsaturation which exhibit positional and geometric isomerization. The literature dealing with the effects of polymerization temperatures on the nutritive values of edible oils has been reviewed by Crampton,

Common, Farmer, Wells and Crawford (1953), and by Pritchard (1954).

Since then Raju and Ragagopalan (1955) have reported on the deleterious effects of feeding rats with diets containing 15 percent of peanut, sesame or coconut oils which had been heated at 270°C. in contact with air. However, although the temperature used suggested that polymerization of the oil occurred, the experiments of these workers are not comparable to experiments where oils were heated in a current of carbon dioxide (Crampton, Common, Pritchard and Farmer, 1956).

Kunitz, Slanetz and Johnson (1955) have reported that cottonseed oil, heated and aerated at 90°C. -95°C. for periods up to three hundred hours, became injurious to rats. Incorporation of fresh oil gave a degree of protection against some of the deleterious effects. Peroxides were not considered to be responsible for the ill effects.

Crampton, Common, Pritchard and Farmer (1956) have compared the nutritive values of various fractions of the ethyl esters prepared from polymerized linseed, soybean and sunflower seed oil. Particular attention was paid to the fraction of the distillable ethyl esters that failed to form urea adducts (the NAFD fraction). The NAFD from linseed oil was highly injurious to the well-being of young growing rats, despite the fact that the material was a bland neutral oil. The corresponding fraction from soybean oil was also injurious, though to a lesser extent than linseed oil NAFD. Sunflower seed oil NAFD was much less injurious than that from the other two oils. The adduct-forming fractions of the distillable esters (AFD) from all three oils were nutritionally innocuous. Thermal damage to the nutritive value of marine oils has been studied by Frahm, Lembke and Von Rappard (1953). These workers reported nutritionally deleterious effects of

heat-polymerized whale oil when fed to mice.

The work described in this thesis included a study of the nutritional effects of various fractions of the esters of polymerized menhaden oil. This study has shown that the NAFD fraction of the distillable esters from heated menhaden oil was toxic to rats to a degree comparable with the toxicity of the similar fractions from heated linseed oil. The adduct forming fraction (AFD) of the distillable esters from the heated oil was nutritionally innocuous. These results have provided some additional evidence for an association between the toxicity of the NAFD fraction and the presence of polyene acids in the original oil.

From this and earlier work it is reasonable to suppose that heated oil is nutritionally injurious because of the presence of polymerized material that is poorly absorbed; and also the presence of monomeric acid radicals incapable of forming urea adducts by reason of some structural feature or features, possibly including cyclization. The occurrence of cyclization reactions during thermal polymerization has been demonstrated by Paschke and Wheeler (1955). These workers proved by the methods of classical organic chemistry that cyclized monomeric acids were formed during thermal treatment of methyl eleostearate. Macdonald (1956), working with triglycerides, was able to show the presence of cyclic monomeric material in the NAFD from polymerized linseed oil. Hydrogenation of the NAFD fraction, followed by urea adduct formation, produced a negligible yield of adduct forming material (approximately 2 per cent). This substantiates the work of Pritchard (1954), who claimed that linseed oil NAFD, though highly unsaturated, contained only 3 per cent of difficultly conjugable material, the remainder consisting of non-conjugable esters.

Essential Fatty Acids

In 1929 Burr and Burr published their classical paper in which they submitted evidence that certain unsaturated fatty acids are essential for growth and survival. This paper marked a departure from the rather general assumption that fats were optional constituents of the diet. Since that time the main submission of Burr and Burr has received general acceptance and it is believed that the essential fatty acid(EFA) requirement of the rat, of man and possibly of many higher vertebrates may be met by supplying relatively small amounts of any of the following unsaturated fatty acids:- linoleic (9, 12 octadecadienoic) acid, linolenic (9, 12, 15 octadecatrienoic) acid and arachidonic (5, 8, 11, 14 eicosatetraenoic) acid. The EFA activity of docosapolyenoic acids from brain glycerophosphatides has been demonstrated by de Jongh and Thomasson (1956). Lack of EFA causes hyperkeratosis in rats (Ramalingaswami and Sinclair, 1953), and leads to disturbed ovulation and many epidermal diseases in both humans and rats (Wooster and Blanik, 1950).

The classification of fatty acids having essential fatty acid action is still controversial. Melnick and Deuel (1954), studying the biological utilization of fatty acid isomers, have shown that iso-oleic acids were not antimetabolites for natural oleic acid, but were utilized as nutrients. These workers were also able to show that isomers of linoleic acid in hydrogenated oil, although resistant to spectrophotometric determinations, nevertheless exhibited essential fatty acid activity. In this connection, Thomasson (1954) questions

whether stearolic acid (9, octadecynoic acid) is an essential fatty acid. Having assayed a large number of fatty acids for essential fatty acid activity, he has put forward the theory that the essential fatty acids are all characterized by the grouping: CH_3. $(CH_2)_4$. CH:CH. CH_2. CH:CH.

This view has been supported by nutritional experiments using weanling male rats fed hydrogenated marine oil. Linoleic acid was found to be an essential dietary constituent. It was suggested that the formation of biologically inert isomers could increase the requirement for essential fatty acids in the diet (Aaes-Jorgensen, Engel, Funch and Dam, 1955).

Data collected by Holman and Peifer (1956) suggest that essential fatty acids are required for the normal utilization of saturated fats. They pointed out that the inclusion of linolenic acids in the diet did not cure skin symptoms. These results add to the evidence showing that linolenic acid has a lower essential fatty acid activity than linoleic acid.

The degree of toxicity exhibited by linseed and menhaden oil NAFD far exceeds the toxicity produced by essential fatty acid deficiency (see Part III, Section II). This result suggests that essential fatty acid deficiency should not be considered the major cause of the toxic effect, although it may well contribute to the toxicity of NAFD fractions.

Occurrence of Branched-Chain Fatty Acids and their Physiological Effects

From the following review of the literature, it will be seen

that branched-chain fatty acids are neither rare, nor are they confined to micro-organisms and marine mammals. It will also be seen that many of the acids contain odd numbers of carbon atoms, and are therefore exceptions to the generalization that the natural fatty acids are even numbered.

Before 1950, no naturally occurring branched-chain fatty acid with the sole exception of iso-valeric, had been reported as existing in natural triglycerides. However, the presence of a branched-chain fatty acid had been demonstrated in the waxy envelope of some acid fast bacilli (Anderson and Chargaff, 1929). This acid was shown to be 10-methylstearic acid by Spielman (1934). Since then, Polgar and Robinson (1943, 1945) have isolated from "tubercle wax" 3, 13, 19-trimethyltricosanoic acid. A 10- or 11-monoethylnonadecanoic acid has been isolated from the acetone soluble fats of Phylomonas tumefaciens (Valeck and Anderson, 1944). Polgar (1954a, 1954b) has shown that two acids, mycolipenic and mycoceranic, from the tubercle bacilli, are trimethyl branched fatty acids.

Degras (wool wax) is also a source of branched-chain fatty acids, which occur mainly as esters of sterols and triterpenoids (Weitkamp, 1945). Hair grease of dogs has been shown to contain 2-methylbutanoic and 3-methylbutanoic acids (Brouwer and Nijkamp, 1952). Branched-chain esters of octadecanol from the coccygeal glands of ducks have also been isolated (Weitzel, 1951). Working with butterfat, Hansen and Shorland (1951a; 1951b) reported the isolation of a C_{17} methyl-branched acid and a multi-branched C_{20} acid. Optically active 12-methyltetradecanoic and 13-methyltetradecanoic acid were isolated from butterfat (Hansen, Shorland and Cooke, 1954). The same authors (1952a; 1955) found a C_{17} dimethyl fatty acid and isopalmitic acid in the tissue fat of oxen. Hansen, Shorland and Cooke (1952b;

1953; 1955) also isolated optically active 12-methyltetradecanoic acid, (+)-14 methylhexadecanoic acid, 13-methyltetradecanoic acid from mutton fat.

Pritchard (1954) has shown that the NAFD from heated linseed oil contains some branched-chain fatty acids. A C_{18} multi-branched fatty acid has been isolated from shark liver oil (Morice and Shorland, 1952), and two unsaturated fatty acids with chain lengths of fifteen and seventeen carbon atoms have been reported by Schlenk (1956) as present in menhaden oil.

It is almost certain that the branched-chain fatty acids follow a metabolic pathway different from that followed by normal straight chain fatty acids. Wick (1941) has demonstrated a ketogenic action of branched-chain fatty acids. He assumed that the larger alkyl groups blocked the normal oxidation reaction. Intraperitoneal injections of 2, 2-dimethylundecanoic acid produced peritonitis and lukemia in rabbits. "Tubercle-like lesions" were produced at the injection site when 2, 2-di-n-octylpropanoic acid was applied (Robinson, 1940). A similar lukemic effect was observed by Buu-Hoi and Ratsimamanga (1943), who injected guinea pigs with 2, 2-dimethyloctadecanoic acid. Autopsy revealed extensive adhesions between organs of the peritoneal cavity as well as tubercular-like lesions in the liver and diaphragm. Guinea pigs injected with an unsaturated branched-chain acid (α,α-dimethyl-ω-tridecyclic acid) exhibited similar effects (Paraf, Deshordes, Buu-Hoi, Ratsimamanga and Cagniant, 1945). Additional proof of the toxic nature of branched-chain fatty acids resulted from studies by Bailey and Polgar (1955) working with methyl substituted α- and β-unsaturated acids.

It is of interest to note that subcutaneous injections of the esters of ∝, ∝- dimethyl-ω- tridecylic acid gave no toxic effect. Pritchard (1954) was also unable to detect any physiological effects from subcutaneous injections of the NAFD esters from heated linseed oil.

These reports indicate that branched-chain fatty acids have definite physiological effects, and these effects are apparently more pronounced with the higher molecular weight acids. A free carboxyl group may be a prerequisite for biological activity.

A highly significant inverse relationship has been shown to exist between the mean molecular weight of the component fatty acids of various oils and their apparent digestibility. A species difference with respect to ability to utilize long chain fatty acids was also indicated in these experiments (Lloyd, Crampton and MacKay, 1957). The biosynthesis of branched-chain fatty acids has been studied by Rudney and Farkas (1955). They were able to show the sequence of reactions by which branched-chain fatty acids, such as β-hydroxy-isovaleric acid and β-hydroxy-β-methylglutaric acid are synthesized from acetyl CoA and acetoacetic acid. Coon (1955) was able to demonstrate that a variety of branched-chain acids were formed in animal enzyme systems from the essential amino acids: leucine, isoleucine, and valine. The importance of branched-chain monomers in the biosynthesis of squalene and cholesterol has been elucidated by Rabinowitz, Dituri, Cobey and Gurin (1955).

Occurrence of Cyclic Fatty Acids and their Physiological Effects

Chaulmoogra, Lukrabo and gorli-seed oils contain high concentrations of cyclic fatty acids (chaulmoogric, hydnocarpic and gorlic). These three cyclic acids are confined to the seed oil of the Flacourtiaceae. Chaulmoogric acid is a C_{18} monoethenoid acid characterized by a five-membered carbon ring at the end of the carbon chain. Hydnocarpic acid is similar to chaulmoogric acid except that it is a C_{16} acid; and gorlic acid differs from chaulmoogric in having a double bond between the sixth and seventh carbon atoms (Shriner and Adams, 1925). Chaulmoogric acid apparently does not form urea adducts (Wells, 1952).

Hofmann and Lucas (1950) isolated from Lactobacillus arabinosis a C_{19} acid called "lactobacillic acid" containing a non-terminal cyclopropane ring. These authors have suggested that the structure is that of a methyleneoctadecanoic acid. The occurrence of fatty acids of this general structure is not limited to the above mentioned Lactobacilli. The kernel oil of the tropical tree Sterculia foetida contains, as a major constituent sterculic acid, having a non-terminal cyclopropene ring at the 9-10 position (Hofmann, Jucker, Miller, Young and Tousig, 1954). Comparison of the physical properties of the dehydrosterculic acid derivatives with similar properties of lactobacillic acid derivatives indicated that the structure of lactobacillic acid resembled closely that of trans-11, 12 methyleneoctadecanoic acid, but differed significantly from that of the 9, 10 isomer. These non-terminal cyclic fatty acids have been shown to form urea adducts with great difficulty (Nunn, 1952).

Cyclic fatty acids of chaulmoogric oil possess bactericidal activity which is not greatly decreased by esterification. Oil from the seeds of Albizia amara, containing arachidic and lignoceric acids, has also been demonstrated to possess bactericidal properties (Sandra, Sud, and Handa, 1956). Other cyclic and branched-chain acids have been synthesized and tested for bactericidal action on B. leprae; and many of them have proven as effective as chaulmoogric acid. It is now established that the bactericidal action on B. leprae is independent of the cyclopentenyl ring structure, but that the principal requirement is high molecular weight, with molecular configuration being of secondary importance.

Bernhard and Müller (1938) have shown that oral administration of chaulmoogric acid and its derivitives produces mild toxicity. This effect has been confirmed by Buu-Hoi and Ratsimamanga (1941). The physiological effects of intraperitoneal and subcutaneous injections of synthetic cyclopentenyl acids and closely related fatty acids have been studied by Sartory, Meyer and Cagniant (1950). They injected rats and guinea pigs with 0.6 ml. and 1.0 ml. of acid respectively. As a result of these studies, it was concluded that a five or six-membered ring possessing an isolated ethenoid bond was responsible for toxicity. Removal of the double bonds by hydrogenation, or addition of another double bond to the ring depressed the toxicity. This is in contrast to the bactericidal effect, where molecular configuration appeared to be of minor importance.

METHODS

Alkali-refining and Bleaching

Whenever necessary, the oils used in this study were alkali-refined with 20° Baumé sodium hydroxide. The acid value of the oil was determined and the correct amount of alkali was calculated from tables (Bailey, 1951d). The alkali was added to the oil at room temperature while stirring vigorously with a mechanical stirrer. When the oil "broke" the stirring was discontinued. The temperature of the oil was raised to 50°C. on a steam bath, then removed and placed in a cold room (6°C.) overnight. The clear oil was decanted and the foots were discarded. The refined oil was washed free of alkali with warm water and dried over sodium sulphate.

The refined oil was bleached with two per cent W/V. activated bleaching clay (Super Filtrol). The oil was heated to 50°C. under a stream of nitrogen, then the bleaching clay was added with stirring. The flask was swirled to assure intimate mixing and then allowed to remain at 50°C. for fifteen minutes. The bleached oil was filtered free of clay on a Büchner flask.

Thermal Polymerization

The polymerization apparatus consisted in part of a two liter three-necked flask having a 45/50 standard taper neck and 29/42 standard taper side necks. These necks accomodated a 360°C. thermometer, a thermoregulator and a gas inlet and outlet tube.

The polymerization flask was heated by a Glas-Col hemispherical mantle. During polymerization, the upper half of the flask was insulated with a layer of glass wool. Temperature control was regulated to $\pm$ 2°C. by means of a thermoregulator (Precision Scientific Company) attached to a mercury relay. A Powerstat variable transformer, set at approximately 85 Volts, was inserted between the 110 V.A.C. power supply and the relay. This provided a better temperature control and acted as a safeguard against relay failure.

A vigorous stream of carbon dioxide was bubbled through the "charge" of oil during the heat-up and cool-down periods as well as during the actual polymerization. Timing was begun when the temperature reached 275°C. When the required heating time had elapsed, the power was shut off and the heating mantle and glass wool insulation were removed from the polymerizer. The gas stream was increased to a violent bubble in order to facilitate removal of the volatile degradation products of pyrolysis. Time required for the heated oil to cool to room temperature was approximately one and one-half hours.

Preparation of Esters

In previous work, esters were normally prepared from heated oils by transesterification using 0.4 per cent sodium metal in anhydrous ethanol (Pritchard, 1954). The defect of this procedure when applied to marine oils, is that a large degree of isomerization can occur. Consequently, in the present studies, acid-catalyzed transesterification with anhydrous ethanol has been employed throughout.

The author is indebted to Dr. C. Y. Hopkins for the details of the procedure, which has proved convenient and efficient. The full details are as follows:- Absolute ethanol (1000 gm.) was weighed with its container and dry HCl bubbled in until 20 gm. HCl had been absorbed. 500 gm. of the oil was then added and the mixture refluxed for twenty-four hours and then cooled, the final cooling taking place in a separatory funnel. The upper layer (A) was removed and distilled from a steam bath until the volume was reduced by half. The concentrate was poured into four volumes of water and to this was added the lower layer (B). The esters were extracted from this mixture by ether which was removed finally under reduced pressure. An inert atmosphere of CO_2 or N_2 should be employed. The prepared esters were stored under CO_2 or N_2 in tightly stoppered flasks in the freezer until required for analysis.

Vacuum Distillation

Distillation temperature limits were established for menhaden and herring oil ethyl esters. The maximum permitted bath temperature was 265°C., although distillation of the monomeric material was usually complete at 220°C. A maximum bath temperature of 240°C. was used when distilling linseed, soybean and sunflower seed oils. The estimated vacuum on the system was 1-3 mm.

"Quickfit" ground glass apparatus was used for preparation of oils for nutritional feeding trials. A two liter distillation flask with a "B 50" neck was connected to a "B 24" condenser by a simple reduction bend. Use of a wide necked distillation flask with a short

reduction bend permits of a more rapid distillation, thus reducing further polymerization at high distillation temperatures. Heat was supplied by a wax bath (Fisher Bath Wax). The distillation flask was half filled with pyrex glass wool to minimize "bumping" of the oils. When distillation was complete, the bath was removed, and the oil allowed to cool without breaking the vacuum. All fractions were protected from oxidation with 0.05 per cent W/W Tenox II antioxidant (Tennessee Eastman Corporation).

A semi-micro distillation was used for the quantitative determination of per cent distillable esters. The apparatus consisted of a small bulb blown on a piece of pyrex glass tubing (7-9 mm. diameter) furnished with a short side arm and bulb to act as a receiver. A "charge" of 0.5 to 0.8 gm. of ester was employed. 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.

An all glass semi-micro flash distillation apparatus was used in the preparation of aromatized NAFD fractions. The material (approximately 8 gm.) to be flash distilled, was introduced, drop by drop, to the hot distillation bulb through a capillary stop cock connecting the cold upper reservoir to the distillation bulb. In order to obtain equal vacuum in the reservoir and the remainder of distillation system, a small bore glass tube connected the distillation bulb to the reservoir. The distillation bulb was furnished with a short side arm and an attached bulb to act as a receiver.

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 the solvent. One ml. of solvent was used for each gm. of urea used. This was not a sufficient quantity of ethanol to dissolve all the urea, but it was found that adduct formation proceeded smoothly and efficiently in the pasty mixture so formed.

When large quantities of urea adduct were required for nutritional trials, it was found that 1000 gm. batches of the ester could be processed conveniently. The full details are as follows:-

One kgm. of the ester was dissolved in 4 liters of absolute ethanol. To this was added, slowly with stirring, 4 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 precipitated adduct was removed on a 3" Buchner 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. The ester fraction was then washed with warm water and dried

under reduced pressure. The addition of small amounts of sodium chloride aided in the separation of the two phases and reduced the loss of ester due to emulsification.

The filtrate plus alcoholic washings, containing the non-adduct-forming esters, was vacuum distilled under reduced pressure to two-thirds of its original volume, using in addition, a bubbler connected to a source of carbon dioxide gas. The concentrated filtrate plus about 30 per cent ether was diluted with a large volume of warm water. This served to throw these esters out of solution without the complication and accompanying loss due to emulsification. The aqueous phase was extracted twice with ether. The combined ethereal solution was also washed with water and the ether evaporated under reduced pressure.

When quantitative measurements of yields were desired, the ester fractions were recovered from the aqueous mixtures by extraction with ether. The ether was then removed under an atmosphere of carbon dioxide and the yields of adduct-forming and non-adduct-forming ester fractions determined by weighing.

Mean Molecular Weights

Mean molecular weights were determined by cryoscopy in purified cyclohexane (Spectro Grade, Eastman Organic Chemicals). Ten ml. of solvent was the volume normally used, and the concentration of the solute was kept below five per cent in all determinations. The cooling unit consisted of an air bath cooled by an ice water jacket. The test tube, containing the solution to be tested, was placed in

the air bath and the solution was stirred with a coiled aluminum wire stirrer. A Beckmann thermometer enabled freezing points to be estimated to within ± 0.002°C. Observed molecular weights were corrected for free fatty acids by the method of Bernstein (1948).

All mean molecular weight determinations were made at a room temperature of 3° to 6°C. The advantages of this procedure are twofold:- (1) evaporation of cyclohexane is at a minimum; and (2) errors caused by constant resetting of the Beckmann thermometer are eliminated.

Iodine Value

The method of Benham and Klee (1950) was used exclusively throughout the present work. This method has a reaction time of one minute and has been found satisfactory for comparative purposes.

Acid Values

A sample of about 5 gm. of oil was dissolved in 100 ml. of a previously neutralized mixture of toluene and ethanol (1:1 by volume). One ml. of the indicator of Kleinzeller and Trim (1944) was added, and the solution titrated with 0.1 N sodium hydroxide using vigorous stirring. The acid values were expressed as per cent oleic acid.

Peroxide Value

Peroxide values were determined by the method of Skellon and Wills (1948). The extent of oxidation of oil fractions prior to and after incorporation in the diet, was measured by a modification

of the procedure as described by Crampton, Common, Farmer, Berryhill and Wiseblatt (1951). Peroxide values are reported as mg. of peroxide oxygen per kgm. of oil.

Refractive Index

Refractive indices were determined at 25°C. with a Hilger Abbé refractometer used in conjunction with a "Precision" constant temperature bath and circulating system (Precision Scientific Co., catalogue No. 6600). A sodium vapour lamp was used for illumination.

Aromatization

Aromatization of NAFD fractions of heated oils to produce the dialkyl benzene derivative was performed by the method of Paschke and Wheeler (1955). This method involves a bromination with N-bromosuccinimide, followed by a dehydrohalogenation with 2, 4, 6-collidine.

Hydrogenation

Nickel by the Raney process (Covert and Adkins, 1932) was used as a catalyst of hydrogenation. On the assumption that the NAFD fraction would contain material difficult to hydrogenate, a large excess (15-20 per cent W/W) of the catalyst was employed. The hydrogenation reaction was continued for thirty hours at a temperature of 40°C. and pressure of one atmosphere. The reaction mixture was constantly agitated with a mechanical stirrer.

Viscosity

Viscosities were determined at 25°C. with the Gardner Bubble Viscometer.

Percentage Unsaponifiable

The unsaponifiable portion was determined by the method of Ennos, Bolton, Jordon, Hilditch, and Simmons (1933).

Saponification Number

Saponification numbers were determined by the method of Rieman (1943), using the"double indicator" procedure. It was found that thermally polymerized marine oils were incompletely saponified when the standard saponification time of thirty minutes was used. In this work, a sixty minute saponification time has been found satisfactory for comparative purposes.

Spectrophotometric Analysis

Absorption spectra were determined using the isomerization procedure recommended by the Spectroscopy Committee, American Oil Chemists' Society (1955). When preparing twenty-one per cent KOH-glycol reagent, the potassium hydroxide should be added very slowly to avoid the accumulation of undissolved alkali. The constant temperature bath used for alkali-isomerization has been fully described previously (Wells, 1952). Only minor changes have been made; these include insertion between the oxygen-absorption system and the gas manifold,

of one tube filled with calcium chloride to remove moisture.

Digestibility

Digestibility was determined by the method of Bolin, King and Klosterman (1952) using chromic oxide (Cr_2O_3) as an index substance.

Ether Extract

The ether extract was determined by the official method of the A.O.A.C. (1955).

Total Digestible Calories

Oxygen bomb calorimetry was used in the determination of total digestible calories.

PART III

EXPERIMENTAL

SECTION I

CHEMICAL ASPECTS OF THE THERMAL DAMAGE TO THE NUTRITIVE VALUE OF MENHADEN OIL

INTRODUCTION

Nutritional trials have shown that part of the reported deleterious effects of thermally polymerized linseed oil in the nutrition of the rat was associated with the monomeric fatty acid fraction recovered by distillation (Wells, 1952). It was also shown that further separation of the distillable monomers of linseed oil by urea fractionation yielded a nutritionally innocuous adduct-forming fraction (AFD).

Comparative studies with soybean and sunflower seed oils have suggested a relationship between the yield and degree of toxicity of the NAFD fraction and the concentration of polyene acids in the original oil (Crampton et al.1956).

The suggestion that "cyclized" monomeric fatty acid radicals might be present in the NAFD fraction from heated linseed oil has been proven correct by Macdonald (1956). Linseed oil NAFD has also been shown to contain non-conjugable unsaturated branched-chain fatty acids (Pritchard, 1954).

These results suggested that it would be of interest to conduct similar studies on an oil containing acids of higher unsaturation. Menhaden oil was chosen for this purpose. The results of preliminary kinetic studies of the thermal polymerization reaction are presented in this section.

Procedure

Samples of commercial alkali-refined menhaden oil were heated at 275°C. in a current of carbon dioxide for various periods of time. The current of carbon dioxide was enough to keep the oil surging freely and the free flow of the gas also served to sweep out volatile products of pyrolysis.

The ethyl esters of menhaden oil were prepared by acid-catalyzed transesterification. Losses during the esterification procedure were negligible, and amounted to less than two percent. The use of acid catalysis was preferred because of the sensitivity of the highly unsaturated fatty acid constituents of menhaden oil to alkali isomerization. The ethyl esters were distilled under vacuum, and a monomeric fraction was collected. The greater part of the distillate was collected in the temperature range 160° - 230°C. under an estimated pressure of two to three mm. of mercury. The upper temperature limit for the monomeric fraction was set at 260°C. in order that the distillate might include any long (C_{22} - C_{26}) fatty acid constituents. A semi-micro distillation was also carried out on each fraction in order to determine accurate percentage yields of monomer and polymer.

One hundred grams of each distillate was treated with urea and the urea adduct and non-adduct forming fractions were collected. The yield of each fraction was expressed as a percentage of the total ester. Ultraviolet spectrograms of the various fractions from heated and unheated menhaden oil were determined. Although information on the structure of fish oils is relatively sparse and inconclusive, it is believed that they are predominantly characterized by a methylene-interrupted type of unsaturation. The lack of knowledge of the structure of the common unsaturated acids of fish oils is the main reason why the alkali isomerization procedure cannot be applied to fish oils for the determination of individual acids with any hope of accuracy.

In the case of thermally polymerized marine oils, it is considered that the determination of fatty acid composition by means of the A.O.C.S. tentative method (Cd) 7-48 is meaningless, even when the method is modified by the use of 21 per cent KOH-Glycol as the isomerizing reagent instead of the 6.6 per cent KOH-Glycol reagent normally used.

The theoretical relationship between mean molecular weight of stand oils and dimeric acid content as presented in Fig. (1) was derived from the following considerations (Wells, 1952): in a hypothetical system containing ten triglyceride molecules, each of molecular weight X, the total weight of the system would be 10X and the mean molecular weight would be X. If union between two fatty acid residues in different triglyceride molecules were to occur, the number of molecules in the system would become nine.

The total weight of the system is still 10X. Therefore the mean molecular weight becomes 10X/9.

The numbers 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 $2/30 \times \frac{100}{1}$ or 6.7 per cent.

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 molecular weight. It makes no difference whether the succeeding stages involve union between monomeric and polymeric glycerides, 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 mol. wt. of heated triglycerides

and M = mean mol. wt. of triglycerides before heating.

The question as to whether or not a random state of acyl group distribution exists over the triglyceride molecule, can be determined by comparing calculated and observed values for the weight fraction of monomeric triglycerides present in the stand oils.

The mathematical relationship applied to the calculation of values for the weight fraction of monomeric glycerides as presented in Table VII was derived from the following considerations

(Hoeve and Sutton, 1956).

If a random state of acyl group distribution exists over the triglyceride molecules, then the probability that a glycerol molecule is esterified with three mono-acids is M^3, where M is the number of acid groups on a number basis residing on monomeric acids. Therefore M is equal to the weight fraction of monomeric acids. The total weight of the oil is somewhat more than the amount of glycerol molecules would indicate, since free acids are present. If this is taken into account, the weight fraction of monomeric triglycerides is $P_b M^3$, when P_b is given by one minus the weight fraction of free acids.

Results and Discussion

Table I presents the chemical and physical characteristics of the menhaden oil and the stand oils prepared from it. The values for the oil itself lie within the accepted range for menhaden oil. The rate of polymerization, as judged by the increase of mean molecular weight with time, was of the same order as that for linseed oil over the first nine hours, and much more rapid than for either sunflower or soybean oils. With longer heating times, the mean molecular weights of menhaden stand oils levelled off and only a negligible increase was observed during the final hours of the polymerization.

The menhaden oil did not exhibit gelling properties even after eighteen hours of polymerization. However, the stand oils exhibited definite thixotropy as determined by appropriate viscosity measurements.

TABLE I

Characterization of commercial refined menhaden oil and of stand oils prepared from it by thermal polymerization at 270°C. in a current of CO_2

Time of Heating (Hours)	Sp. Gr. 25°C.	Ref. Ind. 25°C.	I.V. (B. & K.)	Viscosity (Poise) 25°C.	Mean Mol. wt. (Cryoscopic)	Sap. No.*	Free Fatty Acids (Percent)	Unsap. Percentage
0	0.9251	1.4784	180	0.3	839	188	0.18	1.04
2	0.9436	1.4852	135	3.7	1139	165	0.80	0.95
5	0.9563	1.4877	115	9.7	1347	147	1.30	1.02
9	0.9603	1.4886	113	17.6	1450	124	2.16	1.19
13	0.9641	1.4894	110	> 22.7	1507	107	2.40	1.27
18	0.9667	1.4898	108	≫ 22.7	1515	96	2.63	1.20

* Based on a saponification time of 30 minutes (A.O.A.C. procedure). The average saponification number of all fractions, based on a saponification time of one hour was 189.

The apparent fall in saponification number with time was traced to the increased difficulty of saponifying stand oils; for when the time of saponification was increased to one hour, all the oils gave saponification values close to 189. The "thirty minutes" saponification values are listed in Table I because they may afford a rough measure of the degree of steric hindrance to saponification.

Table II presents the analytical results for the ethyl esters of menhaden oil and the stand oils. The mean molecular weight of the ethyl esters displayed little increase beyond thirteen hours heating time, whereas the values for linseed stand oils continued to increase rapidly. The fall of iodine value and increase in refractive index were of the order and trend to be expected from the increase in mean molecular weight.

TABLE II

Characteristics of the ethyl esters derived from thermally polymerized menhaden oil

Time of Heating (Hours)	Ref. Ind. 25°C.	I.V. (B. & K.)	Free Fatty Acids (Percent)	Mean Molecular Weight (Cryoscopic)	Sp.Gr. 25°C.	Sap. No.*
0	1.4592	172	5.24	317	0.8830	176
2	1.4648	122	4.85	341	0.8950	175
5	1.4669	98	2.65	369	0.9090	172
9	1.4670	92	4.70	389	0.9150	172
13	1.4691	92	3.75	399	0.9170	171
18	1.4700	88	1.25	418	0.9200	180

* Based on a saponification time of one hour.

It may be pointed out that the percentage unsaponifiable remained remarkably constant, so that no errors were being introduced by formation of unsaponifiables by pyrolysis or other side reactions.

Table III presents the analytical data for the distillable esters. The slight residue from the distillation of the esters of unheated oil is presumably a reflection of the content of unsaponifiable. The non-distillable fraction of the stand oil esters increased in amount rather sharply up to nine hours, and then levelled off. This trend conforms with the course of the mean molecular weight values of the whole oils (vide supra).

Tables IV and V show the chemical and physical characteristics of the AFD and NAFD fractions. Percentage yields of the monomeric, polymeric, AFD and NAFD fractions are presented in Table VI.

TABLE III

Characteristics of distillable esters (DE) of thermally polymerized menhaden oil

Time of Heating (Hours)	Yield* (Percent)	Ref. Ind. 25°C.	I.V. (B. & K.)	Free Fatty Acid (Percent)	Mean Molecular Weight (Cryoscopic)	Sap.** No.
0	99.4	1.4560	138	5.75	311	185
2	80.0	1.4525	86	3.75	348	187
5	73.9	1.4495	69	4.50	324	189
9	68.4	1.4475	66	3.15	304	192
18	66.6	1.4465	55	1.95	293	192

* Based on micro-distillation.
** Based on a saponification time of one hour.

All data in this table are expressed as percentage of the total ethyl esters. The high yield and relatively large iodine values of the NAFD fraction conform with the theory that NAFD production is dependent on the nature of the unsaturated fatty acid constituents of the original oil. It can be seen from Table V that esters of the unheated oil, when handled by the standard methods in use in this laboratory, gave an appreciable yield (five per cent) of NAFD with an iodine value of 327. This was probably due to the relative difficulty with which longer chain highly unsaturated fatty acids form urea adducts.

A striking and unexpected feature of the results for the distillable esters (Table III) was the sharp increase in the mean molecular weight value for this fraction at two hours polymerization. There can be little doubt of the reality of this effect for the determinations were repeated on three occasions, and the greatest care was taken in preparing samples for analysis lest the original observation might have been in error. As a further check on the validity of these results, the two-hour thermal polymerization of menhaden oil was repeated. The high value of 348 for the mean molecular weight of the distillable ester fraction was again observed. No analogous feature has been observed in connection with the polymerization of either linseed, soybean or sunflower seed oils. The author is unable to offer any convincing explanation for the high mean molecular weight value of this distillable ester fraction. Various speculative hypotheses were considered but none warrants discussion here, in the absence of further experimental evidence.

TABLE IV

Characteristics of AFD (adduct-forming-distillable) fraction of ethyl esters of thermally polymerized menhaden oil

Time of Heating (Hours)	Yield (Percent)	Ref. Ind. 25°C.	I.V. (B. & K.)	Free Fatty Acids (Percent)	Mean Molecular Weight (Cryoscopic)	Sap.* No.
0	95	1.4557	146	0.76	303	190
2	87	1.4450	67	3.09	298	193
5	81	1.4430	48	3.52	292	195
9	78	1.4422	43	1.98	291	198
18	75	1.4413	45	1.28	287	210

* Based on a saponification time of 30 minutes.

TABLE V

Characteristics of NAFD (non-adduct-forming-distillable) fraction of ethyl esters of thermally polymerized menhaden oil

Time of Heating (Hours)	Yield (Percent)	Ref. Ind. 25°C.	I.V. (B. & K.)	Mean Molecular Weight (Cryoscopic)
0	5	1.4801	327	385
2	13	1.4884	204	333
5	19	1.4854	200	320
9	22	1.4830	177	321
18	25	1.4818	152	313

TABLE VI

Composition of the ethyl esters of thermally polymerized menhaden oil. Results expressed as percentage of total ester

Time of Heating (Hours)	Distillable esters			Non-distillable esters (Polymeric residue)
	Total	Adduct forming (AFD)	Non-adduct forming (NAFD)	
0	99.4 (311)	95 (303)	5 (385)	0.6
2	80.0 (348)	87 (298)	13 (333)	20.0
5	73.9 (324)	81 (292)	19 (320)	26.1
9	68.4 (304)	78 (291)	22 (321)	31.6
18	66.6 (293)	75 (287)	25 (313)	33.4

Note: Figures in parentheses show mean molecular weights (cryoscopic).

From two hours onward, the mean molecular weight of the distillable esters fell off regularly. This regular fall agrees with the hypothesis that longer chain highly unsaturated fatty acids participate preferentially in thermal polymerization.

The measured and calculated values for the dimeric acyl content of the menhaden stand oils are presented in Table VII. These data are graphically presented in Figure 1 which shows the relationship between actual and theoretical dimeric acyl content and mean molecular weight for the series of menhaden stand oils.

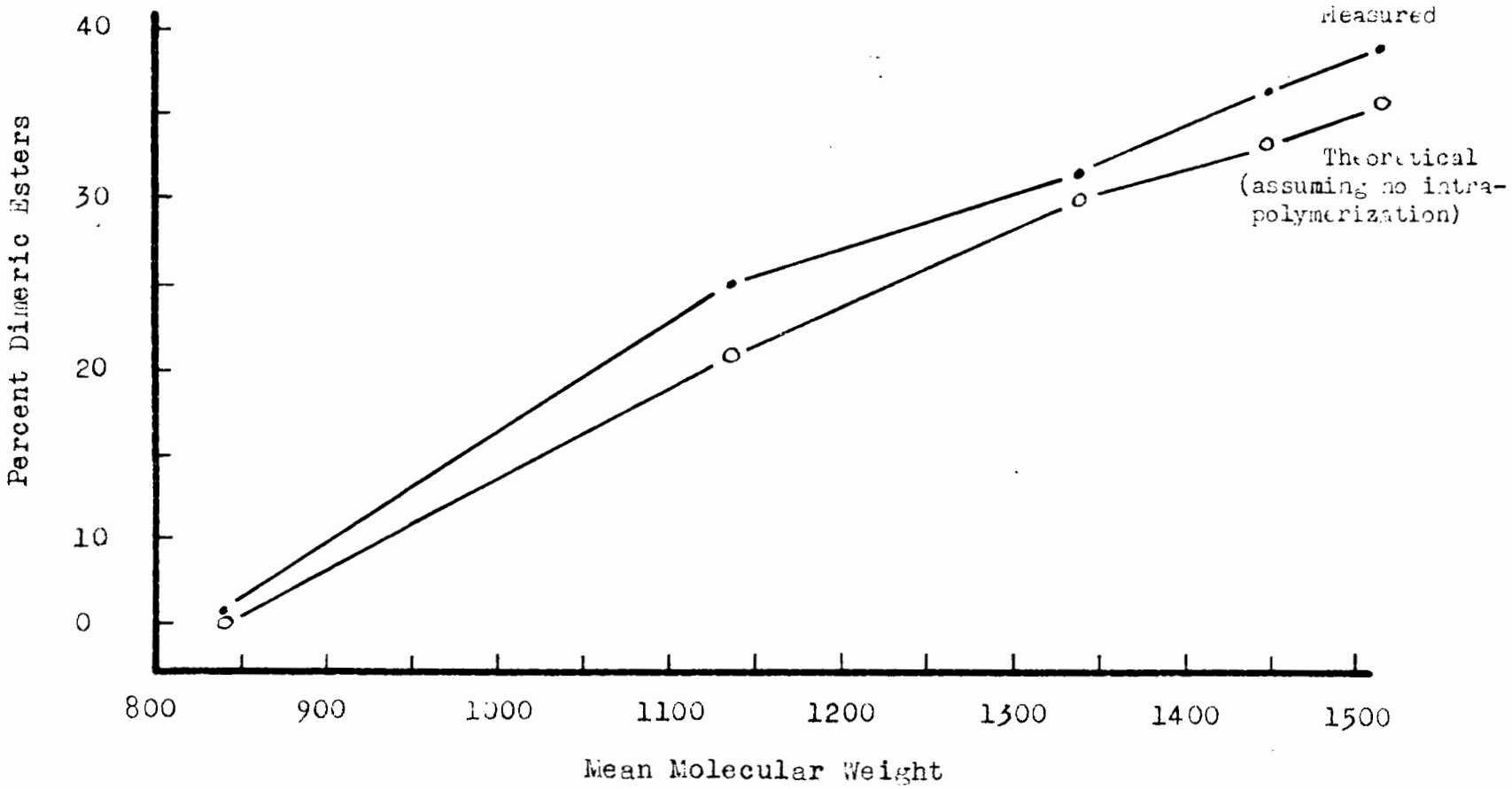


Figure 1. Thermally Polymerized Menhaden Oil: The Relation of Percent Dimeric Esters to the Mean Molecular Weight

TABLE VII

Amount of dimeric acids in polymerized menhaden oil

Mean Molecular weight of stand oils	Amounts of dimeric acids in weight percentage	
	Measured	Calculated
839	0	0
1139	20.0	16.0
1347	26.0	25.0
1450	31.5	28.2
1515	33.5	30.2

The theoretical relationship is based on the assumption that intra-molecular dimerization of fatty acid residues does not occur. The initial rise in dimeric acyl group content without a corresponding increase in the mean molecular weight of the stand oil suggests that, during the initial stage of the thermal polymerization of menhaden oil, dimerization occurs between fatty acid residues within the same monomeric glyceride molecule.

The ratio of polymeric acyl groups to polymeric glycerides is shown in Table VIII. This ratio as a function of degree of polymerization is presented in Figure 2, and illustrates the intra-glyceride reactions of initially non-conjugated oils. The curve for menhaden oil suggests that intra-glyceride reactions play an important part in the initial changes caused by thermal polymerization. Fig. 3 shows the relation of refractive index to

A. Menhaden oil: 275°C.

The following curves have been taken from work published by Sims (1956).

B. Linseed oil: 220°C
C. β Tung oil: 203°C
D. ∝ Tung oil: 270°C
E. Linseed oil: 320°C

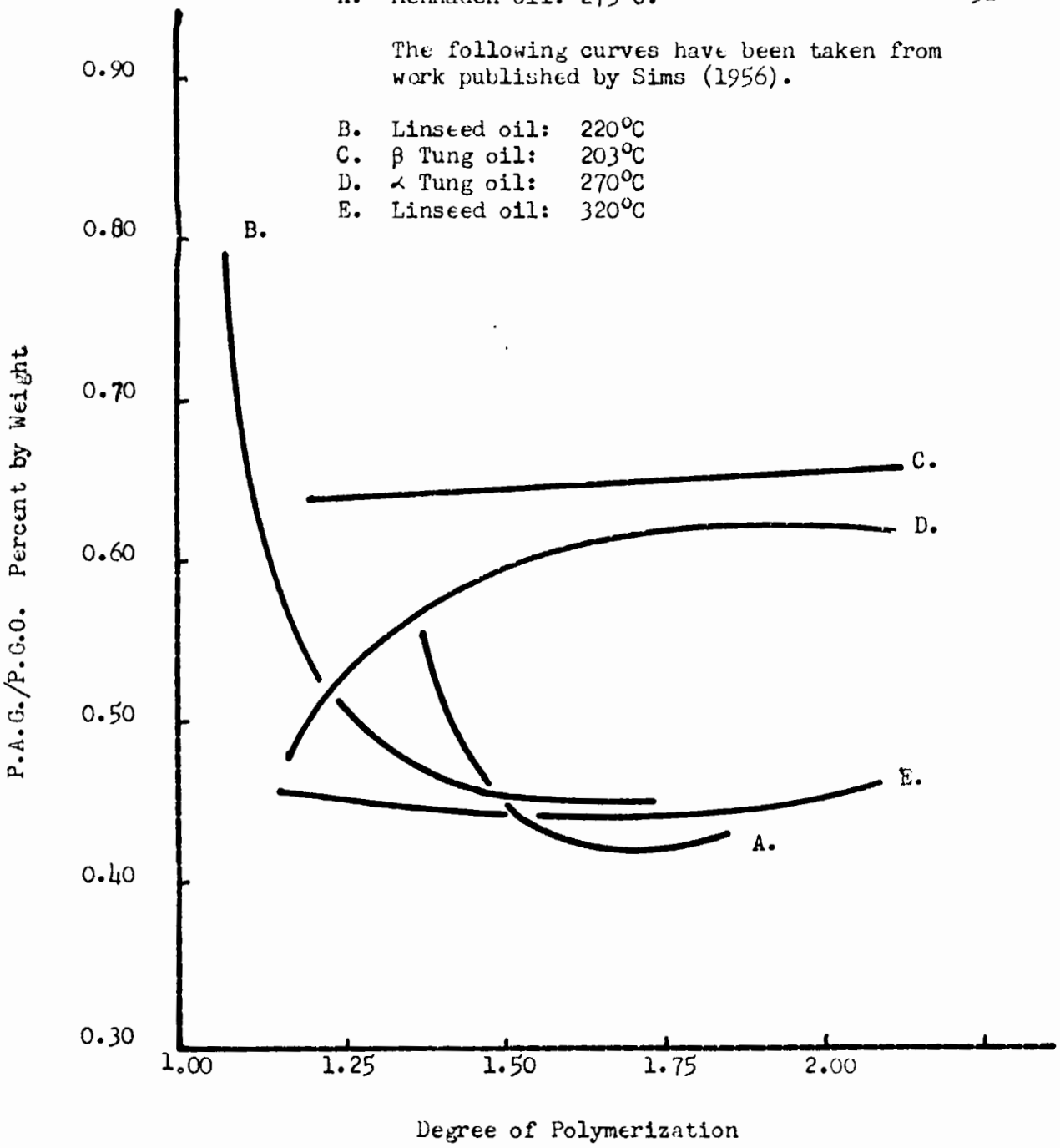


Figure 2. Ratio of polymeric acyl groups to polymeric glycerides as a function of degree of polymerization

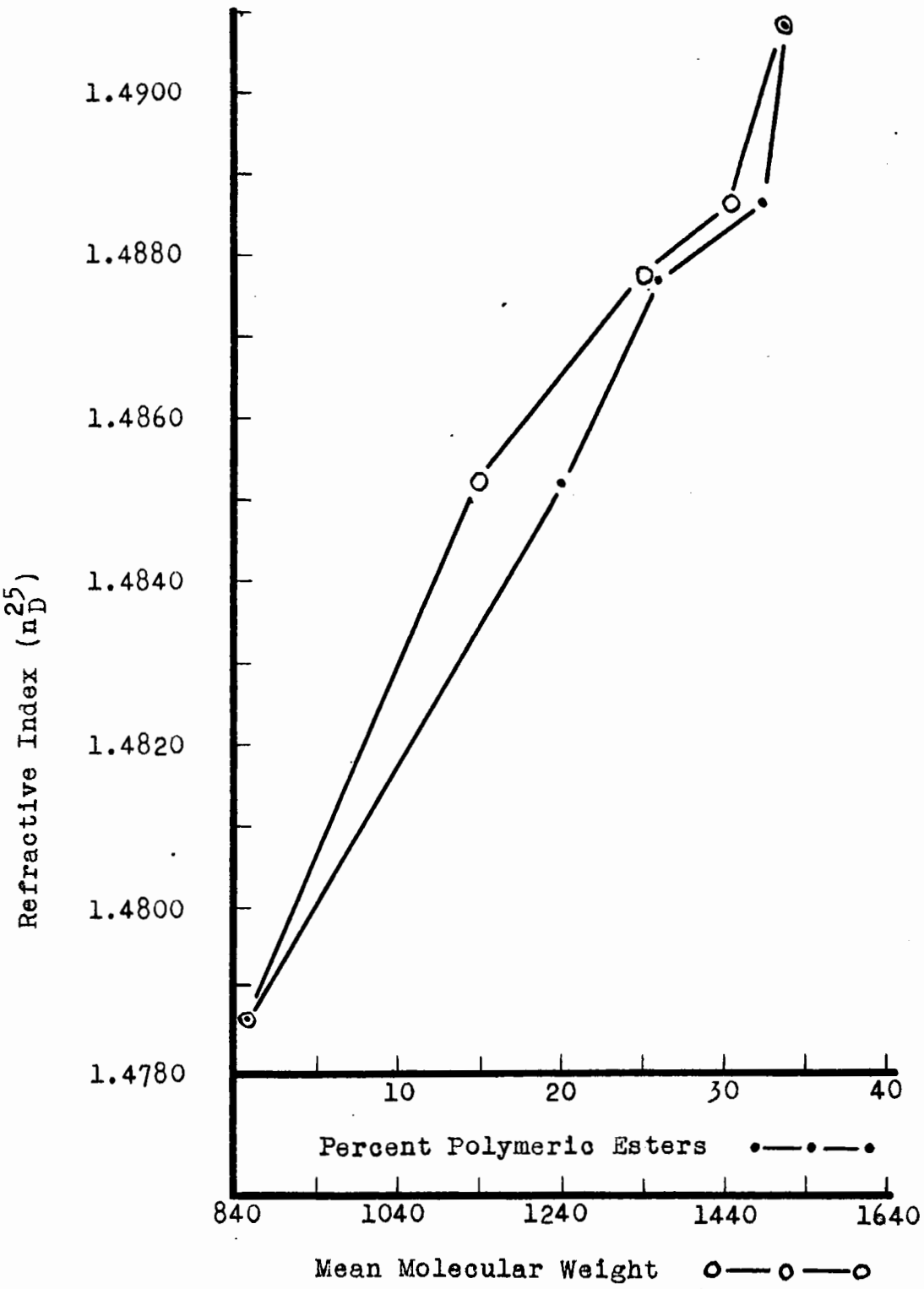


Figure 3. Thermally Polymerized Menhaden Oil: The Relation of Polymer Content and Mean Molecular Weight to the Refractive Index

TABLE VIII

The weight percent of polymeric glycerides and polymeric acyl groups in thermally polymerized menhaden oil

Menhaden oil degree of Polymerization	Polymeric glycerides (wt.percent)	Polymeric acyl groups (wt. percent)	PAG/PGO weight ratio (percent)
1.36	36	20	0.56
1.60	60	26	0.43
1.71	71	31.5	0.44
1.80	80	33.5	0.42

(a) the per cent yield of polymeric ethyl esters, and to (b) the degree of triglyceride polymerization measured in mean molecular weight units. The divergence between these two curves conforms with the hypothesis that intra-molecular reactions play an important role during the initial stage of thermal polymerization of menhaden oil. It seems very probable that this role is of relatively much greater importance in the heat polymerization of menhaden oil, than of sunflower, soybean or linseed oil.

TABLE IX

Amounts of monomeric triglycerides in polymerized menhaden oil

Duration of polymerization at 275°C. (Hours)	Amounts of monomeric glycerides in weight percentage	
	Measured	Calculated
2	64	50
5	40	40
9	29	31
18	20	28

The large differences between calculated and measured values for the weight percentage of monomeric triglycerides, as shown in Table IX, indicate the existence of an initial state of non-random distribution of acyl groups over the glyceride molecule. However, thermal polymerization of menhaden oil at 275°C. for five hours appears to create a state of complete randomization of acyl groups over the glyceride molecule.

Ultraviolet absorption coefficients have been calculated for the following fractions: (1) ethyl esters prepared from menhaden and stand oils, (2) distillable ethyl esters, (3) adduct-forming distillable ethyl esters, (4) non-adduct-forming distillable ethyl esters. The absorption coefficients of these fractions are presented in Tables X, XI, XII and XIII respectively.

Comparison of the ultraviolet spectrograms of unheated oil and whole unheated ethyl esters, indicates that an appreciable degree of conjugation was apparently caused by acid catalyzed transesterification reactions.

The two-hour heat treatment of menhaden oil appears to have increased the concentration of conjugated material. This isomerization is greatest in cases of diene conjugation; and it also occurs, though to a lesser extent, with triene, tetraene and pentaene conjugation.

The data shown in Tables XI, XII and XIII indicate that urea adduct fractionation separated the distillable esters into an adduct-forming-fraction containing relatively more saturated components, and a non-adduct-forming fraction containing highly unsaturated complex fatty acid constituents.

These results also indicate that only minor changes in the composition of the menhaden stand oils, and of fractions derived from it, occurred during the final nine hours of polymerization.

The results presented in this section may be summarized as follows:

(1) The acyl groups of commercial menhaden oil appear to be non-randomly distributed over the glyceride molecule.

(2) Intra-glyceride reactions play an important part during the initial stages of thermal polymerization.

(3) Changes due to thermal polymerization occur rapidly during the initial five hours, but appear to decrease to very minor proportions after nine hours heating time.

TABLE X

Absorption coefficients of the ethyl esters
of menhaden stand oils

Time of Heating (Hours)	"K" conj. constit.	"K" non-conj. constit.	Δ K	"K" conj. constit.	"K" non-conj. constit.	Δ K
		233 mμ			268 mμ	
0	1.31	19.20	17.89	0.38	16.20	15.82
2	5.04	13.00	7.96	1.76	6.38	4.62
5	4.14	9.00	4.86	0.66	2.02	1.36
9	3.30	6.60	3.30	0.39	1.20	0.81
18	2.41	5.14	2.73	0.21	0.66	0.45
		278 mμ			300 mμ	
0	0.32	15.17	14.85	0.15	11.68	11.53
2	0.90	5.72	4.82	0.25	2.84	2.59
5	0.39	1.54	1.15	tr	0.54	0.54
9	trc	0.84	0.84	nil	trc	trc
18	nil	0.48	0.48	nil	nil	nil
		315 mμ			327 mμ	
0	0.10	11.97	11.87	0.06	7.85	7.79
2	0.15	2.53	2.38	trc	1.38	1.38
5	trc	0.34	0.34	nil	trc	trc
9	nil	trc	trc	nil	nil	nil
18	nil	nil	nil	nil	nil	nil
		346 mμ			374 mμ	
0	0.05	6.76	6.71	0.03	0.94	0.91
2	trc	0.96	0.96	nil	trc	trc
5	nil	nil	nil	nil	nil	nil
9	nil	nil	nil	nil	nil	nil
18	nil	nil	nil	nil	nil	nil

TABLE XI

Absorption coefficients of the distillable ethyl esters of menhaden stand oils

Time of Heating (Hours)	"K" conj. constit.	"K" non-conj. constit.	Δ K	"K" conj. constit.	"K" non-conj. constit.	Δ K
		233 mμ			268 mμ	
0	0.83	10.19	9.36	0.21	7.02	6.81
2	2.56	8.36	5.80	0.49	3.18	2.70
5	2.11	5.80	3.69	0.27	1.86	1.59
9	1.50	4.44	2.94	trc	1.20	1.20
18	1.14	2.74	1.60	nil	0.47	0.47
		278 mμ			300 mμ	
0	0.20	6.94	6.74	0.07	5.61	5.54
2	0.35	2.90	2.55	0.06	1.80	1.74
5	0.17	1.49	1.32	0.03	0.61	0.58
9	nil	0.96	0.96	nil	0.42	0.42
18	nil	0.35	0.35	nil	trc	trc
		315 mμ			327 mμ	
0	0.04	6.12	6.08	0.01	2.02	2.01
2	0.03	1.24	1.21	nil	0.37	0.37
5	trc	0.36	0.36	nil	0.12	0.12
9	nil	0.24	0.24	nil	nil	nil
18	nil	trc	trc	nil	nil	nil
		347 mμ			374 mμ	
0	0.01	2.45	2.44	nil	0.33	0.33
2	nil	0.22	0.22	nil	trc	trc
5	nil	0.06	0.06	nil	nil	nil
9	nil	nil	nil	nil	nil	nil
18	nil	nil	nil	nil	nil	nil

TABLE XII

Absorption coefficients of the adduct-forming distillable ethyl esters of menhaden stand oils

Time of Heating (Hours)	"K" conj. constit.	"K" non-conj. constit.	ΔK	"K" conj. constit.	"K" nonconj. constit.	ΔK
		233 mμ			268 mμ	
0	1.22	11.10	9.88	0.39	8.82	8.43
2	1.08	6.53	5.45	0.29	3.85	3.56
5	0.96	4.46	3.50	0.08	1.56	1.48
9	0.60	3.41	2.81	0.05	0.83	0.78
18	0.36	2.51	2.15	trc	0.30	0.30
		278 mμ			300 mμ	
0	0.36	8.20	7.84	0.13	6.17	6.04
2	0.25	3.50	3.25	0.04	2.16	2.12
5	0.07	1.30	1.25	0.01	0.50	0.49
9	0.03	0.68	0.65	nil	0.24	0.24
18	nil	0.20	0.20	nil	trc	tr
		315 mμ			327 mμ	
0	0.05	6.10	6.05	0.02	3.66	3.64
2	0.02	2.10	2.08	trc	0.69	0.69
5	nil	0.42	0.42	nil	0.13	0.13
9	nil	0.18	0.18	nil	trc	trc
18	nil	trc	trc	nil	trc	trc
		347 mμ			374 mμ	
0	nil	3.03	3.03	nil	0.36	0.36
2	nil	0.80	0.80	nil	trc	trc
5	nil	0.08	0.08	nil	nil	nil
9	nil	trc	trc	nil	nil	nil
18	nil	nil	nil	nil	nil	nil

TABLE XIII

Absorption coefficients of the non-adduct-forming distillable ethyl esters of menhaden stand oils

Time of Heating (Hours)	"K" conj. constit.	"K" non-conj. constit.	Δ K	"K" conj. constit.	"K" conj. constit.	Δ K
		233 mμ			268 mμ	
0	2.84	27.60	24.76	0.58	27.60	27.02
2	13.64	24.50	10.86	2.81	9.85	7.04
5	9.77	18.90	9.13	1.32	5.67	4.35
9	6.36	14.50	8.14	trc	3.08	3.08
18	4.80	10.70	5.90	nil	2.22	2.22
		278 mμ			300 mμ	
0	0.49	24.48	23.99	0.21	21.67	21.46
2	1.21	9.14	7.93	0.44	5.14	4.70
5	0.77	4.94	4.17	trc	1.41	1.41
9	tr	3.15	3.15	nil	0.60	0.60
18	nil	1.65	1.65	nil	0.42	0.42
		315 mμ			327 mμ	
0	0.16	22.44	22.28	0.10	16.40	16.30
2	0.09	4.24	4.15	trc	2.64	2.30
5	nil	0.82	0.82	nil	0.33	0.33
9	nil	0.18	0.18	nil	trc	trc
18	nil	0.12	0.12	nil	trc	trc
		347 mμ			374 mμ	
0	0.05	13.70	13.65	trc	2.20	2.20
2	trc	1.87	1.87	nil	trc	trc
5	nil	0.10	0.10	nil	nil	nil
9	nil	trc	trc	nil	nil	nil
18	nil	trc	trc	nil	nil	nil

SECTION II

THE NUTRITIONAL VALUE OF FRACTIONS OBTAINED FROM THERMALLY POLYMERIZED MENHADEN OIL

INTRODUCTION

The nutritive values of certain fractions of the ethyl esters prepared from heat-polymerized linseed, soybean and sunflower seed oils have been compared by Crampton, Common, Pritchard and Farmer (1956). Particular attention was paid to the fraction of the distillable ethyl esters that failed to form urea adducts (the NAFD fraction). Linseed oil NAFD was highly injurious to young rats; and the corresponding fraction from soybean oil was also injurious, though to a lesser estent than that from the linseed oil. Sunflower seed oil NAFD was only slightly injurious nutritionally. The adduct forming fraction of the distillable esters (the AFD fraction) from all three oils was nutritionally innocuous.

Failure of the NAFD fraction to form urea adducts may be due, in part, to formation of cyclic and branched-chain monomeric acids during thermal polymerization. It has been established that "cyclized" monomers are formed during heating of methyl eleostearate (Paschke and Wheeler, 1955b) and of linseed oil (Macdonald, 1956). Confirmatory evidence, from infra-red spectrophotometry, of "cyclized" material in linseed oil NAFD will be presented below (Section III). These results suggested a possible association between the toxicity of the NAFD fraction and the presence of polyene acids in the original oil.

If this view is correct, then NAFD fractions from oils containing acids of higher unsaturation than linolenic should be as toxic, or more toxic, than the NAFD from linseed oil. Menhaden oil was chosen for the purpose of such studies.

Procedure and Results

The commercial alkali-refined menhaden oil used in this work had the following characteristics:- sp. gr. (25°C.), 0.9251; refractive index (25°C.), 1.4784; iodine no. (Benham and Klee, 1950), 180; saponification no., 188; mean molecular weight (cryoscopic), 839; unsaponifiable, 1.04 percent. Typical data for the composition of the mixed fatty acids of menhaden and linseed oil are presented in Table XIV. The menhaden oil was heated in batches of 500 gm. for 15 hours at a temperature of 275°C. in the same manner as reported in Section I. The fifteen hours heating time was selected on the basis of the results of the preliminary kinetic studies reported in Section I. The heated oil was converted to the ethyl esters by acid catalyzed transesterification as described in Part II. Fractionation of the esters by vacuum distillation and urea adduct formation was performed as described in Part II. For convenience of reference, the system of fractionation and the abbreviations used are set out in Fig. 4. Samples (1200 gm.) of each fraction were prepared for the feeding trials. The percentage yields and characteristics of the fractions of ethyl esters of heat polymerized menhaden oil used in the feeding trials are presented in Tables XV and XVI.

TABLE XIV

Fatty acid composition by weight of menhaden and linseed oil

Fatty acid components	Menhaden[1] % W/W	Linseed[2] % W/W
Saturated acids		
C 12	Trace	-
C 14	6.8	-
C 16	15.5	6.3
C 18	3.1	2.5
C 20	-	0.5
C 22	-	-
Total saturated	25.4	9.3
Unsaturated acids[3]		
C 14	0.1(-2.0)	-
C 16	14.9(-2.1)	19.0(-1.0)
C 18	23.7(-2.1)	24.1(-2.0)
C 20	17.5	47.4(-3.0)
C 22	10.8	-
C 24	4.0	-
C 26	1.2	-
over C 26	2.4	-
Total unsaturated	74.6	91.7

1 Smith and Brown (1946) cited by Bailey (1952)
2 Rose and Jamieson (1941) cited by Bailey (1952)
3 Figures in parentheses refer to the average number of double bonds per molecule.

TABLE XV

Yields of fractions of ethyl esters of heated menhaden oil used in feeding trial

Diet No.	Ester Fraction	Yield as percent of total esters of heated oil	
		Linseed** (12 hr. at 275°C)	Menhaden (15 hr. at 275°C)
1	Adduct-forming fraction of total esters (AF)	46 (293)**	45 (299)
2	Non-adduct-forming fraction of total esters (NAFD)	54 (472)	45 (497)
3	'Distillable' esters (DE)	60 (294)	60 (296)
4	Adduct-forming fraction of distillable esters (AFD)	49 (293	34 (290)
5	Non-adduct-forming fraction of distillable esters (NAFD)	11 (300)	20 (319)
6	Non-distillable esters (NDE)	40 (550)	-
7	Whole esters of unheated oil (WE)	-	100 (314)

* Data for linseed oil quoted from Crampton et al. (1953).
** Figures in parentheses are cryoscopic mean molecular weights determined in cyclohexane.

TABLE XVI

Characteristics of fractions of ethyl esters of menhaden oil used in feeding trial

Diet No.	Ester Fraction	I.V. (B. & K.)[1]	Free Fatty Acids (Percent)	Mean[4] Molecular Weight (Cryoscopic)	Sap.[2] No.	Peroxide[3] value mgm peroxide O_2/kgm oil (a)	(b)
1	AF	1.4483	86	299	183	276	6712
2	NAF	1.4964	151	497	151	0	1047
3	DE	1.4517	84	296	186	0	861
4	AFD	1.4420	45	290	189	360	1792
5	NAFD	1.4659	143	319	169	0	1215
7	WE	1.4598	156	314	182	0	1100

[1] Iodine numbers determined by the method of Benham and Klee (1950).

[2] Saponification numbers determined by the method of the A.O.A.C. (1955).

[3] Peroxide values determined by the method of Skellon and Wills (1948).
(a) Value of ester fraction before its inclusion in the diet.
(b) Value determined on oil fraction extracted from the diets by cold chloroform at the conclusion of the 28-day feeding trial.

[4] Mean molecular weights determined cryoscopically in cyclohexane.

Fractionation of Ethyl Esters

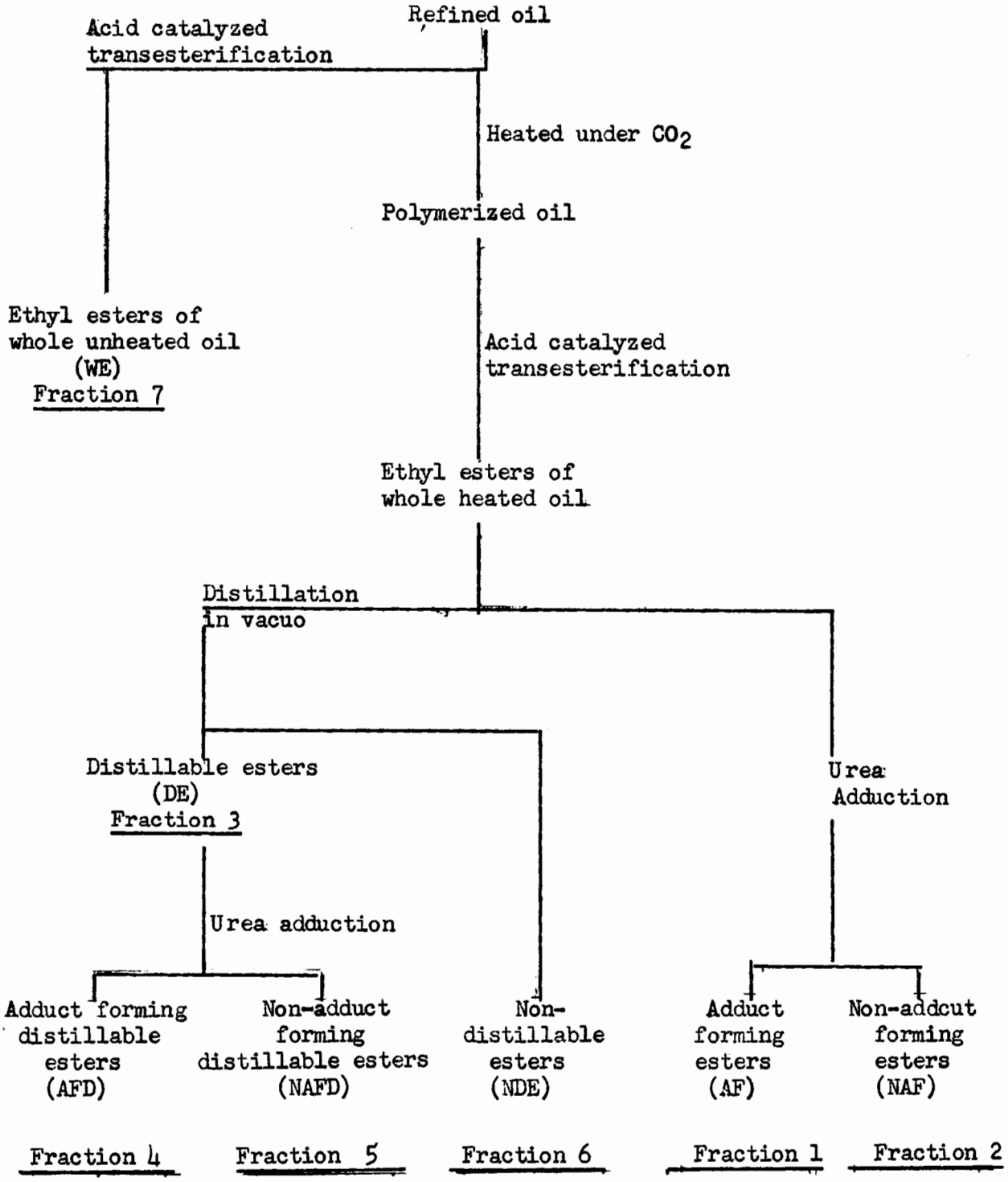


Figure 4. Flow sheet illustrating preparation of fractions of heated menhaden oil used in feeding trials.

A peroxide determination was conducted on each ester fraction prior to its incorporation into the rat diets. At the completion of the feeding trial, the ester fractions were extracted from the various diets and their respective peroxide values determined. All fractions were protected with 0.025 per cent Tenox II antioxidant during processing operations as well as before incorporation into the diets. The diet formulation is given in Table XVII.

TABLE XVII

Composition of diets used in nutritional experiment

Ingredient	10% Ester diet	20% Ester diet
Whole wheat	54	44
Skimmilk	19	19
Casein	12	12
Ester fraction	10	20
Dried yeast	3	3
Daicalcic phosphate ("20.FOS)	1	1
Iodized salt	0.50	0.50
Ferrous sulphate	0.10	0.10
Vit-A & D supplement	0.15	0.15
Chromic oxide*	0.25	0.25

* The chromic acid was used as a tracer for digestibility determinations.

TABLE XVIII

Effects of ethyl esters of heated menhaden oil on survival and live weight gain of rats and the digestibility of ether extracts of the diets

Diet No.	Ester Fraction	% of rats surviving 28-day test		Av. live weight gains for 28-day period		Av. feed intake per rat per 28-day period		Apparent digestibility of ether extract		Av. live weight gain per 1000 Cals. digested	
		20%	10%	20%	10%	20%	10%	20%	10%	20%	10%
		%	%	gm	gm	gm	gm	%	%	gm	gm
1	AF	100 (100)	100 (100)	118 (101)	113 (107)	238 (266)	286 (300)	94 (96)	87 (95)	105 (61)	95 (61)
2	NAF	0 (20)	90 (100)	- (-32)	3 (39)	- (141)	132 (205)	- (75)	73 (77)	- (9)	37 (50)
3	DE	80 (90)	100 (100)	2 (4)	46 (66)	95 (118)	152 (228)	95 (97)	88 (92)	43 (43)	67 (63)
4	AFD	100 (100)	100 (100)	111 (57)	122 (108)	233 (193)	261 (299)	94 (96)	91 (96)	92 (54)	103 (62)
5	NAFD	10 (0)	60 (0)	-50 -	-13 -	81 -	96 -	85 -	87 (93)	- -	- -
7	WE	100 -	100 -	61 -	98 -	165 -	233 -	93 -	94 -	73 -	87 -
LSD (P = 0.05)				18 (23)	18 (23)	30 (36)	30 (36)			18 (16)	18 (16)

NOTE: Figures in parentheses relate to comparable data for linseed oil cited from Crampton et al. (1953).

The experimental animals comprised twelve groups each of ten weanling male rats. Six of these groups received each a different ester fraction at a level of ten per cent of the diet; and the other six groups received the same ester fractions at a level of twenty per cent. The various diets were offered ad libitum for a twenty eight day period. The age of the test animals varied from twenty to thirty days. The results for survival, feed intake, live weight gains, digestibility of the ether extract and gain per 1000 digested Calories are presented in Table XVIII. This table also includes comparable data for similar fractions from heated linseed oil (cited from Crampton, Common, Farmer, Wells and Crawford, 1953).

Discussion

Survival of rats. The percentage of rats surviving the 28 day feeding period is shown in Table XVIII. The only deaths recorded were in lots where the diets contained either non-adduct forming monomers or dimeric and higher polymers. However, the menhaden oil NAFD seemed to be slightly less toxic than that from linseed oil.

The ester fraction which consisted entirely of urea adduct forming monomers or esters of the whole unheated oil had no harmful effect on survival.

Gain in weight of rats. The results leave little doubt that esters of both "cyclic" monomeric and polymeric acids produced by the thermal polymerization reaction are undesirable components of rat diets. The weight gain of rats on diets containing esters of whole unheated menhaden oil were definitely sub-optimal and significantly lower than

on diets containing AF or AFD fractions. This deleterious effect may be due to the highly unsaturated fatty acid constituents.

Food intake. Diets containing fractions No.1 and No.4 were apparently eaten at comparable rates. The "cyclic" monomer (fraction 5) was less readily eaten than the other fractions; and in general, the results suggest that the only fraction of really poor acceptability was fraction No.5.

Digestibility of ether extract. There was evidence that the non-adduct forming monomers and polymers were poorly digested. The apparent digestibility of all other fractions was above 90 per cent.

Efficiency of utilization of dietary calories (gain in grams per 1000 digested Calories). The data suggests that the rats were unable to utilize diets containing "cyclized" monomeric material. The data also suggest that the efficiency of utilization of dietary calories is less, in the case of diets containing linseed oil, as compared to menhaden oil.

Deficiency symptoms. Approximately 40 per cent of all rats under test exhibited signs of deranged metabolism (dermatitis, necrotic tail and scaly paws). However, these symptons were distributed throughout the test animals in such a way, that no correlation between specific diets and incidence of deficiency symptons could be made.

Post-mortem examination. No macro-anatomical abnormalities could be observed as a result of autopsies performed on rats that died during the course of the 28 day feeding trial.

The results presented in Table XVIII may be summarized in the general statement that the various fractions from heated menhaden oil

had about the same effect on the well-being of the rats as had similar fractions from linseed oil in previous comparable experiments. The slight apparent superiority of menhaden oil NAFD was not sufficiently marked to warrant any assertion as to its superiority over the NAFD from linseed oil. The menhaden oil NAFD, however, was markedly inferior nutritionally to the NAFD from soybean or sunflower seed oil.

SECTION III

ON THE CHEMICAL NATURE OF THE NON-ADDUCT-FORMING FRACTION OF THE DISTILLABLE ETHYL ESTERS OF THERMALLY POLYMERIZED MENHADEN, HERRING, LINSEED, SOYBEAN AND SUNFLOWER SEED OILS

INTRODUCTION

Clingman, Rivett and Sutton (1954) have shown that the heat treatment of various esters and of sunflower seed oil yields cyclized dimeric material.

The presence of cyclic monomers among the products of the heat treatment of glycerides and fatty esters has often been suggested, but until recently the evidence for them has been indirect. Wells and Common (1953) suggested that the failure of the monomeric distillable esters to form urea adducts might be due to the presence of a non-terminal ring structure. Phthalic acid has been detected by means of colour reactions among the products of the oxidation of heated linseed oil (Champetier and Petit, 1945). Boelhouwer, Jol and Waterman (1952) used a graphical-statistical method of ring analysis to show that the monomeric products from the heat treatment of linseed oil contained ring compounds. Mehta and Sharma (1956) concluded that intra-acyl cyclization is the chief cause of the initial rapid drop in iodine value without a corresponding increase in molecular weight which takes place during the early stages of heat bodying of linseed oil.

Direct chemical evidence for the existence of six-membered rings in the monomeric fraction from thermally polymerized methyl eleostearate has been obtained by Paschke and Wheeler (1955b). Until recently no such evidence has been obtained for cyclic compounds in the monomeric products from non-conjugated esters or glycerides. However, Macdonald (1956) was able to provide spectroscopic and chemical evidence for the existence of a compound containing an unsaturated six-membered ring in linseed oil NAFD.

Holman and Aaes-Jorgensen (1956) were able to demonstrate that trans isomers of linoleic acid further depressed the growth and worsened the skin conditions of young rats fed on an EFA deficient diet. These trans acids were also found to have been deposited in the fat of the test animal.

There is also evidence that aromatic hydro-carbon chains may interfere with the protein metabolism of the rat via direct binding of the hydrocarbons to the proteins through the S-H or S-S groups (Souto and Boixados de Souto, 1956).

Part of the deleterious effect of heated linseed oil on the nutrition of the rat is associated with the NAFD fraction, and the yield and degree of toxicity of this fraction appears to be related to the presence of polyene acids in the original oil (Crampton et al., 1956). With these relationships in mind, it was considered of interest to examine the NAFD fraction from heat treated, menhaden, herring, linseed, soybean and sunflower seed oils.

In the present investigation, spectroscopic evidence for the presence or absence of cyclic monomers in these oils (vide supra)

has been obtained by methods involving aromatization *via* allylic bromination and dehydrohalogenation.

PROCEDURE

Preparation of NAFD: Alkali refined and bleached oils were thermally polymerized, and the non-adduct-forming distillable ethyl ester fractions were prepared. All methods used have been fully described in Part II. However, the linseed NAFD fraction from the first urea adduct separation was put through a further urea adduct fractionating procedure as follows: The filtrate plus washings from the first urea adduct separation was reduced in volume by fifty percent (corresponding to approximately 7% W/V solution of NAFD). The concentration of urea in this solution was adjusted to approximately 50% W/V. This slurry of urea, alcohol and oil was allowed to stand overnight at room temperature and was then placed in a freezing unit at -10°C. for a further six to eight hours. The crystalline urea material was removed by filtration on a Büchner funnel and the precipitated urea was washed free of NAFD material with ethanol saturated with urea. The crystalline urea material was then decomposed in hot water and the adduct-forming fraction was recovered in the usual way (see Part II).

A volume of ethanol, equivalent to the amount used when washing the crystalline urea material, was removed under vacuum from the combined solution of washings plus filtrate. This urea adduct fractionation procedure was then repeated until no appreciable yield of adduct-forming material was obtained.

Aromatization of the NAFD Fractions: The following procedure was applied to each NAFD fraction. The amounts of NAFD used varied from five to ten grams, but the molar ratio of NAFD to reactants etc. remained constant. Approximately nine grams (0.03 mole) of the NAFD fraction were dissolved in ninety ml. of benzene. To this was added an equivalent molar quantity of N-bromosuccinimide. The mixture was heated to reflux temperature for two hours. The solution was cooled, and most of the succinimide was precipitated on the walls of the flask. The solution was decanted from the solid succinimide, which was then washed with fresh benzene, and these washings were added to the benzene solution. The combined solution was transferred to an ampule (approximately 150 ml. capacity) and the benzene was stripped off under vacuum. One hundred ml. of freshly distilled 2, 4, 6 collidine was added to the residue (presumably the bromo-diene) and the ampule sealed with a flame while under a water pump vacuum. The sealed ampule was then submerged into Fisher bath wax at 150°C. for four hours, presumable to dehydrohalogenate the diene to the benzene derivative. The ampule was cooled and opened and the excess collidine was stripped off at 60°C. under water pump vacuum. The final removal of the residual collidine was achieved by using a high mechanical vacuum. The residue of collidine-hydrobromide plus some succinimide was extracted several times with Skellysolve B by decantation. The Skellysolve solution was washed five times with dilute HCl, five times with water, five times with more dilute HCl and finally five times with water. The solution was dried by filtration through a column of exsiccated sodium sulphate (Na_2SO_4). The solvent was stripped off to give a dark oil. This oil

was flash distilled (Part II) at a bath temperature of 240°C. in the case of linseed, soybean and sunflower seed oils and at a bath temperature of 260°C. in the case of menhaden and herring oils. The flash distillation procedure was carried out at an estimated pressure of two to three mm. of mercury. The clear coloured distillate darkened rapidly on exposure to light and air.

Hydrogenation of NAFD: Linseed oil NAFD was hydrogenated as described in Part II, using Raney nickel as the catalyst for hydrogenation.

Infrared analysis: Infrared absorption spectrograms were determined for the monomeric distillable ethyl ester fractions, the NAFD fractions and the "aromatized" NAFD fractions of menhaden, herring, linseed, soybean and sunflower seed oils. The phase used was a liquid oil film with an approximate thickness of 0.01 mm.

Oxidation of the dialkyl-benzene to ortho phthalic acid: Two attempts were made to isolate phthalic acid from aromatized linseed oil NAFD by means of the oxidation procedure described by Paschke et al.(1955), but neither attempt was successful.

RESULTS AND DISCUSSION

Tables XIX and XX present the physical and chemical characteristics of the various oils and derived ethyl ester fractions. The values for menhaden, herring, linseed, soybean and sunflower seed oils lie within the accepted ranges for these oils. The rates of polymerization of the three vegetable oils and of menhaden oil were of the order to

be expected from the results of previous work in this laboratory. The fall of iodine value and the increase in refractive index were also of the order and trend to be expected from the corresponding increases in the mean molecular weight values for the various stand oils.

An unexpected and peculiar feature of the results for the herring oil was the low viscosity and relatively small increase in the mean molecular weight values for the stand oils, even with a polymerization time of twenty hours. There can be little doubt of the reality of this effect, because the thermal polymerization procedure was repeated and the greatest care was taken in controlling the temperature of polymerization.

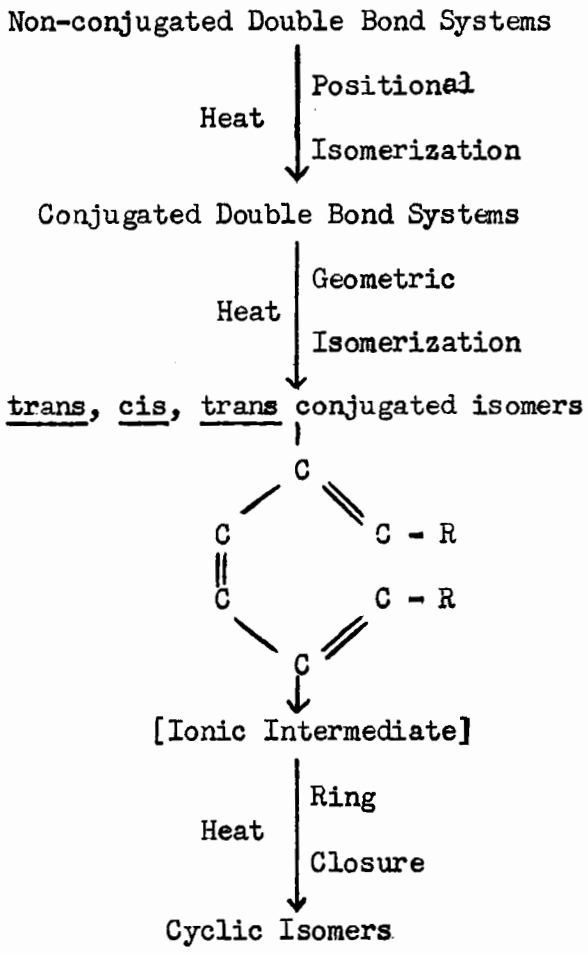
The composition of the ethyl esters of the thermally polymerized oils is shown in Table XXI. Yields of the various fractions are reported as percentages of the total ethyl esters. These yields conform with results of previous work done in this laboratory. However, the seven per cent yield of herring oil NAFD is remarkably low, but not surprising in view of the apparently small degree of polymerization.

The yields of linseed oil NAFD before and after exhaustive urea adduct fractionation is shown in Table XXII. The observed difference of two per cent in the yield of NAFD demonstrates the efficiency of the single urea adduct fractionation procedure as followed routinely in this laboratory.

The failure of NAFD to form urea adducts suggests that it has a non-linear structure. At the same time, it was considered possible that geometric isomerization about the plane of the double bond might

contribute to this failure. Hydrogenation of linseed NAFD, however, yielded a product that still failed to form urea adducts;and hence the non-linearity of the carbon skeleton (other than that associated with geometric isomerization of the double bonds) appears to be sufficient to prevent urea adduct formation (Table XXII).

Examination of the Fisher-Hirschfelder molecular models of unsaturated fatty acids shows that cyclization is sterically possible in numerous ways. However, in the case of a conjugated triene, the sole requirement necessary for the attainment of a pre-cyclization position appears to be a cis configuration at the central double bond. The central double bond of a conjugated system has the smallest amount of double bond character, and less activitation energy is necessary for its isomerization. A possible cyclization mechanism could be as follows:-



A model of this hypothetical intermediate (ionic) exhibits no steric hindrance.

The alkyl groups of the cyclized material would be either cis or trans to each other about the ring, with the trans form probably predominant. The cyclized material could consist of a mixture of double bond positional isomers formed after isomerization.

Since N-bromosuccinimide is a free radical reagent, it is necessary to bear in mind the possibility that during the bromination procedure, free-radical-induced cyclization may have given rise to the aromatized NAFD material. However, if such a cyclization had occurred in the aromatization of the NAFD fractions, a higher concentration of aromatic material might have been expected to result from these experiments.

It is probable that cyclic isomers of the common unsaturated fatty acids are of more significance in industrial operations and are more widely distributed in processed fatty material than might have previously been supposed.

The infrared spectra of the distillable esters, the NAFD and the "aromatized" NAFD fractions of the five oils under study, have been determined. The pertinent sections of these curves are presented in Figures 5 - 19. The infrared spectra of the various ethyl ester fractions of heated menhaden, herring, linseed and soybean oil exhibit a characteristic peak at 660 cm^{-1}. This peak is more pronounced in the cases of linseed and menhaden oil, but it is to be observed also in herring and soybean oils. Characteristic infrared absorption bands at 970, 940 (sh)*, 855, 755 (sh)*, 745 and 660 cm^{-1} indicate that the "aromatized" NAFD fractions of linseed, menhaden, herring

* (sh):- shoulder

TABLE XIX

Characteristics of various fractions of heated and unheated oils

Oil fraction	Viscosity 25°C. (poise)	Ref. Ind. 25°C.	I.V. (B. & K.)	Mean Molecular Weight (Cryoscopic)	Free Fatty Acid (Percent)
Linseed Oil					
Heating time of 12 hours					
(1) Unheated oil	0.50	1.4795	176	881	0.15
(2) Heated oil	12.00	1.4857	132	1498	2.45
(3) Ethyl esters of heated oil	- -	1.4640	-	-	1.10
(4) Distillable ethyl esters	- -	1.4575	140	287	1.11
(5) Non-adduct-forming distillable ethyl esters	- -	1.4691	169	305	1.32
Soybean Oil					
Heating time of 20 hours					
(1) Unheated oil	0.30	1.4765	122	870	0.9
(2) Heated oil	9.50	-	105	-	2.4
(3) Ethyl esters of heated oil	- -	1.4690	103	329	1.7
(4) Distillable ethyl esters	- -	1.4587	108	288	0.8
(5) Non-adduct-forming distillable ethyl esters	- -	1.4698	139	295	0.6
Sunflowerseed Oil					
Heating time of 26 hours					
(1) Unheated oil	0.30	1.4716	133	736	0.06
(2) Heated oil	6.00	1.4775	117	1608	2.42
(3) Ethyl esters of heated oil	- -	1.4598	113	339	0.92
(4) Distillable ethyl esters	- -	1.4530	100	-	2.64
(5) Non-adduct-forming distillable ethyl esters	- -	1.4600	110	298	2.90

TABLE XX

Characteristics of various fractions of heated and unheated oils

Oil fraction	Viscosity 25°C. (poise)	Ref. Ind. 25°C.	I.V. (B. & K.)	Mean Molecular Weight Cryoscopic)	Free Fatty Acid (Percent)
Menhaden Oil Heating time of 18 hours					
(1) Unheated oil	0.30	1.4784	180	839	1.04
(2) Heated oil	≫22.70	1.4898	110	1515	1.27
(3) Ethyl esters of heated oil	- -	1.4700	-	418	2.20
(4) Distillable ethyl esters	- -	1.4460	55	293	2.70
(5) Non-adduct-forming distillable esters	- -	-	152	313	1.63
Herring Oil Heating time of 10 hours					
(1) Unheated oil	0.50	1.4738	138	894-315*	0.05
(2) Heated oil	3.30	1.4800	94	1177	2.41
	4.70**	1.4804**		1228**	
(3) Ethyl esters of heated oil	- -	1.4600	91	346	0.84
(4) Distillable ethyl esters	- -	1.4500	62	302	1.31
(5) Non-adduct-forming distillable ethyl esters	- -	1.4830	150	331	1.00

* Value for the ethyl esters.
** Value for a heating time of twenty hours.

TABLE XXI

Composition of ethyl ester fractions of thermally polymerized menhaden, herring, linseed, soybean, and sunflowerseed oils. Results expressed as percentage of total ester

Type of oil	Time of heating (hours)	Distillable esters*			Non-distillable esters (polymeric residue)
		Total	Adduct-forming (AFD)**	Non-adduct-forming (NAFD)	
Menhaden	18	68	43.0	25.0	32
Herring	10	69	62.0	7.0	31
Linseed	12	70	57.7	12.3	30
Soybean	20	65	56.0	9.0	35
Sunflowerseed	26	56	48.0	8.0	45

* These values were based on a macro-distillation procedure.

** These values were based on the weight of the NAFD fraction.

TABLE XXII

Composition of ethyl ester fractions of linseed oil, thermally polymerized at 275°C. for a period of twelve hours. Yield of distillable esters expressed as percentage of total ester:- 70 percent*

Fraction		Percent of total esters	
		AFD	NAFD**
(1)	First urea fractionation	57.70	12.30
(2)	Second urea fractionation	1.15	11.15
(3)	Third urea fractionation	0.15	11.00
(4)	Fourth urea fractionation	0.00	11.00
	Total with no hydrogenation	59.00	11.00
First urea fractionation of hydrogenated NAFD from fraction (4)		1.50	9.50
	Total with hydrogenation	60.50	9.50

* Value based on macro-distillation.

** Values based on the weight of the corresponding AFD fractions.

and soybean oils contain a proportion of a disubstituted aromatic compound, most likely a benzene derivative. The constituents of the monomeric ethyl ester fraction from heated oils that cause this absorption at 660 cm^{-1}, are concentrated by the urea fractionation, and appear in the NAFD fraction.

This peak at 660 cm^{-1} is apparently absent from the spectra of the comparable fractions from sunflower seed oil (Figures 17, 18 and 19). This is consistent with the supposition that linolenic acid, or higher unsaturated acids of marine oils (normally absent in sunflower seed oil) act as precursors for the constituent that absorbs at 660 cm^{-1} and is formed in consequence of thermal treatment.

The absence of this absorption peak from the infrared spectrum of the brominated-debrominated NAFD fraction of sunflower seed oil (Figure 19), may be considered as indirect evidence that a minimum of free radical induced cyclization occurs as a result of using N-bromo-succinimide as the brominating agent.

However, the "aromatized" NAFD fraction of sunflower seed oil contained about twenty to thirty percent solid crystalline material at room temperature. The infrared spectrum of this semi-solid fraction differs from the corresponding fractions from the other four oils in that it exhibits characteristic absorption maxima at 690, 720 and 733 (sh)* cm^{-1}. An absorption band of low intensity at 800 - 805 cm^{-1} and well developed twin peaks at 965 and 985 cm^{-1} can be also observed. These twin peaks take the place of the absorption band at 970 cm^{-1}, which appears in the infrared spectra of all fractions of the other oils (Fig. 5 - 18), and which is probably due to <u>trans</u>

* (sh) :- shoulder

disubstituted double bonds. It is also possible that the presence of a two phase system may cause these double peaks to occur in the infrared spectra of sunflower seed oil (Fig. 19). The "aromatized" NAFD fractions, with the exception of that from sunflower seed oil, display an absorption band at 745 cm^{-1} that is characteristic of an ortho-disubstituted benzene derivative.

Macdonald (1956) has shown that the peaks at 970 and 660 cm^{-1} are not present in the spectrum of unheated linseed oil acids. However, the spectrum of the adduct-forming distillable esters of linseed oil also exhibits the peak at 970 cm^{-1} but lacks the peak at 660 cm^{-1}.

With the exception of sunflower seed oil, the absorption band at 660 cm^{-1} can thus be considered a distinguishing feature of the infrared spectra of the various NAFD fractions (Fig. 6, 9, 12 and 15).

The strong absorption band at 970 cm^{-1} in the infrared spectra of the various fractions of heated oils, indicates that all fractions contain double bond material with trans configuration. There is also a marked absence of absorption at 3030 cm^{-1}, which indicates the absence of any significant amount of double bond material with cis configuration. In this connection it is of interest to note that the distillable monomeric ethyl ester fraction of menhaden and linseed oils (Part III, Section II) when incorporated into the diets of young rats, produced a significantly lower weight gain than that produced by the unheated oils. This result is in agreement with work published by Holman and Aaes-Jorgensen (1956) on the anti-metabolic effects of trans-isomers upon E F A deficiency in rats.

The results of nutritional experiments with heated sunflower seed oil (Pritchard, 1954) can also be explained on the basis of E F A deficient rat diets. However, although this E F A deficiency exists in thermally treated linseed and menhaden oils, it cannot satisfactorily explain their degree of toxicity and especially the speed with which the ill effects appear. Evidence has been presented (vide supra) indicating that this increased toxicity of the distillable ethyl ester fraction of heated linseed, menhaden and soybean oils, may be caused primarily by cyclic material, created by thermally-induced cyclization reactions.

The results presented in this section may be summarized as follows:

1) The non-linearity of the carbon skeleton of linseed oil NAFD (not associated with geometric isomerization of the double bonds) is apparently sufficient to prevent urea adduct formation.

2) The single urea adduct fractionation procedure as commonly employed in this laboratory, is approximately ninety-eight percent efficient.

3) Unsaturated constituents of the monomeric ethyl ester fraction of heated oils apparently adopt a trans configuration about the plane of the double bond.

4) The NAFD and "aromatized" NAFD fractions from the ethanolysis of heated menhaden, herring, linseed and soybean oils exhibit an infrared absorption band at 660 cm^{-1}. This absorption band is also present in the monomeric distillable ethyl ester fractions, but is not present in the various spectra from comparable fractions of heated sunflower seed oil.

5) Characteristic infrared absorption bands at 970, 940 (sh)*, 855, 775 (sh)*, 745 and 660 cm^{-1} indicate that the "aromatized" NAFD fractions of linseed, menhaden, herring and soybean oils contain a certain amount of disubstituted aromatic compound.

6) The concentration of the disubstituted aromatic constituent, appears to be greatest in cases of linseed and menhaden oil, but markedly less in the case of herring oil. Even less of this aromatic constituent appears to be present in the case of soybean oil.

7) The absorption band at 660 cm^{-1} is not present in the infrared spectra of the unheated ethyl ester fraction or the AFD fraction of heated linseed oil (Macdonald, 1956).

8) Infrared spectral analyses indicate that the disubstituted aromatic constituent is not present in the brominated-debrominated NAFD fraction from heated sunflowerseed oil.

* (sh.):- shoulder

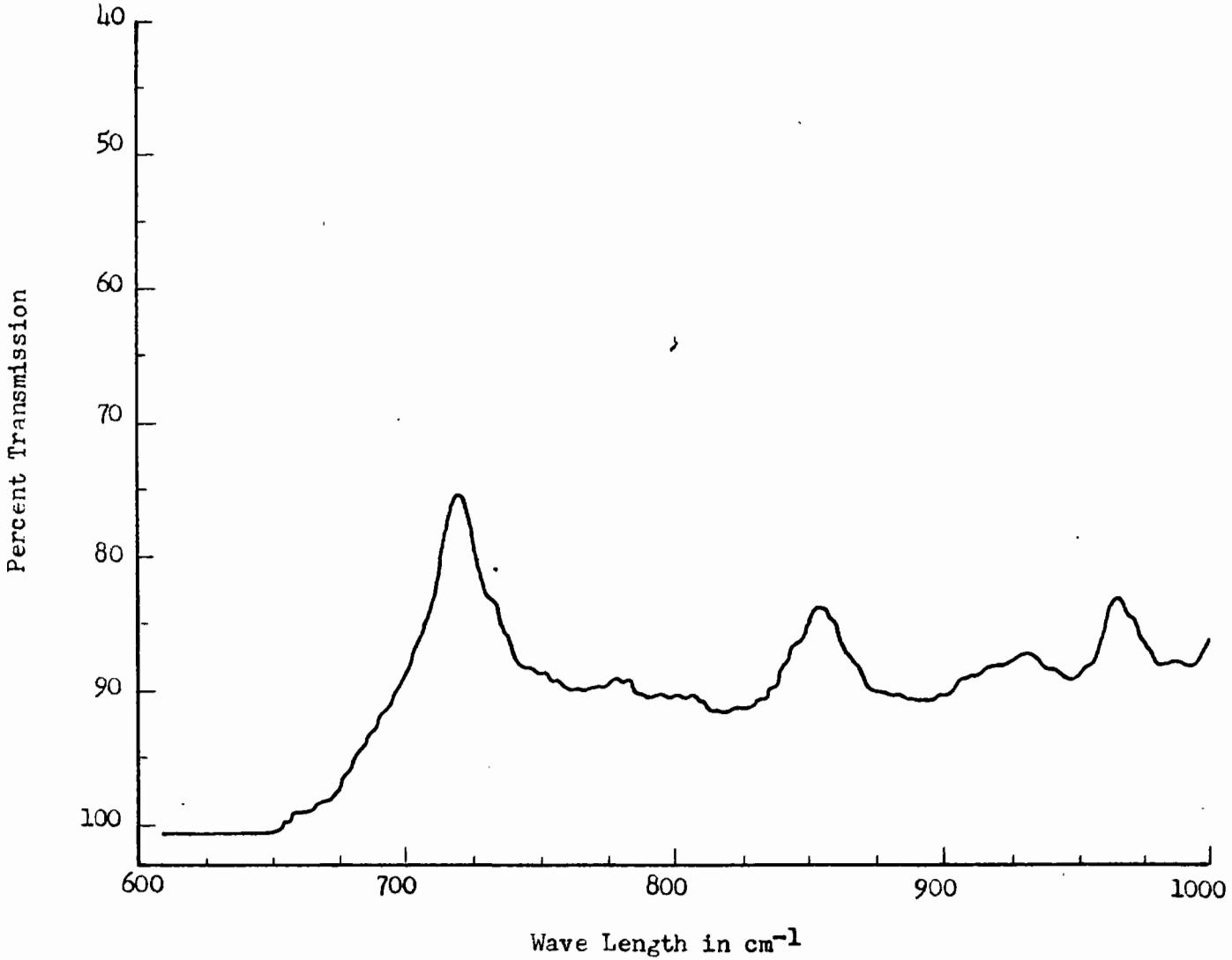


Figure 5. Menhaden Oil: Distillable Ethyl Ester of Heated Oil

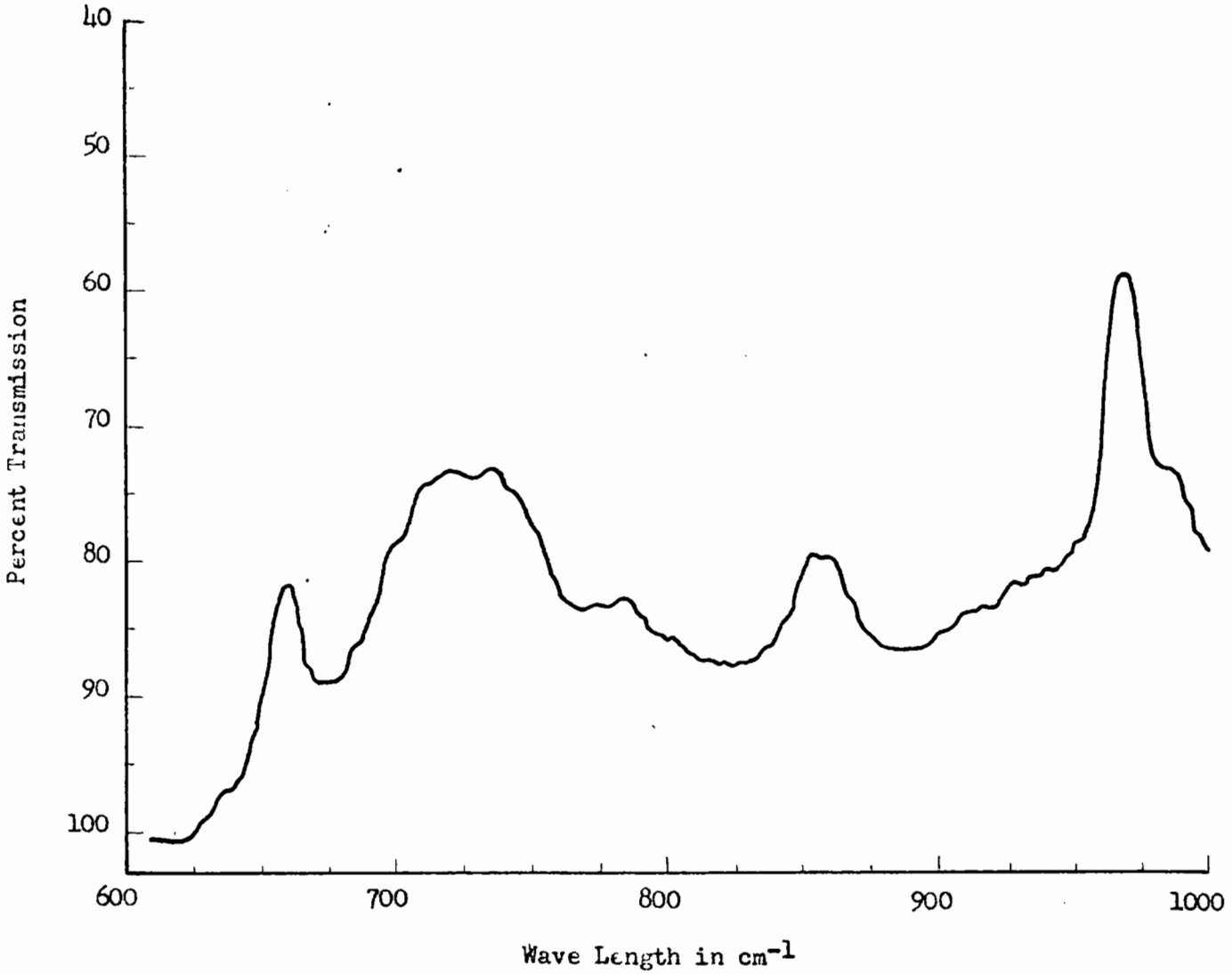


Figure 6. Menhaden Oil: NAFD Fraction of Heated Oil

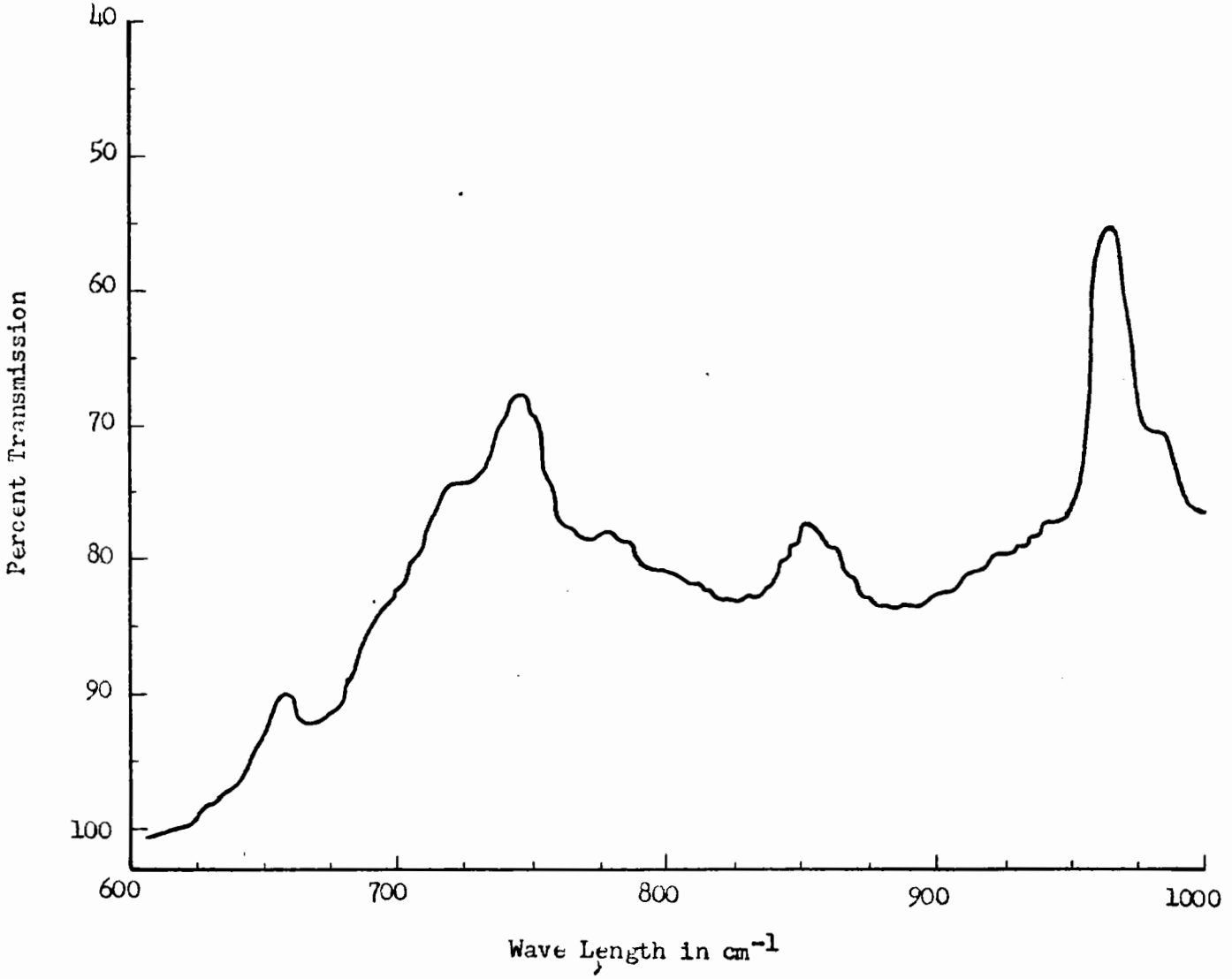


Figure 7. Menhaden Oil: "Aromatized" NAFD Fraction of Heated Oil

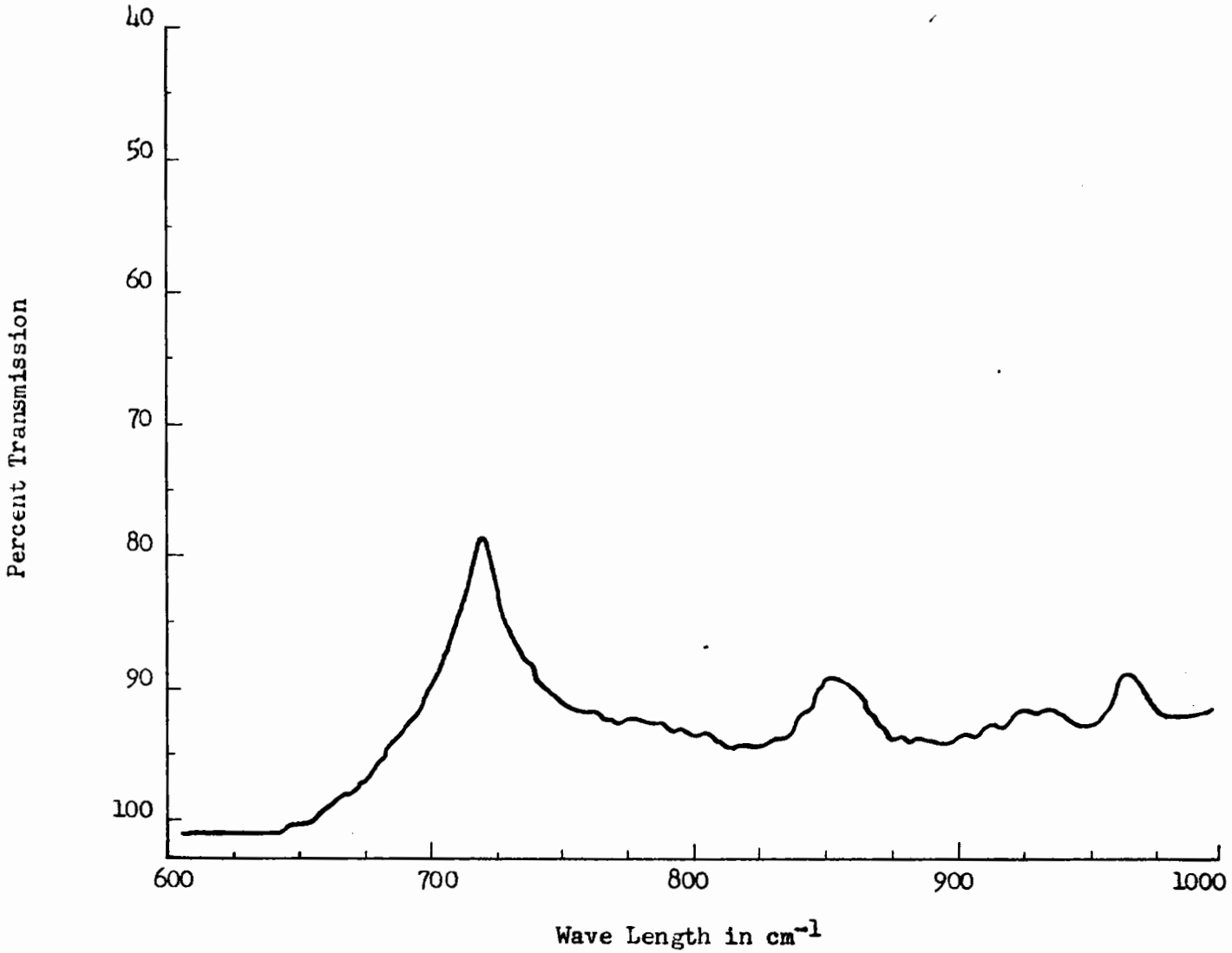


Figure 8. Herring Oil: Distillable Ethyl Ester Fraction of Heated Oil

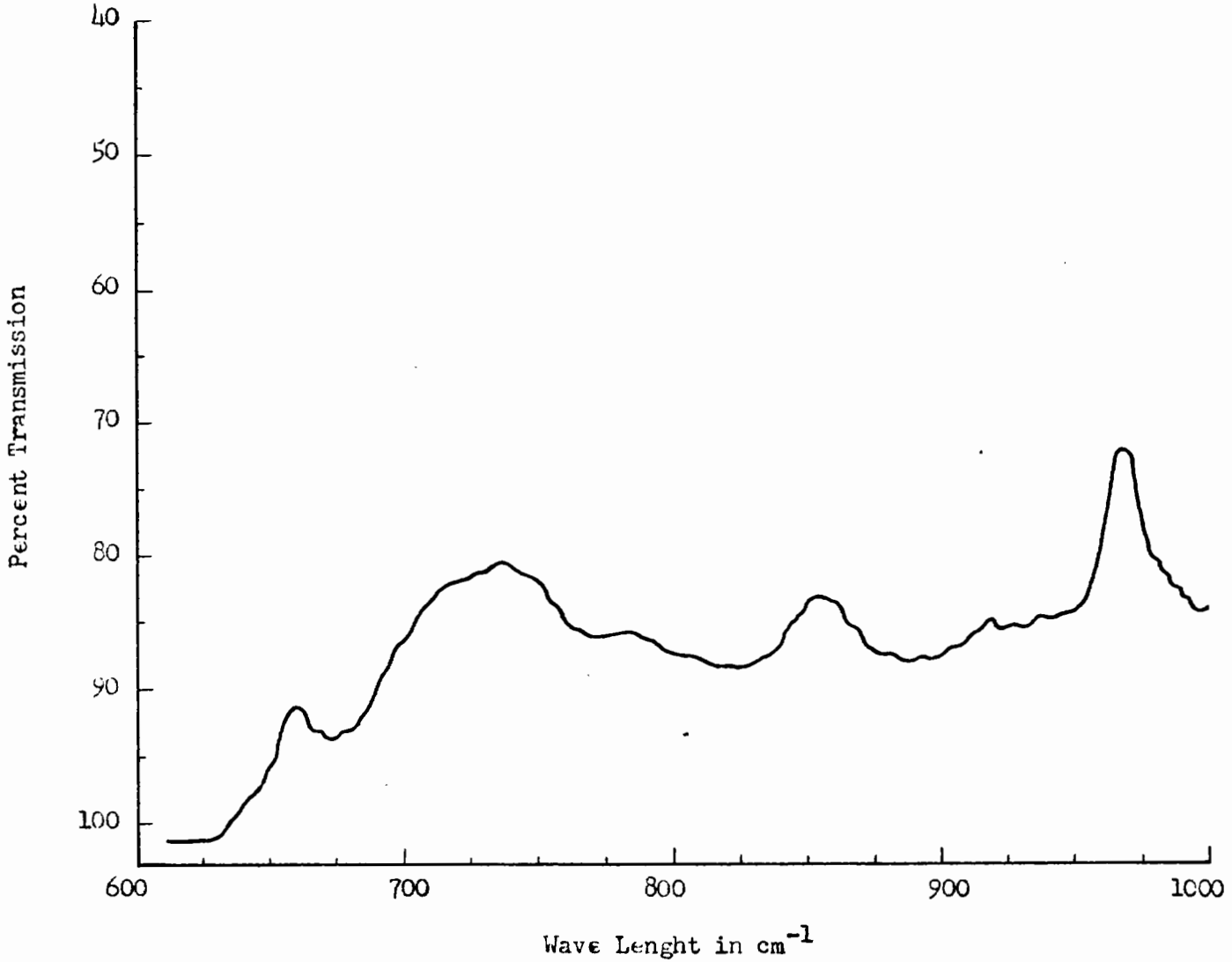


Figure 9. Herring Oil: NAFD Fraction of Heated Oil

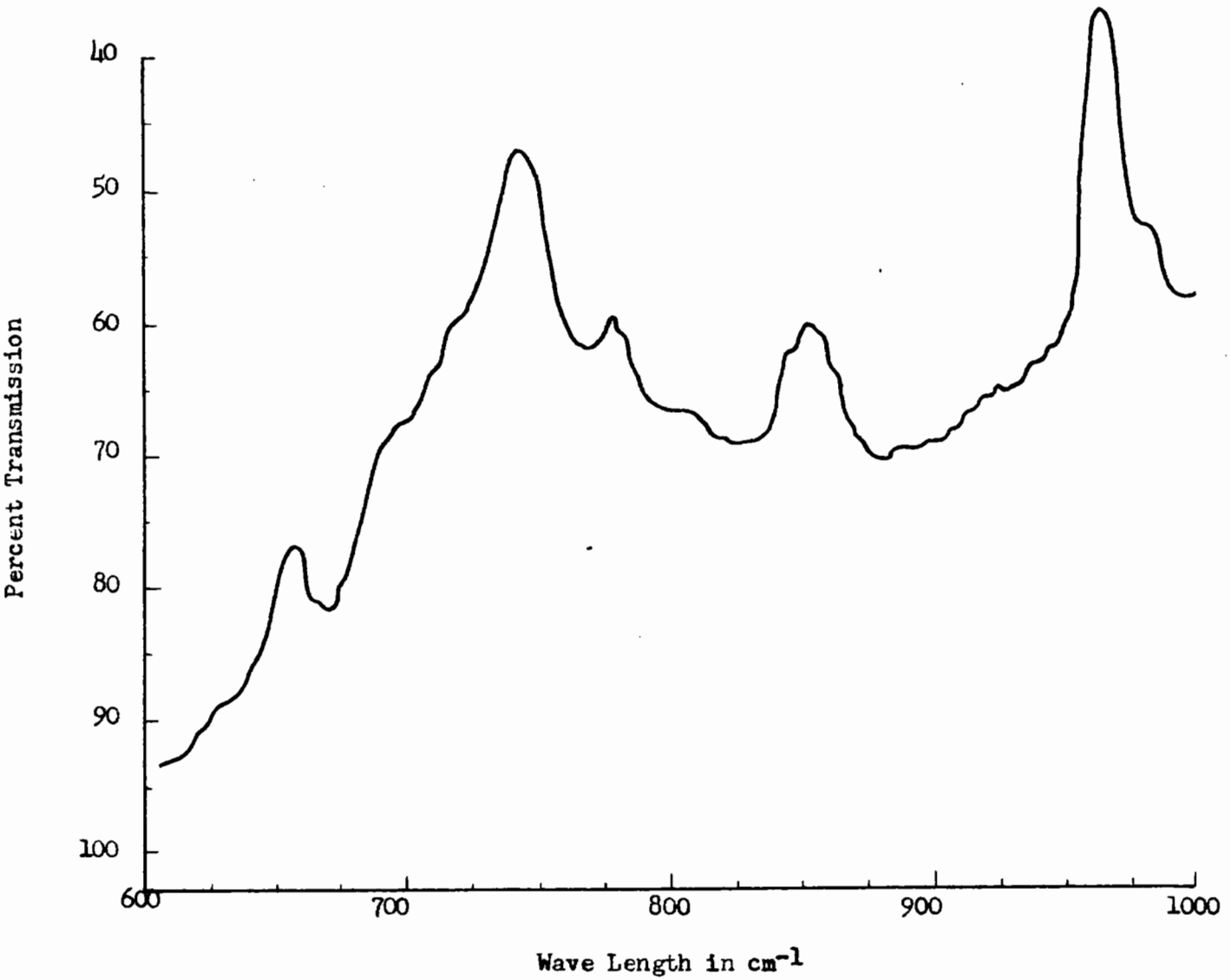


Figure 10. Herring Oil: "Aromatized" NAFD Fraction of Heated Oil

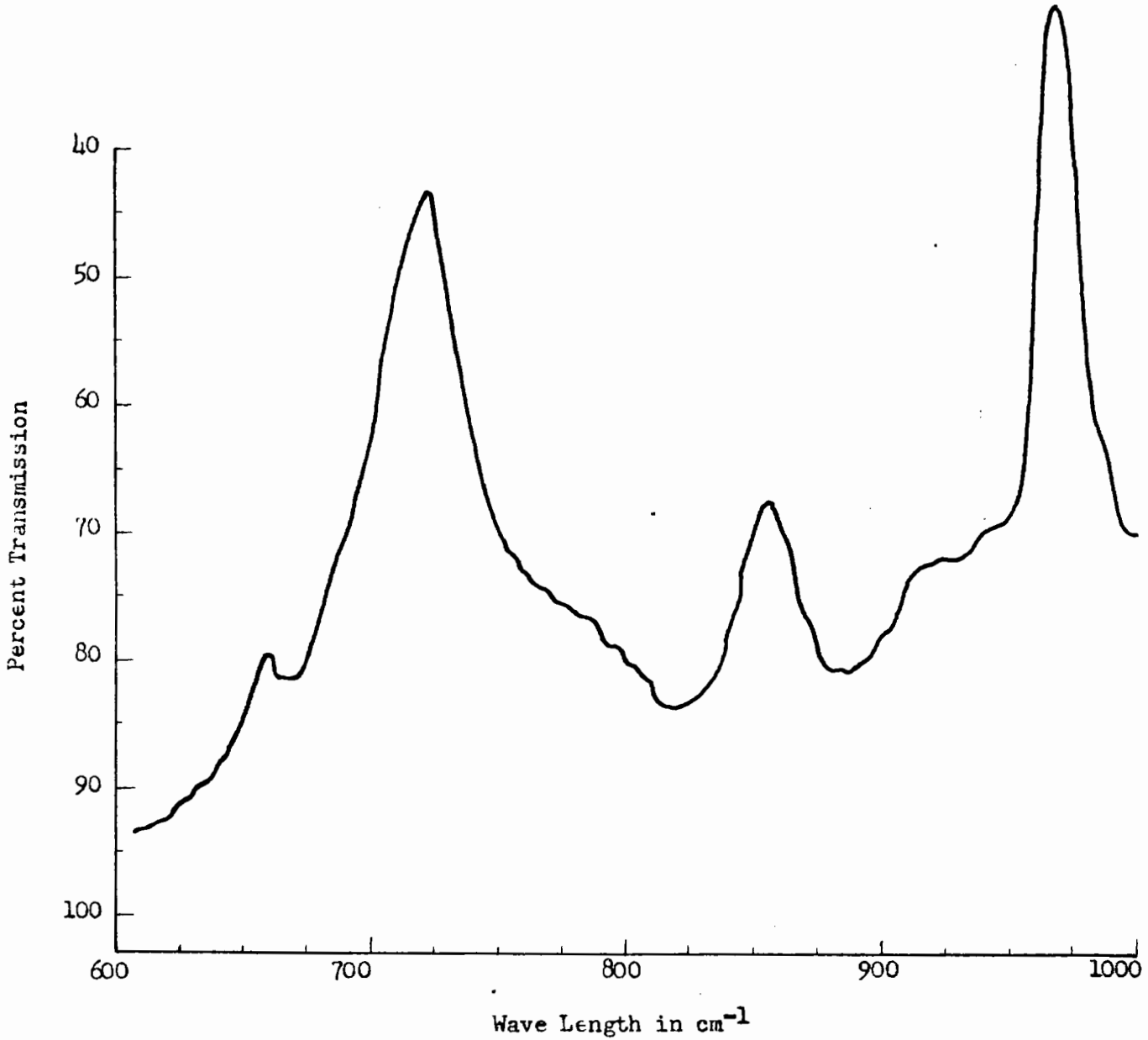


Figure 11. Linseed Oil: Distillable Ethyl Ester of Heated Oil

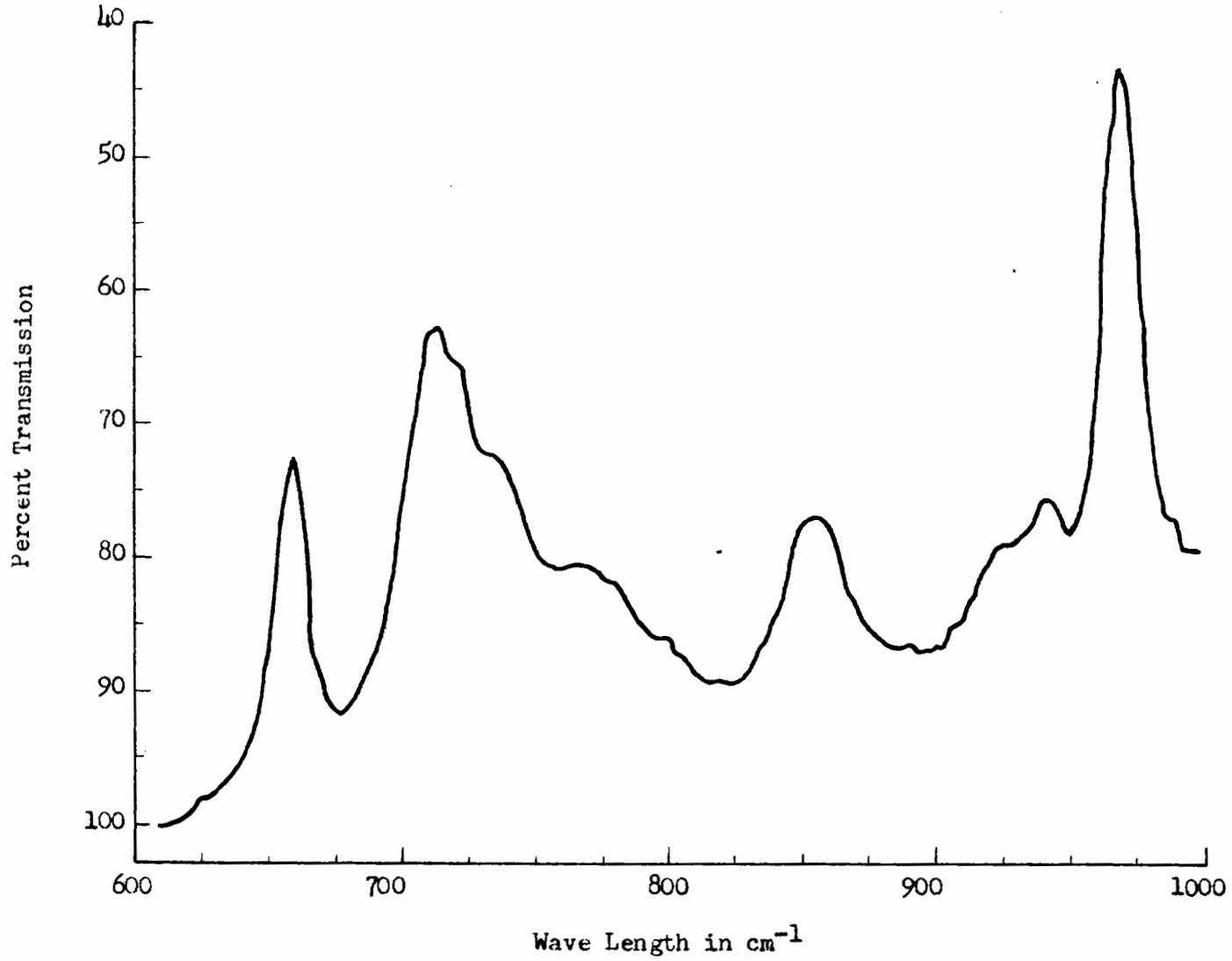


Figure 12. Linseed Oil: NAFD Fraction of Heated Oil

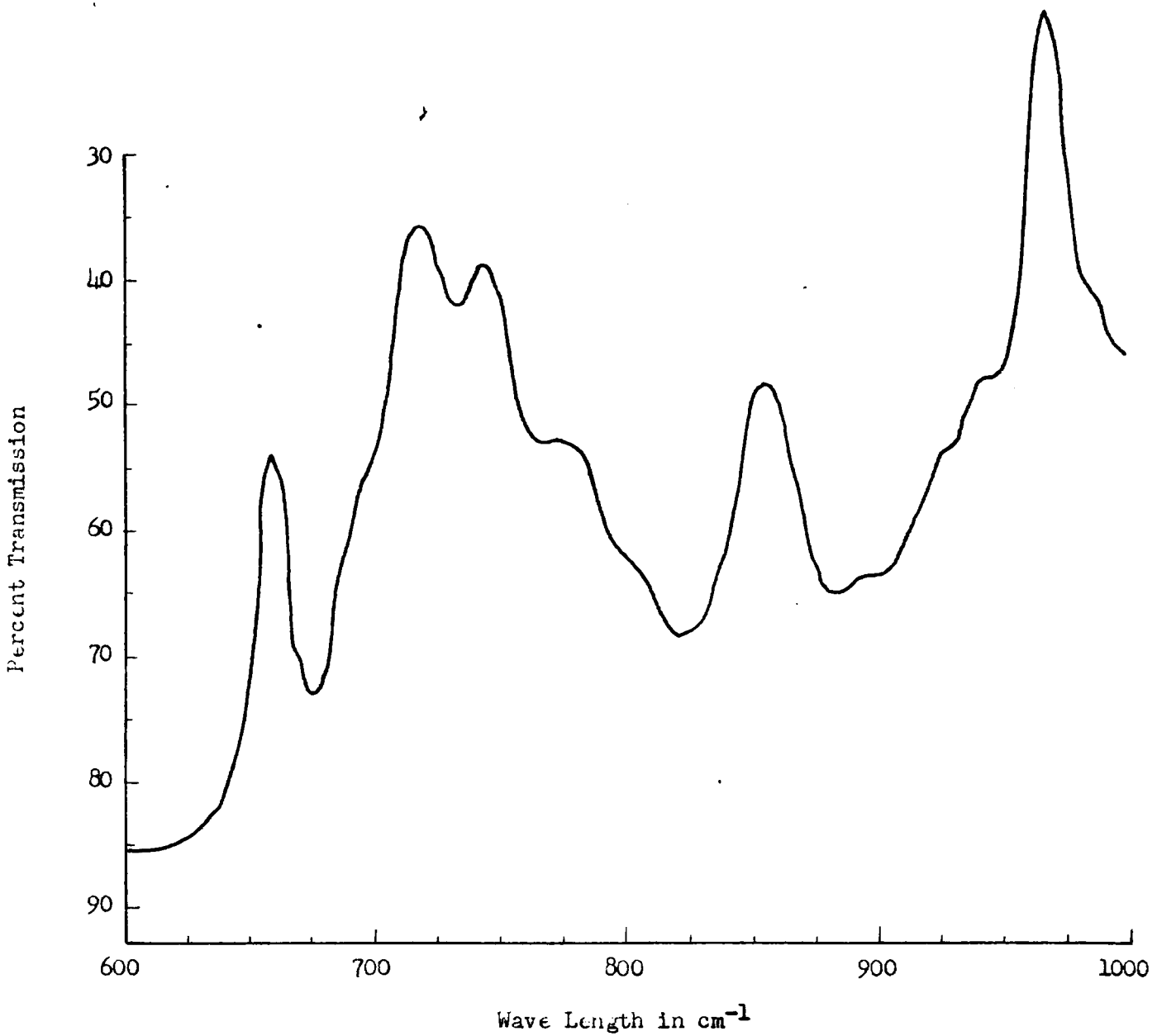


Figure 13. Linseed Oil: "Aromatized" NAFD Fraction of Heated Oil

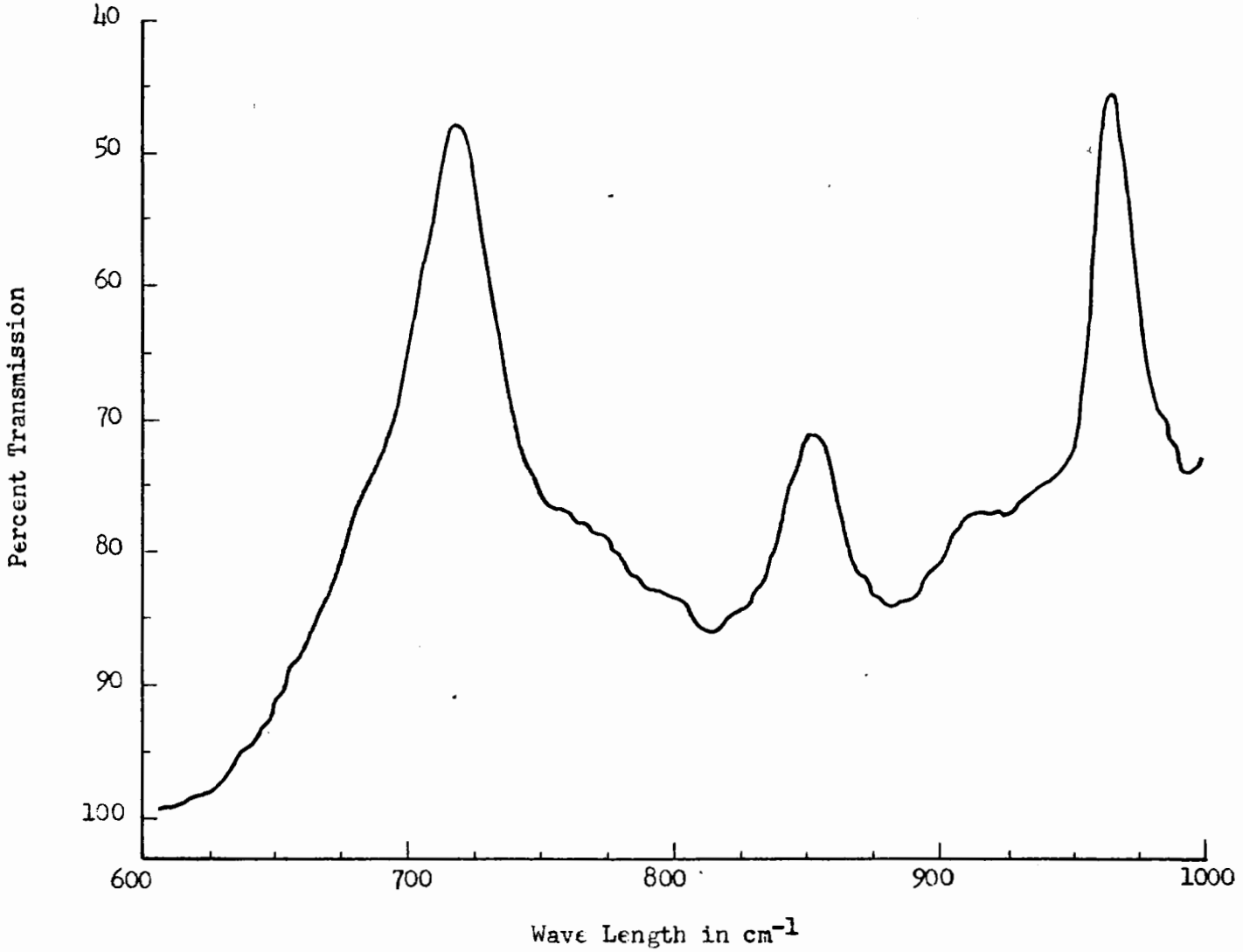


Figure 14. Soybean Oil: Distillable Ethyl Ester Fraction of Heated Oil

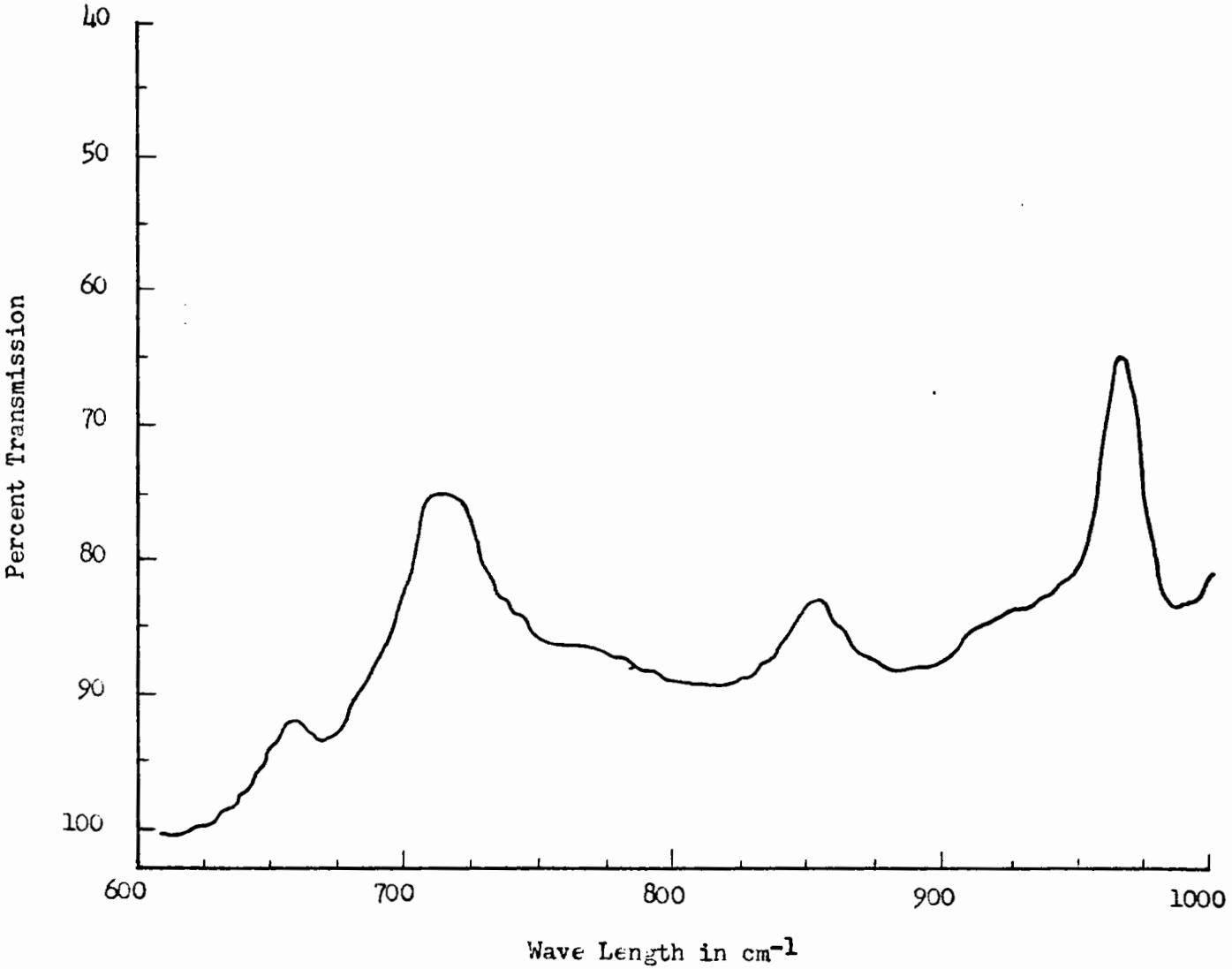


Figure 15. Soybean Oil: NAFD Fraction of Heated Oil

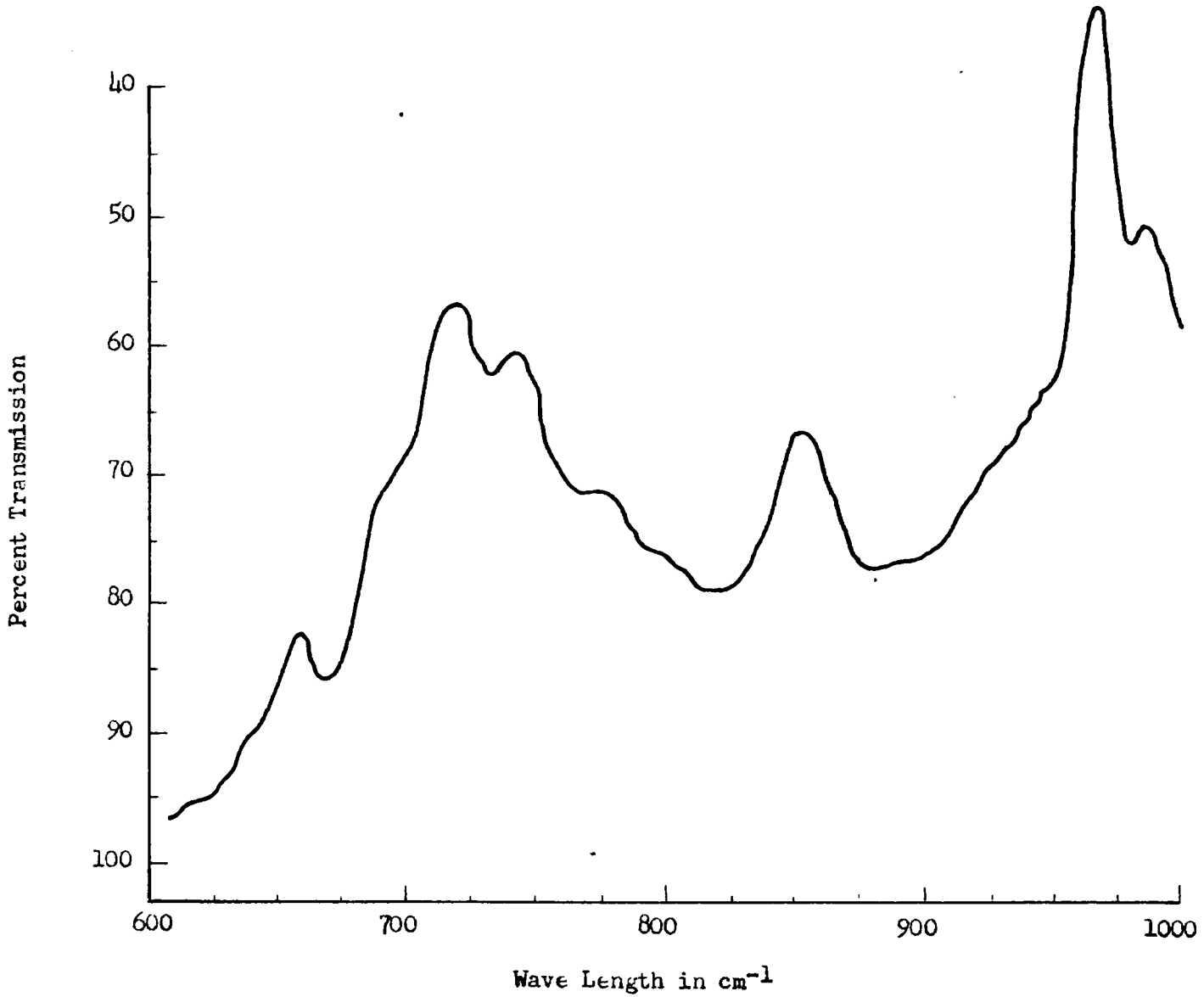


Figure 16. Soybean Oil: "Aromatized" NAFD Fraction of Heated Oil

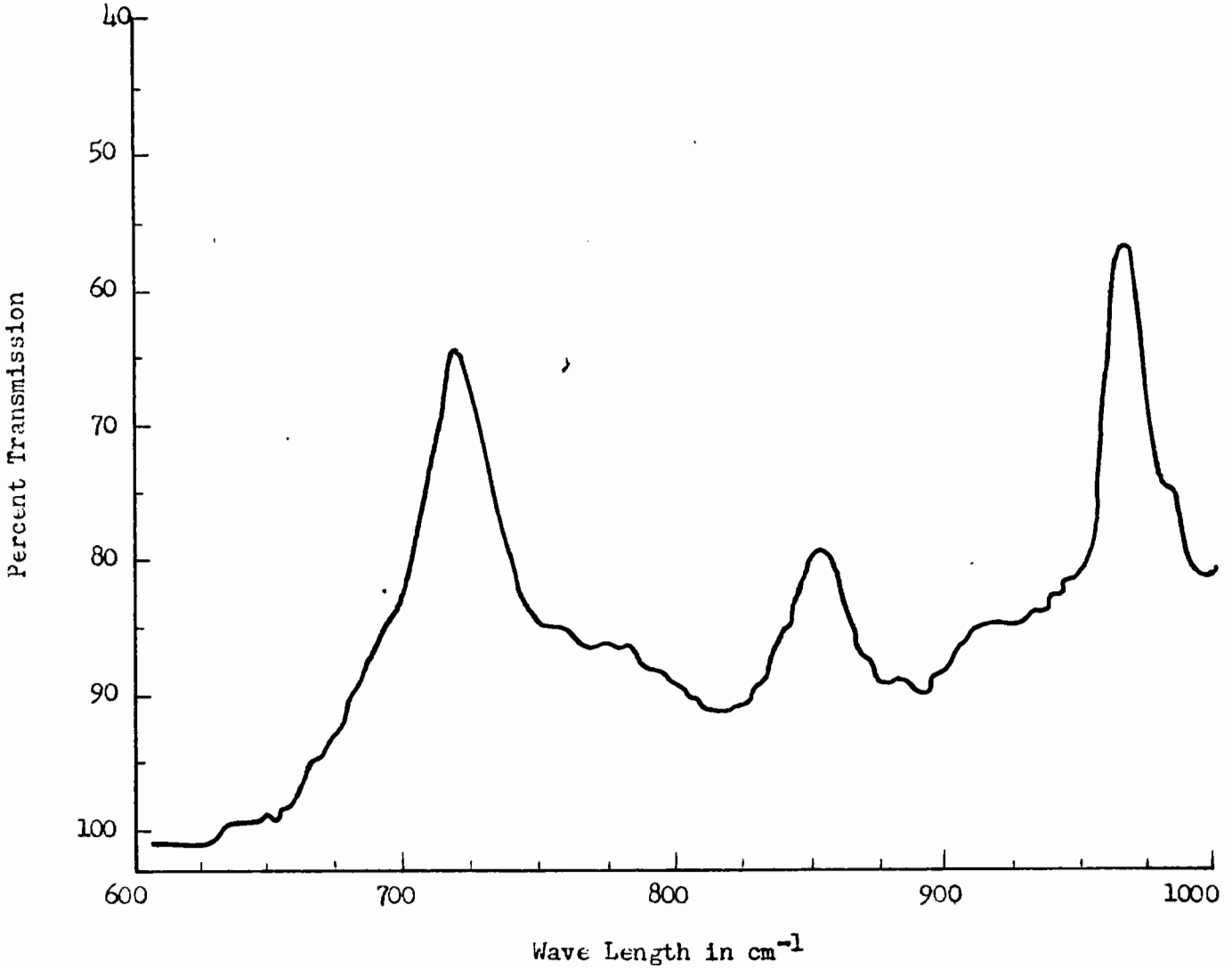


Figure 17. Sunflower Seed Oil: Distillable Ethyl Ester Fraction of Heated Oil

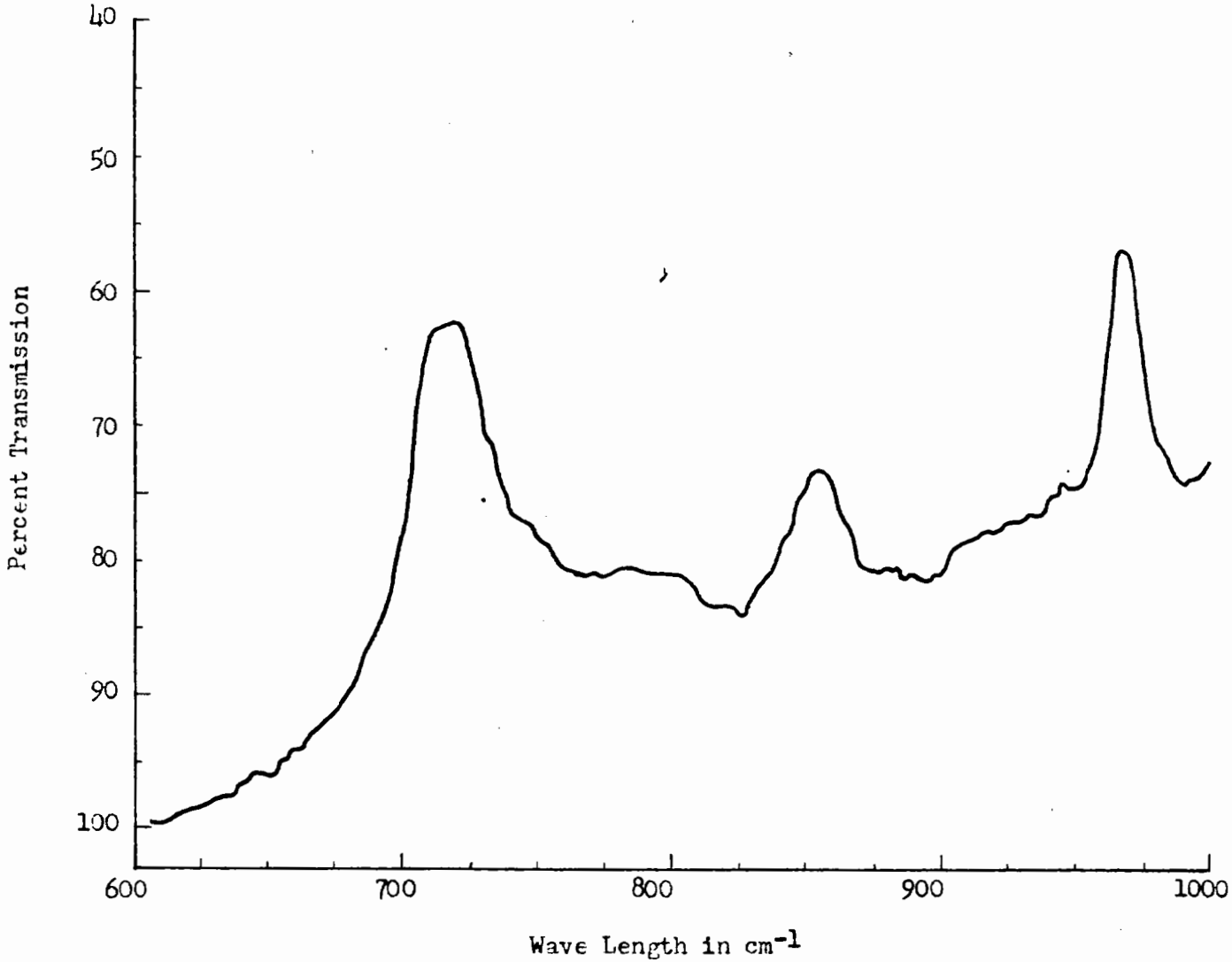


Figure 18. Sunflower Seed Oil: NAFD Fraction of Heated Oil

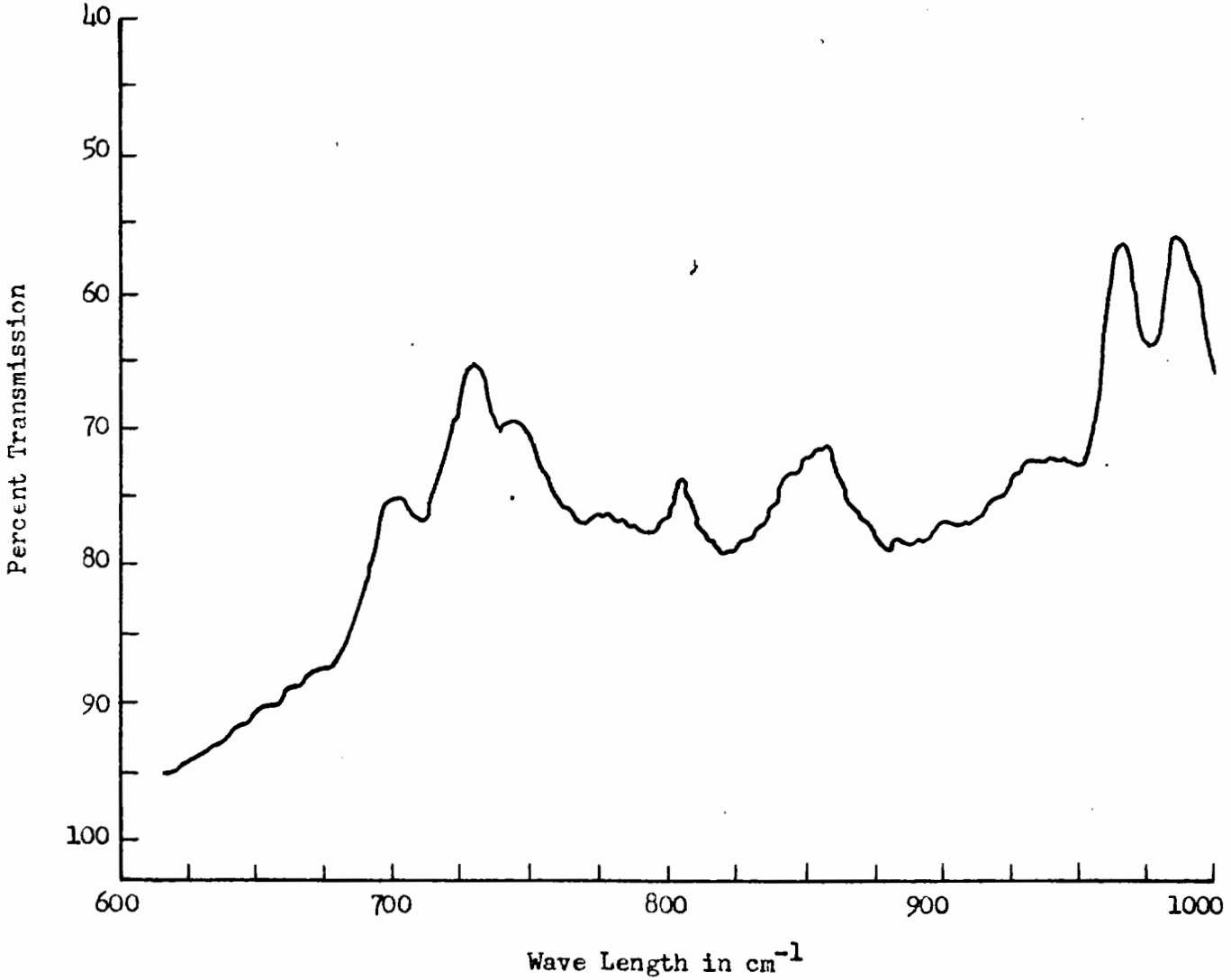


Figure 19. Sunflower Seed Oil: "Aromatized" NAFD Fraction of Heated Oil

SUMMARY

1. The distribution of acyl groups over the triglyceride molecules of menhaden oil appears to be non-random.
2. Intra-glyceride reactions play an important part during the initial stages of thermal polymerization of menhaden oil.
3. Changes due to thermal polymerization of menhaden oil occur rapidly during the initial five hours, but appear to decrease to very minor proportions after nine hours heating time.
4. The non-adduct-forming fraction (NAFD) of the distillable esters from heated menhaden oil was toxic to rats to a degree comparable with the toxicity of a similar fraction from heated linseed oil.
5. The adduct-forming fraction(AFD) of the distillable esters from heated menhaden oil was nutritionally innocuous.
6. The non-linearity of the carbon skeleton of linseed oil NAFD (not associated with geometric isomerization of the double bonds) appears to be sufficient to prevent urea adduct formation.
7. Infrared spectroscopic analyses indicate the presence of a disubstituted cyclic constituent with a characteristic absorption band at 660 cm^{-1} in the thermally polymerized menhaden, herring, linseed and soybean oils, but absent in thermally polymerized sunflower seed oil.

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