A STUDY OF SUNFLOWER OIL

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A STUDY OF SUNFLOWERSEED OIL

The advent of World War II cut off from the North American continent supplies of imported vegetable oils. This focused attention on a search for new plant sources, increased production from available sources and alteration of available vegetable oils by physical and chemical processes to meet industrial demands. This resulted in stimulation of the vegetable oil industry. The maintenance of this industry against competition from oil exporting countries requires production of superior oil products at costs which will not be excessive.

In addition to production of oils in competition with imported oils, there has been an increased demand for modified oils specifically adapted for certain purposes, e.g., fast-drying oils. The modification of vegetable oils may be aided by a knowledge of the acids and glycerides that are present.

Sunflower seed (Helianthus annus) is grown extensively in Russia, Argentine, China, Hungary and United States. Commercial production of sunflower seed in Canada has increased rapidly since 1941 as a war measure. The two main varieties are Sunrise and Mennonite, which are adapted to regions of a relatively long frost free period. Approximately 20,000 acres were seeded in Manitoba in

1945, and two oil expeller mills have been built in Saskatchewan and Manitoba.

The oil is an edible, semi-drying oil used extensively for cooking and salad oils, shortenings and, to a limited extent, for paints and varnishes. Jamieson (63) gives the following fatty acid composition: oleic 33.4, linoleic 57.5, palmitic 3.5, stearic 2.9, arachidic 0.6 and lignoceric 0.4. Linolenic acid is not detected by chemical analyses.

The present investigation was undertaken to determine:

(1) the relation of iodine value to fatty acid composition,

(2) the glyceride structure by fractional crystallization,

(3) if changes in the glyceride structure induced by physical and chemical treatment, such as molecular rearrangements, could be detected by fractional crystallization.

HISTORICAL

Natural fats and oils are esters of polyhydric alcohols and fatty acids. Seed fats are composed of glycerol esters, mainly triglycerides. The component fatty

acids may vary in chain length, degree of unsaturation, and the presence or absence of hydroxyl groups in the chain. In general, fats and oils are composed of a mixture of fatty acids with one to four of the acids occurring in major proportions (over 10%) and the remaining acids in minor proportions (less than 10%). A complete study of a fat or oil involves the qualitative and quantitative determination of the component fatty acids and glycerides.

Hilditch has compiled a monograph (44) giving the detailed fatty acid composition of some one hundred fats of aquatic origin, four hundred and twenty of plant species, and about eighty fats of animal origin. The qualitative and quantitative determination of the component glycerides is a very difficult problem. A great deal of research has been done but the results are mainly qualitative. Since triglyceride molecules are units composed of three fatty acids, it is conceivable that quantitative separation of these units would be more difficult than for the individual component acids.

Fatty Acid Composition

Determination of fatty acid composition involves preliminary separation of the fatty acids into two fractions, saturated and unsaturated. In the absence of erucic and hydroxy acids, this resolution may be achieved by either one

of two methods:

(1) Lead salt separation; differences in solubility of the lead salts of saturated and unsaturated acids form the basis for this separation. The lead salt-alcohol method was proposed by Twitchell (87) and the lead salt-ether method by Jamieson (63).

(2) Fractional crystallization; saturated fatty acids crystallize readily from fat solvents at temperatures in the order of $0^{\circ}C.$, while markedly lower temperatures, $-20^{\circ}C.$ to $-70^{\circ}C.$, are required for unsaturated acids. Some workers claim a more efficient separation by crystallization than by the lead salt method (3,13,17, 22,24,26,86).

Following separation, the saturated and unsaturated fractions are converted to methyl esters and subjected to fractional distillation in vacuo. The details of construction and operation of apparatus have been investigated thoroughly (1,4,46,65,67,74,76,91). This procedure serves to separate acids which vary in length of chain, but does not give an efficient separation of C_{18} unsaturated acids.

The fractionated acids may be qualitatively identified by melting point, molecular weight and saponification values. The composition of the mixture of C_{18} unsaturated

acids may be found by determination of the iodine and thiocyanogen values, and use of the equations outlined by Jamieson (63). The iodine and empirical thiocyanogen values for oleic, linoleic, and linolenic acids have been secured from the pure acids isolated from natural sources. Some doubt exists as to the empirical values for linoleic and linolenic acids. This is due to the difficulty of isolating these acids in the pure state without subjecting them to chemical treatment which may cause rearrangement of the double bonds.

The spectrophotometer has proved valuable for the detection of minute quantities of dienoic and trienoic acids and natural conjugated isomers of these acids. This method is subject to the same limitations as the iodine and thiocyanogen values in that there are doubts as to the purity of the acids isolated from natural sources. The details of apparatus and operations may be found in the work by Zschiele and coworkers (61,94). The analysis for fatty acid constituents may be found in a paper by Brode et al (12) and by Brice and coworkers (10,11,18,72).

Molecular distillation has been used for the quantitative separation of fatty acids (30,33,42), but has not proved to be as efficient as fractional distillation of the esters.

If the saturated acids are not required in detail, the fatty acid composition may be determined by the fol-

wing procedures:

(1) Lead salt precipitation of the saturated acids.

(2) Determination of iodine and thiocyanogen values.

(3) Application of the equations outlined by Jamieson (63) to determine the unsaturated acids.

ıe above procedure is applicable to a vegetable oil which ontains unsaturated acids in major proportions and in which o erucic or hydroxy acids occur.

eparation of the Glycerides

Natural fats and oils may contain two classes of riglycerides, simple and mixed. It has been found experiıentally that there is a tendency to the formation of a ıaximum amount of mixed triglycerides (68). The mixed triglycerides may be subdivided into symmetrical and nonsymmetrical classes. It is at once obvious that mixed triglycerides will be more difficult to separate into homogeneous fractions than the component fatty acids. To illustrate the complexity of triglycerides, consider an oil composed of three fatty acids, stearic (S), oleic (O), and linoleic (L). These are C_{18} fatty acids which differ only in degree of unsaturation. Denoting glycerol by the symbol (G) and using the symbols given above for the fatty acids, the following eighteen combinations are possible:

Simple triglycerides - G(OOO), G(SSS), G(LLL)

Symmetrical mixed triglycerides -

G(OSO), G(OLO), G(SOS)

G(SLS), G(LSL), G(LOL)

Unsymmetrical mixed triglycerides -

G(OOS), G(OOL), G(SSO)

G(SSL), G(LLS), G(LLO)

G(OSL), G(SOL), G(OLS)

Ignoring the configuration, these triglycerides may be grouped into ten general classes differing only in component acids. These classes would be: G(OOO), G(SSS), G(LLL), G(SSO), G(SSL), G(LLO), G(LLS), G(OOS), G(OOL) and G(OLS).

The separation of such molecular species as

(a) trilinolein and oleodilinolein

(b) triolein and linoleodiolein

(c) stearodilenolein and stearooleolinolein

(d) oleodistearin and linoleodistearin

will be very difficult. These molecular pairs differ only by one double bond. However, it may be possible to effect a general separation into fully saturated, monounsaturated, diunsaturated and triunsaturated glyceride fractions. If an efficient separation into these general fractions is possible, each fraction would contain from one to four individual triglycerides. Since sunflowerseed oil contains a mixture of palmitic, stearic, myristic and shorter chain

saturated acids (63), the glyceride composition will be more complex than the structure given above.

Early workers in the field of glyceride separations employed high vacuum distillation (66) with some success. In 1909 Caldwell and Hurtley (23) were successful in distilling some of the glycerides of butter. High vacuum distillation was not successful in the separation of unsaturated, higher molecular weight glycerides due to polymerization and rearrangements.

Distillation of these glycerides is possible in a molecular still, but the degree of separation attained is insufficient to characterize molecular species. Hickmann (41) was able to secure an over-all separation of 42 units in iodine value for five fractions of linseed oil. Riemenschneider, Swift and Sando (77) fractionated cottonseed oil into 15 fractions with an over-all spread of 20 units in iodine value. Data for the molecular distillation of corn oil, castor oil and soyabean oil may be found in a monograph by Embree (30). The over-all fractionation in terms of iodine values is small relative to the glycerides which are present. It has been shown that a greater over-all separation may be achieved by fractional crystallization.

Early work on fractional crystallization is reviewed by Hilditch (60). Amberger, Klimont, Bömer and others (17) isolated simple and mixed glycerides of lauric,

myristic, palmitic, stearic and oleic acids. In some cases hundreds of crystallizations were necessary to isolate a pure glyceride from natural sources. It is notable that the early work was done on relatively saturated fats and oils. The quantitative fractionation of the individual glycerides was found to be impossible.

Since 1936 Hilditch and coworkers have extensively used fractional crystallization from acetone at 0°C. to separate oils into fractions of different solubility. The number of fractions varied from two to seven depending on the fat that was studied. Each fraction, assumed to contain a relatively small number (two or three) of triglycerides of similar composition, was subjected to chemical analyses and the glyceride structure was computed from the data. Hilditch and coworkers applied this method to a wide variety of solid seed fats (5,6,19,20,21,36,47,48,49,51,52) and to a number of animal fats (39,53,54,55).

The application of low temperature crystallization, (-20°C. to -70°C.), to liquid seed fats and unsaturated fatty acids is of more recent origin. Hilditch and coworkers secured low temperatures by placing dry ice directly in the sample to be fractionated (56,57,58). Hilditch and Maddison fractionally crystallized cottonseed oil (57) into six portions having iodine values of 38.3 to 140.0, and olive oil (58) into six fractions having iodine values ranging from

67.8 to 100.7 by this method. Other workers such as Brown, Bailey, etc. (7,13,14,31,32,69) preferred to use a flask containing the sample in a bath maintained at low temperatures by means of dry ice and a stirrer. Riemenschneider, Swift and Sando (77) crystallized cottonseed oil into seven fractions having iodine values from 52.5 to 148.3. Bull and Wheeler (22) investigated the separation of soyabean oil and soyabean oil fatty acids by a single crystallization using acetone and acetone-water mixtures as solvents.

Low temperature crystallization has given a wider and more efficient separation than either high vacuum or molecular distillation. However, the chemical analysis revealed a complex mixture of fatty acids in each fraction. It is evident that segregation into glyceride molecular species has not been achieved.

Determination of Glyceride Structure

Hilditch and coworkers began their study by working on solid seed fats which contained predominately saturated acids, lauric, myristic, palmitic and stearic. Unsaturated acids such as oleic and linoleic occurred in minor proportions. A detailed estimation of glyceride structure embodied some or all of the following procedures, depending on the component acids of the fats that were to be studied.

(1) Crystallization of the fat into two to five fractions.

(2) Esterification and distillation of each fraction to determine the component acids.

(3) Oxidation by $KMnO_4$ - acetone to determine the amount of fully saturated glycerides.

(4) Hydrogenation and crystallization to determine the amount of tri-C_{18} glycerides.

(5) Elaidinization and fractional crystallization of the elaido-glycerides.

(6) Bromination and separation of the bromo-adducts by fractional crystallization.

In 1925 Armstrong and Hilditch (2) used $KMnO_4$ - acetone oxidation to determine the position of double bonds in unsaturated acids. Hilditch then proposed the use of this reagent to determine the amount of fully saturated glycerides in an oil. The unsaturated acids were oxidized at the double bond to shorter chain acids. Fractional crystallization of the oxidized oil revealed the amount of fully saturated glycerides that were present. Since the early work was on relatively saturated oils, this procedure revealed the major proportion of the glycerides. The amount of tri-C_{18} glycerides was determined by hydrogenation of the oil, followed by fractional crystallization. It was found possible to separate the saturated glycerides, mono palmito - distearin and tripalmitin, by fractional crystallization. Using these procedures, Hilditch, Meara and co-

workers postulated glyceride structures for a number of solid seed fats (5,6,19,20,21,36,47,48,49,51,52).

The same authors investigated animal fats using similar procedures (39,53,54,55). From the data accumulated over a series of such investigations, Hilditch concluded that the fatty acids were more or less "evenly" distributed among the glycerides. He found that animal fats which contained 60% or more of stearic acid had a greater proportion of tristearin than did corresponding vegetable fats (44).

Working on the assumption that liquid seed fats also had an "even distribution" of fatty acids, research was extended to include oils which contained major proportions of unsaturated fatty acids. Gunde and Hilditch (37) used the following procedures to determine the glyceride structure of olive oil:

(1) Crystallization of the oil into several fractions.

(2) Determination of the fully saturated glycerides in the more saturated fractions by $KMnO_4$ - acetone oxidation and crystallization.

(3) Elaidinization with Se as a catalyst to form the elaido-glycerides from the oleo-glycerides, followed by crystallization to determine triolein.

The elaidinization procedure proved difficult since

linoleic acid is polymerized by this treatment. However, since the amount of linoleic acid in olive oil is small, an estimation of the glyceride structure was possible.

In 1942 Bomer and Kappeller (9) applied the elaidin reaction to soyabean, peanut and olive oils. Fractional crystallization of the elaidinized olive oil revealed large amounts of trielaidin, some elaidodipalmitin but no elaidodistearin. Peanut oil yielded chiefly trielaidin and some elaidodipalmitin. These workers did not attempt the complete determination of glyceride structure, but confined their research to the isolation and identification of major component glycerides.

The subsequent work published by Hilditch and coworkers made extensive use of the theories of "even distribution". Hilditch and Maddison fractionally crystallized cottonseed oil (57) and determined the component acids in each fraction. Eight possible glyceride structures were calculated using the rules of even distribution. One calculated structure was selected as being the most likely to occur. The remaining seven structures were ruled out by reference to non-occurrence of like structures in previous research. In 1941 Hilditch and Maddison repeated the elucidation of the glyceride structure of olive oil (58) using fractional crystallization. The structure was calculated in the same manner as for cottonseed oil. The results con-

firmed the structure previously deduced from the elaidinization procedure (37).

The rules of "even distribution" and the calculations of glyceride structure were published by Hilditch and Meara (45) in 1942. These rules are as follows:

(1) If any individual acid (e.g. oleic) forms one-third or more of the total molecules of fatty acids present in a fat, that acid will occur once (or more than once) in nearly all the triglyceride molecules.

(2) Any minor component acid which does not form more than 10% of the total molecules of fatty acids will contribute only one acyl group to such triglyceride molecules as may contain it.

The "found" and "calculated" glyceride structures for a number of fats and oils which were investigated by Hilditch and coworkers are included in the paper. It is at once apparent that the deduction of the correct glyceride structure for a number of fats and oils is dependent on the order in which the fatty acids are selected for distribution.

Few references have been made to the configuration of the mixed triglycerides. Some evidence (48,49,57,59) indicates that mixed triglycerides occur in the symmetrical form.

Vidyarthi and Mallya (90) determined the glyceride structure of Niger seed oil, a semi-drying oil of iodine value 129.2, by the following procedures:

(1) $KMnO_4$ - acetone oxidation.

(2) Hydrogenation to determine C_{18} glycerides.

(3) Bromination and crystallization of the bromo-adducts.

One fraction comprising 2% of the oil was ascertained to be trilinolein by hexabromide value. The authors claimed this to be the only trilinolein present. Two other fractions comprising 45% of the oil had iodine values of 147.4 and 147.6 respectively. Since oleodilinolein has an iodine value of 144.1, it would appear that these fractions contain some trilinolein. However, a more efficient separation into fractions was achieved. The bromination-debromination procedure is subject to limitations due to the difficulty in obtaining complete bromination and the uncertainty as to molecular rearrangements during the debromination procedure.

Determination of the glyceride structures for liquid seed fats, those containing major proportions of unsaturated acids, is not fully quantitative. The methods outlined above have yielded only tentative structures. It appears that direct quantitative determination of glyceride structure can only be achieved by some method that separates

the oil into molecular species. Furthermore, a particular glyceride structure is specific for each individual oil sample, since iodine value and fatty acid composition are dependent on the environment under which a plant variety is grown (75,82).

Synthetic Triglycerides

Synthetic triglycerides may be divided into simple triglycerides, symmetrical and non-symmetrical mixed triglycerides. The synthesis of mixed triglycerides is described in a review by Daubert and King (25). This topic will not be reviewed since the research involved the preparation of simple triglycerides.

The early attempts to synthesize simple triglycerides consisted of heating glycerol with an excess of the fatty acid or fatty acid chloride. Since the reaction $RC(=O)-OH + ROH$ is an equilibrium, removal of water drives the reaction to completion.

For the lower members of the fatty acid series, water may be removed by the addition of a drying agent such as PCl_5 (73). KOH is used to remove the hydrochloric acid produced by using the acid chloride (40).

In 1928, Garner (34) prepared simple triglycerides by heating equivalent quantities of fatty acids and glycerol in an atmosphere of CO_2 at 200°C. for 6 hours. Verdake (89)

obtained a good yield by heating glycerol with a slight excess of the fatty acid, using Zn dust as a catalyst. The method proposed by Wheeler, Riemenschneider and Sando (92) yielded simple triglycerides of unsaturated acids. The authors heated an excess of the unsatured fatty acid with glycerol using p-toluene sulfonic acid as a catalyst. A stream of purified nitrogen was passed through the reaction mixture to remove the water produced and to prevent oxidation of the unsaturated acids. These workers produced triolein and trilinolein in 94% yield with analytical values in good agreement with the accepted values.

Molecular Rearrangements and Interchanges

Various investigators have secured patents covering rearrangements and interchanges to produce vegetable oils with different properties than the starting materials. The term molecular rearrangement will be used to indicate a rearrangement in oils without the addition of fatty acids or alcohols. Molecular interchanges are induced by the addition of alcohols or fatty acids and heat treatment with or without the addition of a catalyst.

Molecular interchanges are of two types:

(1) acid interchange, wherein the fatty acids present in the fat or oil are substituted by some selected fatty acids. In 1925 Schwartz (84)

invented a procedure for substituting the fatty acids of cocoanut oil by acetic acid, using sulfuric acid as a catalyst. This resulted in the production of mono- and diacetins. Barsky in 1939 (8) patented a process for the substitution of the lower members of the fatty acids present in an oil by a fatty acid having at least two more carbon atoms than the lowest fatty acid to be replaced. The mixture was heated in vacuo at a temperature above the vaporization point of the acid to be replaced. The replaced acids were distilled, yielding a product different from the starting material. In 1945 Eckey produced three patents (27,28, 29) covering processes for the modification of the molecular structure of fats and oils. These processes consist essentially of the addition of fatty acids or fatty acid esters, heat treatment with catalysts, and removal of the replaced acids by fractional distillation or alkali refining.

(2) alcohol interchanges, wherein the glycerol present in the natural oil is replaced by a monohydric or other polyhydric alcohol. Royce (79)

formed mono- and di-glycerides by adding glycerol and a basic catalyst to the oil and heating the mixture. Goss and Johnstone (35) patented a process in which the oils are subjected to alcoholysis forming the methyl or ethyl esters of the fatty acids. The esters are fractionated on the basis of unsaturation using solvent extraction and are then re-esterified to produce glycerides which differ from those contained in the original oil.

Wright and coworkers (93) reported the action of various monohydric and polyhydric alcohols on the triglycerides using interchange catalysts. The amount of glycerol produced was used as a measure of the extent to which the reaction had proceeded. Ester interchange between polyhydric alcohols and the fatty acid esters of monohydric alcohols were also investigated.

It has been found possible to effect internal rearrangements of glycerides by heating oils with catalysts. Van Loon (88) patented a process covering catalysts for rearrangements and interchange reactions. Bailey and coworkers (7) subjected peanut oil to molecular rearrangement to effect changes in glyceride structure. The rearrangement procedure was effective in changing the glycerides which contributed to difficult filtration in the fractional crystallization of

this oil.

In summary, the glyceride structure of a vegetable oil may be altered by

(1) addition of a fatty acid and acid interchange,

(2) addition of an alcohol and alcohol interchange,

(3) addition of an ester and ester interchange,

(4) molecular rearrangement within the oil itself.

FATTY ACID COMPOSITION OF SUNFLOWER OIL

Selection of an oil for specific industrial uses is usually based on the iodine value of the oil. For example, high iodine value oils are preferred for paints and varnishes, low iodine value oils for shortenings and soaps. This implies that the iodine value is characteristic of the component acids. Sallans (81) and Painter (75) found a regular variation in the fatty acid composition of linseed oils with iodine value. Highly significant correlations of iodine values with thiocyanogen values and with component acids of the samples were obtained. From these relations equations for estimating fatty acid composition from iodine values were derived (81). Sallans (81) found significant differences in linseed oils due to varieties and environmental factors.

The study of component acids of sunflower oils was undertaken to determine if similar relations existed. The following relations were studied:

(1) effect of varietal and environmental factors on the composition of the oil.

(2) relation between iodine and thiocyanogen values.

(3) relation between iodine values and fatty acid composition.

(4) comparison of component acids determined from

iodine - thiocyanogen and iodine - saturated acid equations.

Materials and Methods

Samples of sunflower seed selected for this study comprised 12 varieties, selections, strains and hybrids, listed in Table I of Appendix A. These consisted of Sunrise, three Mennonite strains, three selections made at Scott Experimental Station from Mennonite, and 5 hybrids produced by crossing Sunrise with inbred Mennonite lines. These varieties were grown at 6 stations; University of Saskatchewan, Indian Head, Morden, Melfort, Swift Current and Lethbridge, as a variety trial by the Forage Crops Laboratory, Saskatoon.

Analytical Methods

The samples were hulled in the laboratory huller described by Sallans and Sinclair (80), and the meats were ground in a set of rolls. The oil was expressed at room temperature with a Carver laboratory press at hydraulic pressure of 15,000 lb. per square inch. Pressings were made in a standard 2 1/4 inch cylinder. The oil was clarified by centrifuging for 10 minutes at 3000 r.p.m., transferred to a 50 ml. flask and stored in a refrigerator at $0^{\circ}C$. A working schedule was so arranged that not more than 7 days

elapsed between pressing the oil and the completion of analyses.

The iodine values were determined by the standard Wijs method with one hour reaction time (63). For the thiocyanogen values the method outlined by Mathews, Brode and Brown (71) was used except that a reaction temperature of 21°C. ± .05 was employed. An 0.2 N. solution was used with a reaction time of 24 hours. The temperature was maintained by a closed constant temperature bath, employing a heater controlled by a sealed mercury in glass thermoregulator balanced against an inflow of cold water from the laboratory main. No decomposition of the reagent occurred in this procedure.

Saturated acids were estimated by the modification of the Twitchell lead-salt-alcohol method (63) except that HNO_3(1:3) was used to decompose the lead salts instead of HCl. The Kerr-Sorber method as modified by Jamieson (63) was employed for the determination of unsaponifiable matter.

Calculations

The most accurate and reliable determination of oleic, linoleic and linolenic acids is a modification of Kaufmann's method (64). This was based on the assumption that iodine values indicate total unsaturation of the acids, while thiocyanogen values reflect total unsaturation of

oleic acid, one-half that of linoleic and two-thirds that of linolenic acid. It is possible to set up simultaneous equations involving iodine values, thiocyanogen values, and unsaturated acids which may be solved for the component acids. Employing theoretical values for oleic and linoleic acids Kaufmann's original equations would be as follows:

$$89.9\,x + 181.1\,y = 100 \text{ I.V.}$$

$$89.9\,x + 90.55\,y = 100 \text{ T.V.}$$

$$x + y = u$$

where x = oleic acid, %; y = linoleic acid, %; I.V. = iodine value; T.V. = thiocyanogen value; and u = total unsaturated acids, %. These simultaneous equations may be solved for the component acids.

It has been shown that Kaufmann's assumption regarding thiocyanogen absorption is not strictly correct (71,78). The development of low temperature fractional crystallization (17) for the isolation of unsaturated fatty acids has resulted in modification of thiocyanogen values for linoleic and linolenic acids (71,78). It was found that the experimental thiocyanogen values for linoleic and linolenic acids were lower than the theoretical values proposed by Kaufmann. Substitution of these empirical values in the equations given above and solution of the equations has given good agreement with known mixtures of the fatty acids (71,78).

Sallans (81) used the following equations for the determination of the component acids in linseed oil:

$$89.9\ x + 181.1\ y + 273.1\ z = 100\ \text{I.V.}$$

$$89.9\ x + 97.0\ y + 166.7\ z = 100\ \text{T.V.}$$

$$x + y + z = u$$

where z = linolenic acid, %; and the other symbols have the same significance as those given above. In this case since oleic, linoleic and linolenic acids are present, equations for these acids all involve iodine value, thiocyanogen value and unsaturated acids. Hence only one equation representing each acid can be derived.

The mean values for iodine number, thiocyanogen number, and unsaturated acids, of the 72 samples used in this study were calculated. When these data were substituted in the above equations, a value, 0.1%, was obtained for linolenic acid. In view of the errors of the method, this percentage is negligible. This confirms the work of Jamieson (63), who states that sunflower oil contains no linolenic acid. Accordingly the equations can be modified to the following form which omits the term for linolenic acid:

$$89.9\ x + 181.1\ y = 100\ \text{I.V.}$$

$$89.9\ x + 97.0\ y = 100\ \text{T.V.}$$

$$x + y = u$$

where x = oleic acid, %; y = linoleic acid, %; I.V. = iodine value; T.V. = thiocyanogen value; u = unsaturated

acids = 95.5 - (saturated acid, % + unsaponifiable matter, %). The above simultaneous equations may be solved in three ways. One solution involves iodine and thiocyanogen values from which percentages of oleic, linoleic and saturated acids may be calculated. The second solution involves iodine value and unsaturated acids, from which the percentage of oleic and linoleic acids may be calculated. The third solution involves thiocyanogen value and unsaturated acids from which oleic and linoleic acid percentages may be calculated. However, since thiocyanogen values are empirical, whereas iodine values are theoretical, this latter method is not considered reliable. In addition the constants obtained are larger than for either of the other two solutions. Larger constants increase the magnitude of the errors, and equations involving thiocyanogen values and saturated acids will not be as reliable as those from the other two solutions.

Since linoleic acid can be determined from either one of two equations, a check on reliability of the empirical thiocyanogen value for this acid can be obtained. The mean value for linoleic acid from 72 observations based on iodine value and unsaturated acids should check with that obtained for the same samples using iodine and thiocyanogen values. The data showed that agreement was not obtained when the value 97.0 was used for the thiocyanogen value of linoleic acid. Computation indicated that a value of 97.4 is required

to secure equivalent values from both equations. Since iodine values are reliable and quantitative (63) and unsaturated acids are based on direct quantitative measurements, it appears that the empirical thiocyanogen value is most likely to be in error. Since the value 97.0 is the mean value determined on linoleic acid isolated from oils by either bromination or numerous crystallizations (71,78) there is good reason to believe that either slight racemization or oxidation may have occurred during preparation. In these circumstances the author believes that the value 97.4 is a closer approximation to the thiocyanogen value of linoleic acid as it occurs in natural glycerides. Hence this value was used in computing values given in this thesis.

The equations were as follows:

$$89.9\ x + 181.1\ y = 100\ \text{I.V.}$$

$$89.9\ x + 97.4\ y = 100\ \text{T.V.}$$

$$x + y = u$$

Solutions of these simultaneous equations for the various acid components are as follows:

Oleic acid, % = 2.4067 T.V. - 1.2944 I.V.

Oleic acid, % = 1.9857 u - 1.0964 I.V.

Linoleic acid, % = 1.1947 (I.V. - T.V.)

Linoleic acid, % = 1.0964 I.V. - 0.9857 u

u = unsaturated acids, % = 95.5 - (saturated acids, % + unsaponifiable matter, %)

Saturated acid, % = 95.5 - (oleic acid, % + Linoleic acid, % + unsaponifiable matter, %).

The unsaturated acid percentages are calculated from experimentally determined saturated acids and unsaponifiable matter on the basis that 95.5% of the oil is composed of fatty acids and unsaponifiable matter. The remaining 4.5% of the oil consists of glycerol. The saturated acid percentage given above is calculated using the oleic and linoleic acid percentages computed from iodine and thiocyanogen values.

Discussion of Data

Data on iodine value, thiocyanogen value, saturated acids, unsaponifiable matter, and the calculated oleic, linoleic and saturated acids of the samples are given in Tables II-XI, Appendix A. The station and variety means for these data are shown in Tables I and II. Analyses of variance for the data are given in Table XII, Appendix A. These analyses show that all properties of the oil differed significantly for both varieties and stations. The necessary differences between variety and station means for the 5% level of significance were calculated from the analyses of variance and are included in Tables I and II.

TABLE I

Varietal Means for Oil Properties

Variety	Iod. No.	SCN No.	Sat. Acids Exp.	Sat. Acids Calc.	Unsapon. Matter	Unsat. Acids	IV-TV Oleic %	IV - u Oleic %	IV-TV Linoleic %	IV-u Linoleic %
Sask. Hybrid No. 1	139.6	82.2	9.5	9.3	.61	85.4	16.9	16.2	68.5	69.2
Sask. Hybrid No. 2	141.8	83.1	8.1	8.1	.62	86.7	16.7	16.7	70.1	70.0
Sask. Hybrid No. 3	139.9	82.4	8.9	9.0	.57	86.1	17.2	17.5	68.7	68.6
Sask. Hybrid No. 4	142.3	83.2	8.1	8.2	.57	86.8	16.1	16.4	70.6	70.6
Sask. Hybrid No. 5	141.8	83.0	8.3	8.4	.59	86.6	16.3	16.4	70.2	70.1
Mennonite (Nosthern)	140.8	83.2	8.2	8.2	.54	86.8	17.9	18.0	68.8	68.8
Mennonite (Manitoba)	141.1	83.1	8.0	8.2	.58	86.8	17.5	17.7	69.2	69.1
Mennonite (Winkler)	140.2	83.1	8.4	8.2	.52	86.6	18.5	18.2	68.3	68.4
Sunrise	137.8	81.4	9.9	10.1	.67	84.9	17.4	17.6	67.4	67.3
Scott Selection No. 1	140.7	83.2	8.2	8.0	.58	86.7	18.3	18.1	68.6	68.4
Scott Selection No. 2	140.6	83.5	8.0	7.9	.53	87.0	18.8	18.5	68.3	68.5
Scott Selection No. 3	141.0	83.4	8.1	8.0	.51	86.9	18.2	18.0	68.8	68.9
Necessary Difference 5% Level	.81	.49	.37	.54	.07	.39	1.00	.90	.83	.82

TABLE II

Station Means for Oil Properties

Station	Iod. No.	SCN No.	Sat. Acids Exp.	Sat. Acids Calc.	Unsapon. Matter	Unsat. Acids	IV-TV Oleic %	IV-u Oleic %	IV-TV Lino-leic %	IV-u Lino-leic %
Univ. of Sask.	141.9	83.4	8.2	8.1	.53	86.8	17.0	16.8	69.9	70.0
Indian Head	141.7	82.9	8.7	8.5	.58	86.2	16.3	15.8	70.1	70.3
Morden	139.4	83.3	8.1	7.9	.55	86.8	20.0	19.6	67.1	67.2
Melfort	142.7	83.6	7.8	7.8	.66	87.0	16.5	16.3	70.5	70.7
Swift Current	139.0	82.3	8.7	8.9	.59	86.3	18.3	18.7	67.7	67.6
Lethbridge	139.2	81.8	9.3	9.6	.54	85.6	16.9	17.4	68.5	68.3
Necessary Difference 5% Level	.57	.35	.26	.39	.05	.28	.70	.64	.59	.58

Varietal Differences

From Table I it is seen that the varietal means covered a narrow range in iodine values and that a number of the varieties differ significantly in all properties. This is especially true of Mennonite and Sunrise, which at present constitute the main commercial varieties. However, Sunrise is a late maturing variety adapted to the southern areas of the prairie provinces, where there is a longer frost-free period. The data for Sunrise are thus not as reliable since the test covered six stations and definitely immature samples were obtained from the more northerly stations. The effect of Sunrise parentage can be noted in Sask. hybrids 1 and 3. These have lower iodine values than the other hybrids and are crosses with Sunrise. The Mennonite strains from Rosthern, Manitoba, and Winkler show iodine values which do not differ significantly from the Mennonite selections made at Scott.

Data for the other properties listed in Table I indicate that real differences exist between certain pairs of varieties and in general where two varieties differ significantly in iodine value they also differ in their component fatty acids. This suggests that there is a relation between iodine value and the fatty acid composition of sunflower oil.

Environmental Differences

Significant variations in oil properties between stations are found in Table II. On the basis of increasing iodine values the stations may be placed in three groups, (a) Morden, Swift Current and Lethbridge; (b) University of Sask. and Indian Head; and (c) Melfort. This classification corresponds to the relative northern location of the groups. A similar relation of iodine value to location may be found in reports on Linseed (75, 81). It has been observed that the more unsaturated oils occur in more northerly and cooler climates. For example, linseed oil produced in United States has an average iodine value of approximately 175 units, whereas Canadian linseed averages 185 units. Sallans (81) found that the most unsaturated linseed oil was secured from northern Alberta, a high value of 202.5 being reported. The differences in the remaining properties follow the same general arrangement as the iodine values.

In Tables I and II there is good agreement between calculated and experimental saturated acids, and between the values for oleic and linoleic acids by two methods of calculation. This factor is important to the study of fractional crystallization. Calculation of component acids by iodine values, thiocyanogen values and unsaponifiable matter is less time consuming than the separate determination of

saturated acids by the lead-salt-alcohol procedure. Since iodine and thiocyanogen values can be determined in duplicate on less than 1 gram of oil, while a single determination of saturated acids and nonsaponifiable matter requires 10 grams, replicated analyses can be made on small samples.

Correlations and Regressions

Total simple correlation coefficients between iodine values and oil properties were computed. These data are presented in Table III.

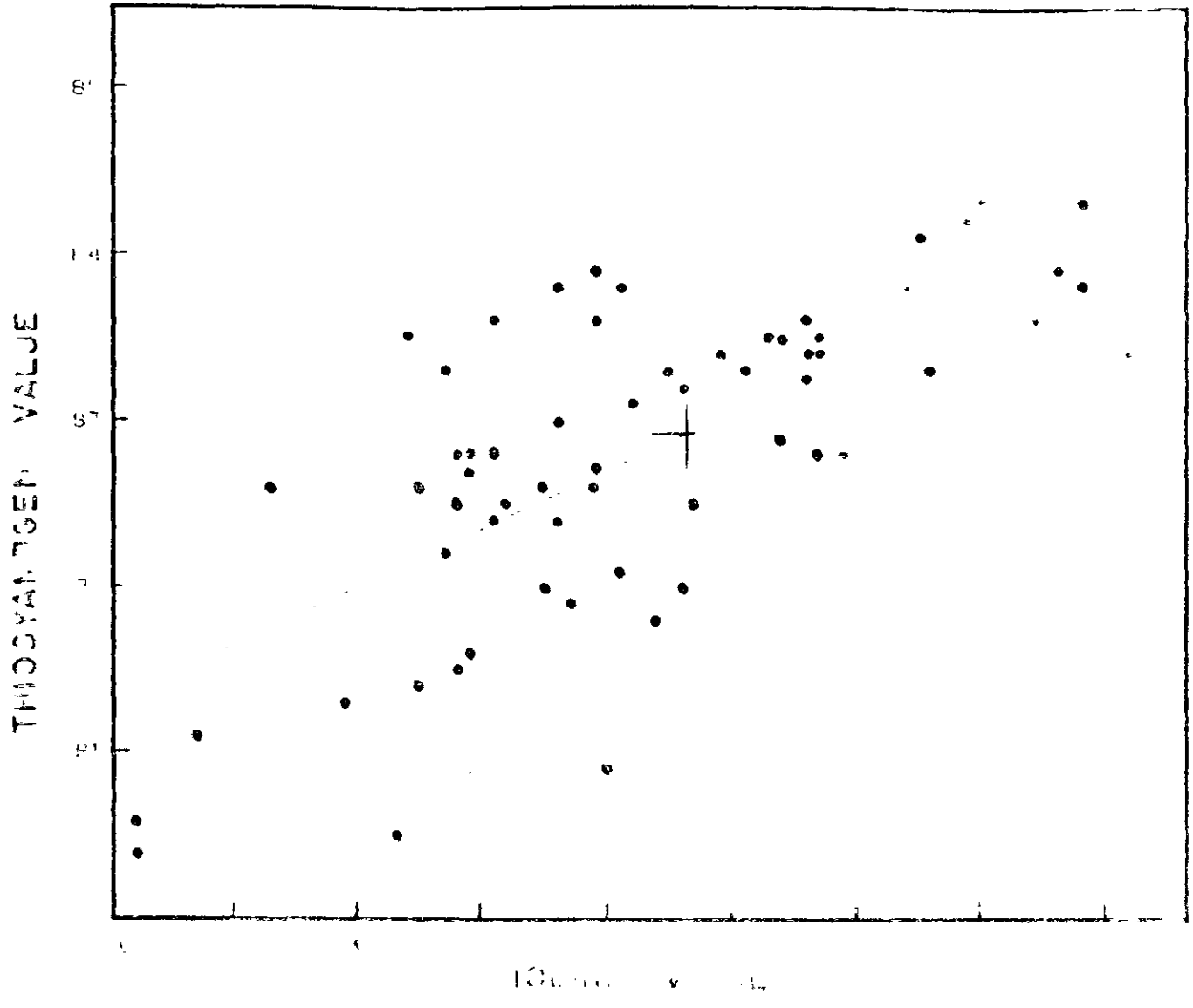
TABLE III

Correlation Coefficients between Iodine Values and Oil Properties

Oil Property	Thiocyanogen Value	Linoleic Acid, IV-TV	Linoleic Acid, IV-u	Oleic Acid, IV-TV	Oleic Acid, IV-u	Saturated Acid, (Exp.)	Saturated Acid, (Calc.)
Iodine Value	.7400**	.9012**	.8874**	-.5105**	-.6152**	-.6645**	-.6685**

** Denotes that the 1% level of significance was attained.

The correlation coefficient between iodine and thiocyanogen values (.7400**) was lower for sunflower oil than the corresponding coefficient (.9616**) found by Sallans (81) for linseed oil. This appears due to immaturity of some of the sunflower samples, especially the Sunrise variety and


THICKNESS VALUE

Sunrise hybrids. The relation between iodine and thiocyanogen values is shown in Fig. 1 by a scatter diagram. It should be noted that Sunrise gave points which varied markedly from the straight line. This characteristic is also found in subsequent figures illustrating the other correlations.

The straight lines in all figures represent the regression equations or lines of best fit computed from least squares. These equations are in effect prediction equations by means of which oil properties may be estimated directly from iodine values. Standard errors were computed for each property, and are appended to each prediction equation.

The prediction equation for thiocyanogen values is T.V. = .3496 I.V. + 33.74, S.E. = $\pm$ .61, where T.V. = thiocyanogen value; I.V. = iodine value and S.E. = standard error. Thus the thiocyanogen values may be determined from iodine values with a maximum error of estimate of 1.83 units.

Highly significant correlation coefficients (.90** and .88**) between iodine value and linoleic acid computed by both equations were obtained with no significant difference between the two coefficients. This means that linoleic acids estimated from iodine and thiocyanogen values are as reliable as linoleic acids estimated from iodine values and unsaturated acid. The relation between iodine value and

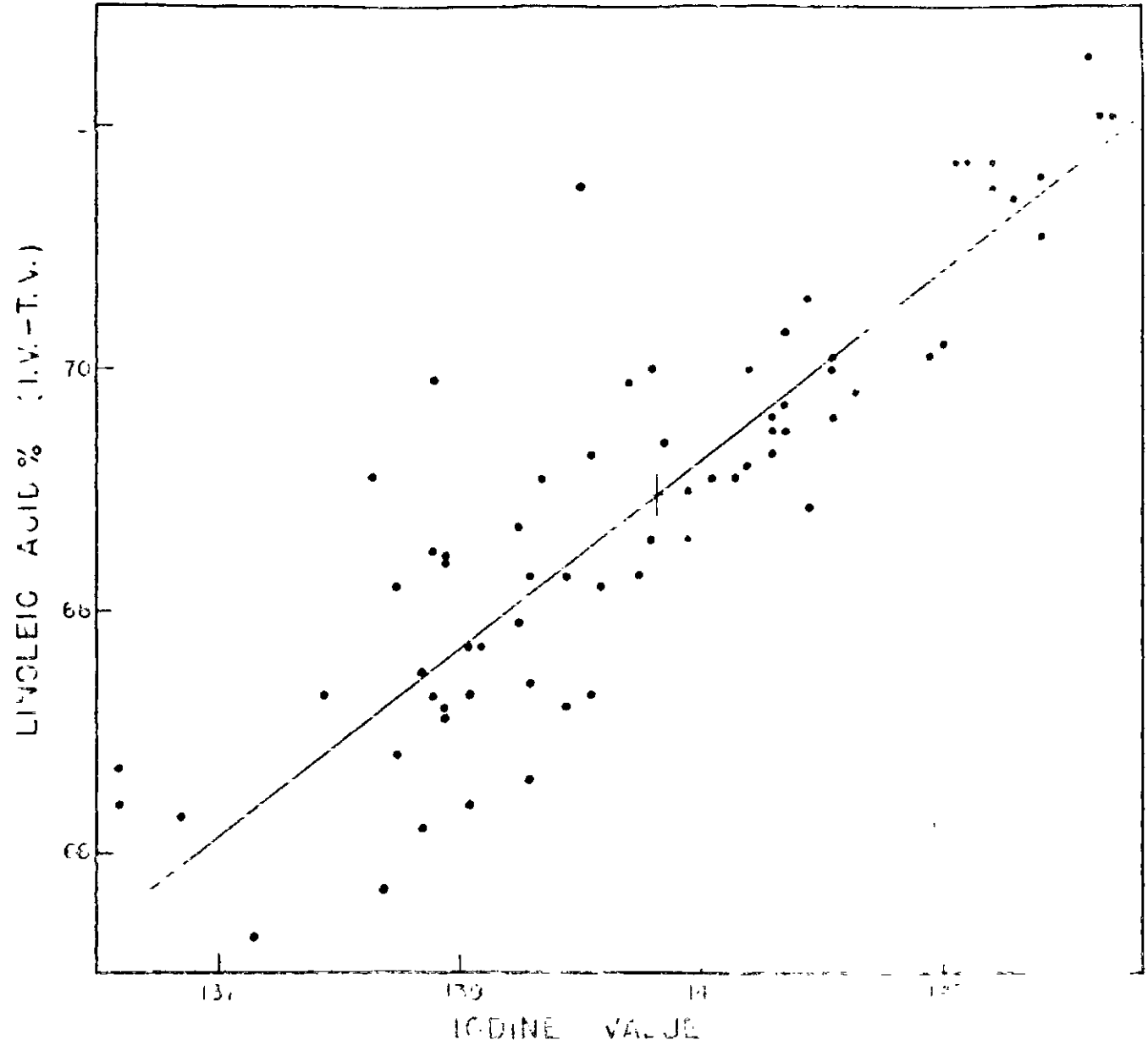


FIG. 1. REGRESSION OF PERCENTAGE LINOLEIC ACID ON IODINE VALUE

linoleic acid calculated from iodine value--thiocyanogen value equations is presented graphically in Fig. 2. A similar graph would be obtained by plotting iodine values against linoleic acids calculated from the iodine value--saturated acid equations. It should be noted that linoleic acid increases with the iodine value of the samples. The prediction equations were as follows:

Linoleic acid, % = .778 I.V. - 40.458; S.E. = $\pm$ .71 obtained from iodine value--thiocyanogen value equations: and, Linoleic acid, % = .773 I.V. - 39.751; S.E. = $\pm$ .80 from iodine value--unsaturated acid equations.

Oleic acid gave significant negative correlation coefficients with iodine values, (Table III). This signifies that oleic acid content decreases with increasing iodine value of the oil. The data on the 72 samples are presented graphically in Fig. 3, where iodine values are plotted against oleic acid calculated from the iodine--thiocyanogen equation. A similar graph would be obtained for oleic acid calculated from the iodine value--unsaturated acid equation. It will be noted that the points on this graph are more scattered than for linoleic acid, (Fig. 2). A greater error is to be expected in the calculation of oleic acids, due to the larger coefficients used in the equations. Prediction equations were as follows:

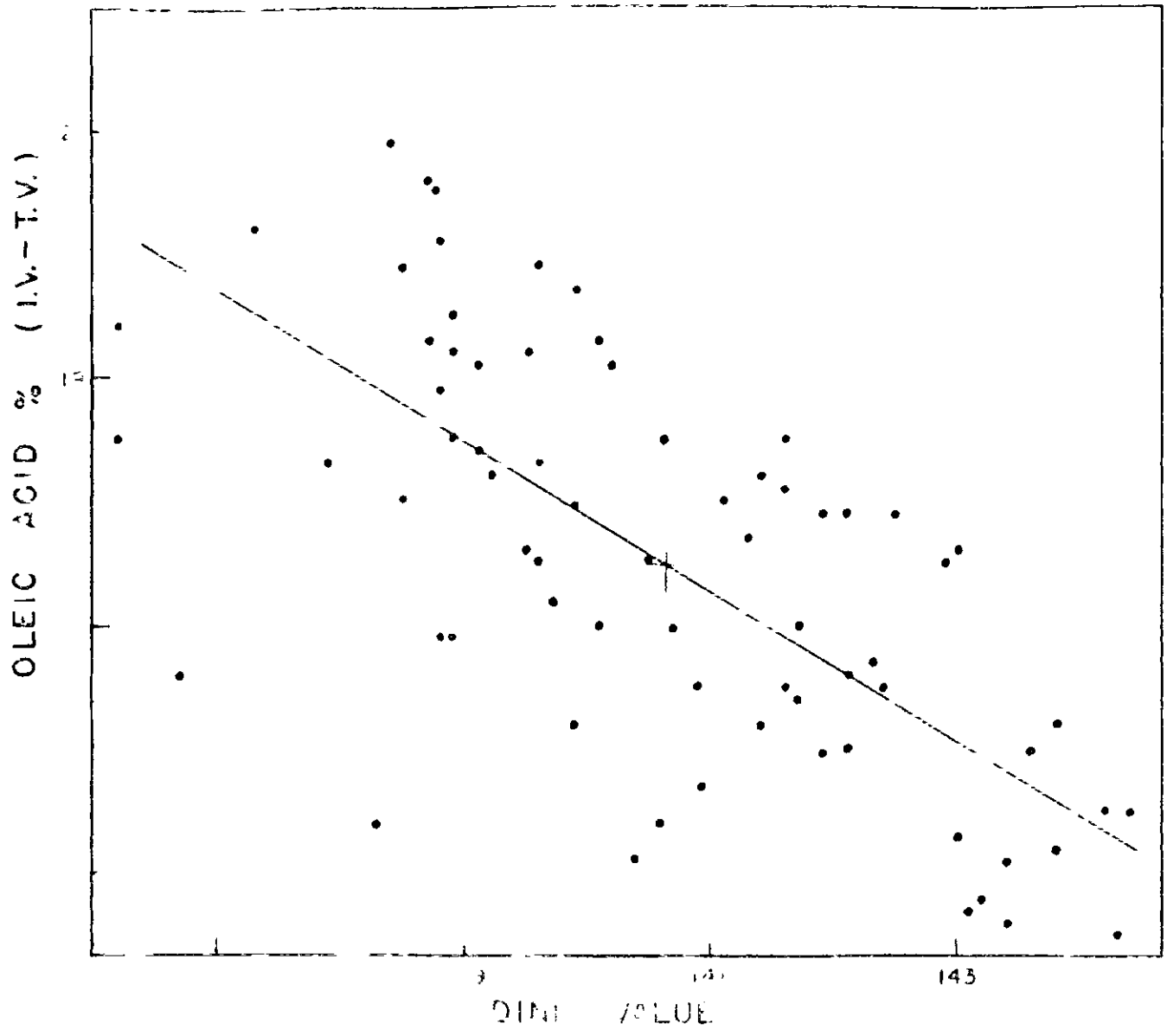


FIG. 1. RELATIONSHIP BETWEEN PERCENTAGE OLEIC ACID ON IODINE VALUE

Oleic acid, % = -.447 I.V. + 80.330; S.E. = ± 1.50, from the iodine--thiocyanogen equations; and, Oleic acid, % = -.524 I.V. + 91.216; S.E. = ± 1.34 from the iodine value--unsaturated acid equations.

Calculated and experimental saturated acids show significant negative correlation coefficients of -.6645** and -.6685** respectively with iodine value. The saturated acid content of the samples decreases with increasing iodine value. The relation is presented graphically in Fig. 4, where the calculated saturated acids are plotted against iodine values.

From Tables V and VI, Appendix A, it will be noted that good agreement was secured between experimental and calculated values for saturated acids. The graph for experimental saturated acids and iodine value would be very similar to Fig. 4. Prediction equations for saturated acids from iodine values as derived from experimental and calculated saturated acids were similar:

Saturated acids, % = -.277 I.V. + 47.3353; S.E. = ± .62 from iodine value and experimental saturated acids: and, Saturated acids, % = -.332 I.V. + 55.191; S.E. = ± .99 from iodine value and calculated saturated acids.

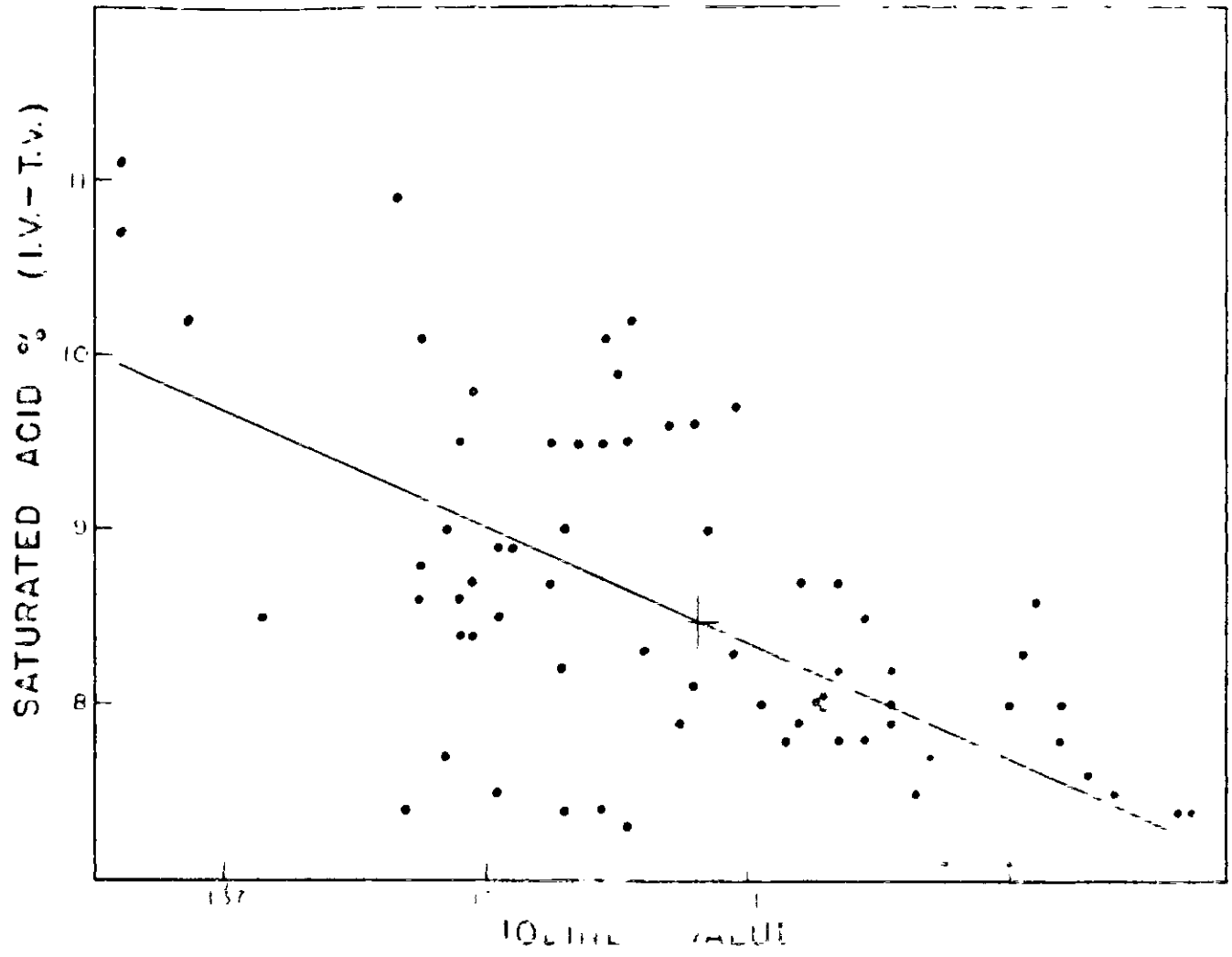


FIG. 4. REGRESSION OF PERCENTAGE SATURATED ACID ON IODINE VALUE

FRACTIONAL CRYSTALLIZATION OF SUNFLOWER OIL

The glyceride composition of an oil cannot as yet be determined from the component fatty acids. This is due to the large numbers of probable glyceride structures which may be computed for any given set of fatty acids. The ideal solution would be a quantitative separation into individual glycerides. The methods for glyceride separation have been reviewed in a previous section and it was seen that fractional crystallization offered the best separation and has been more widely used than either high vacuum or molecular distillation. This is especially true for oils which contain predominately C_{18} unsaturated acids. Quantitative separation into individual glycerides by this method is unlikely in view of the extensive crystallization procedures that were used by Amberger, Klimont and Bomer to obtain pure triglycerides from relatively saturated oils. However, Hilditch and coworkers (57,58) have found that the separation achieved by this method was sufficient for an approximate calculation of glyceride composition.

Accordingly, it was decided to apply the method to sunflower oil to determine the extent to which the procedure would separate the oil into molecular species, whether changes in glyceride structure induced by molecular rearrangements could be detected, and to facilitate a cal-

culation of glyceride composition of sunflower oil.

Materials and Methods

Sunflowerseed oil was obtained from Mennonite seed, 1944 crop. The seed was dehulled in the laboratory huller described by Sallans and Sinclair (80), and the hulls were separated from the meats in a fanning mill. The meats were ground in a set of rolls, and the oil expressed with a Carver laboratory press at a hydraulic pressure of about 15,000 pounds per square inch. The oil was centrifuged after pressing to remove extraneous matter and then stored in a refrigerator. Fresh pressed oil was used for each run.

Crystallization Apparatus

The following apparatus was employed for the isolation of the fatty acids and fractionation of oils. An electric refrigerator was used to secure temperatures of -5°C. and -16°C. Temperatures from -20 to -70°C. were obtained by immersing the flask containing the sample and solvent in an insulated bath of Skellysolve "F". Dry ice was added to the bath with stirring until the desired temperature was reached. Large volumes of solvent and oil were mechanically stirred until crystal formation began, otherwise the flasks were agitated by swirling at inter-

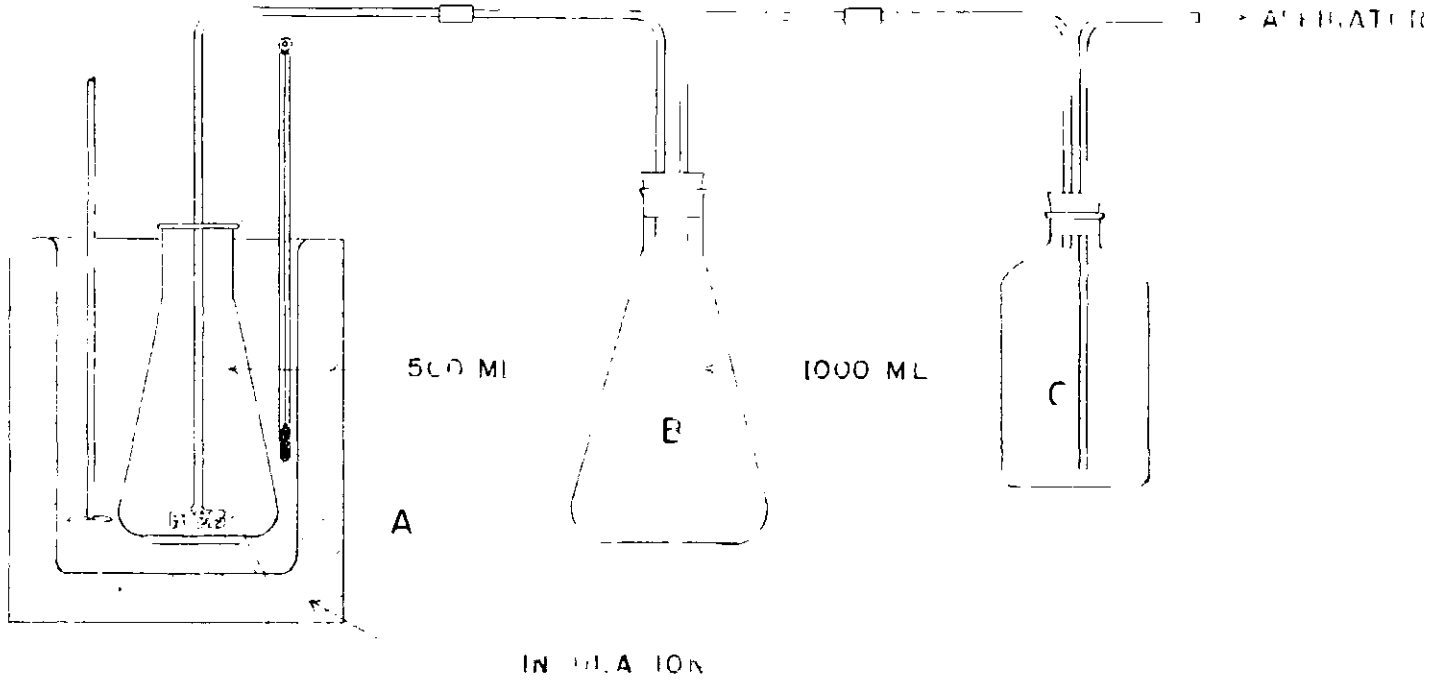


FIG. 5 CRYSTALLIZATION AND FILTRATION APPARATUS

vals. The samples were held at the desired temperature for two hours and filtered by use of the suction system illustrated in Fig. 5. The system consisted of a glass disc (A) packed with fine grade asbestos, connected through a water trap (C) to a water aspirator. The filtrate was drawn from the precipitate into a flask (B). The precipitate was washed thoroughly two or three times with solvent cooled to the same temperature. This procedure is essential for removal of occluded mother liquor in the crystals.

Preparation of Fatty Acids

Synthetic simple triglycerides were prepared from oleic, linoleic and palmitic acids. Palmitic acid was purchased from Eastman Kodak Company. Oleic and linoleic acids were isolated by fractional crystallization from the fatty acids of olive and sunflower oils respectively. The mixed fatty acids were obtained from the oil by a modification of the procedure outlined by Jamieson (63).

The oil was saponified by heating a mixture of 50 g. oil, 40 ml. 36% NaOH, and 40 ml. 95% ethyl alcohol for one hour on a steam bath. One litre of hot water was added and the alcohol removed by distillation. The mixture was cooled and acidified with dilute HCl (1:1). A few drops of methyl orange was added to indicate complete acidification. The mixture was heated until the fatty acid layer was

liquefied and clear. The water layer was removed, and Skellysolve "F" was added to dissolve the fatty acids. The solvent layer was washed free of HCl, and separated from the water layer. The solvent was then removed by distillation in vacuo. Methyl orange was used as an indicator in the solution to ascertain when complete acidification occurred and thus eliminate emulsions during washing of the fatty acids. Addition of Skellysolve "F" facilitated washing and separation of the fatty acids.

The first preparation of linoleic acid was made by the procedure of Frankel and Brown (32), except that Skellysolve "F" was used as the petroleum solvent. The procedure, yields and iodine values were as follows:

(1) 300 g. fatty acids was crystallized from acetone (75 g./l.) at -20 and -50°C. to remove the saturated acids. The acetone was removed by distillation in vacuo, yielding 68 g., I.V. 171.7.

(2) 68 g. dissolved in Skellysolve "F" (65 g./l.), crystallized at -48°C. Yield 40 g., I.V. 172.5.

(3) 40 g. dissolved in 8 litres Skellysolve "F", crystallized at -70°C. Yield 38 g., I.V. 175.4.

(4) 38 g. dissolved in 9 litres Skellysolve "F",

crystallized at -60°C. Yield 21.7 g., I.V. 177.0.

The linoleic acid was judged to be 95.5% pure on the assumption that oleic was the only impurity.

The mutual solubilities of oleic and linoleic acid in Skellysolve "F" at different temperatures were calculated from the above data, and the following procedure was used for the second preparation of linoleic acid:

(1) The fatty acids (750 g.) were crystallized from acetone (1 g./10 ml.) at -20 and -50°C. The acetone was removed by distillation in vacuo. Yield 134.4 g.

(2) 134.4 g. dissolved in 4 litres Skellysolve "F" and crystallized at -55°C. Yield 91.3 g., I.V. 175.9.

(3) 91.3 g. dissolved in 3 litres Skellysolve "F", crystallized at -55°C. Yield 59.5 g., I.V. 177.9.

(4) 59.5 g. dissolved in 1 litre of Skellysolve "F", crystallized at -55°C. Yield 57.1 g., I.V. 178.8.

(5) 57.1 g. in 1 litre of Skellysolve "F", crystallized at -55°C.

(6) Precipitate redissolved in 1 litre of Skellysolve "F", crystallized at -55°C. Yield

49.1 g., I.V. 179.4.

The yield of linoleic acid may be improved by reducing the solvent to fatty acid ratio in steps 1, 2, and 3 of the above procedure.

Oleic acid was isolated by the procedure of Shinowara and Brown (85) except that acetone and Skellysolve "F" were used as solvents. The procedure was as follows:

(1) 260 g. fatty acids were crystallized at -20°C. from 3990 ml. acetone. The filtrate was crystallized at -55°C. The precipitate made up to 2200 ml. in acetone was recrystallized at -20°C. to remove saturated acids.

(2) The filtrate was crystallized from acetone at -55°C. The solvent was removed by distillation in vacuo. Yield 100 g., I.V. 89.04.

(3) 100 g. dissolved in 2 litres Skellysolve "F", crystallized at -55°C.

(4) The fatty acids crystallized in (3) were dissolved in 1 litre Skellysolve "F" and crystallized at -20°C. I.V. 87.6.

(5) The fatty acids from (4) were recrystallized from 800 ml. acetone. Yield 60 g., I.V. 89.32.

The oleic acid was 99.33% pure if saturated acids

were the only adulterant.

Preparation of Simple Triglycerides

From the fatty acids described in the preceding section, tripalmitin, triolein and trilinolein were prepared according to the procedure of Wheeler, Riemenschneider and Sando (92). After esterification, the triglycerides were purified by the neutral oil procedure outlined in Jamieson (63). A slight reddish color in the triolein and trilinolein was partially removed by chromatography with Al_2O_3.

The iodine and thiocyanogen values of the prepared acids and triglycerides are summarized in Table IV. It will be noted that these are not pure as judged by iodine and thiocyanogen values. Further purification was not warranted in view of the intended use of the triglycerides.

Molecular Rearrangement

Samples of sunflower oil and of synthetic oil composed of equal weights of triolein, trilinolein and tripalmitin were subjected to the rearrangement procedure outlined by Bailey et al. (7). The oil was placed in a large pyrex test tube equipped with a thermometer, mercury in glass regulator, and inlet tube for nitrogen, as illustrated in Fig. 6. The nitrogen was bubbled through the inlet tube to provide stirring and to prevent oxidation of the oil by

TABLE IV

Iodine and Thiocyanogen Values of Acids and Triglycerides.

Sample	Iodine Value		Thiocyanogen Value	
	Observed	Theoretical	Observed	Empirical
Linoleic Acid - 1st Prep.	177.0	181.1		
Trilinolein	167.1	173.2		
Linoleic Acid - 2nd Prep.	179.4	181.1	98.1*	96.6
Trilinolein	168.1	173.2	93.8*	92.1
Oleic Acid	89.3	89.9	87.1	89.9
Triolein	84.3	86.0	82.7	86.0
Tripalmitin	0.4	0.0	0.4	0.0

* The high thiocyanogen value may be due to the presence of a small amount of linolenic acid in the sunflower oil.

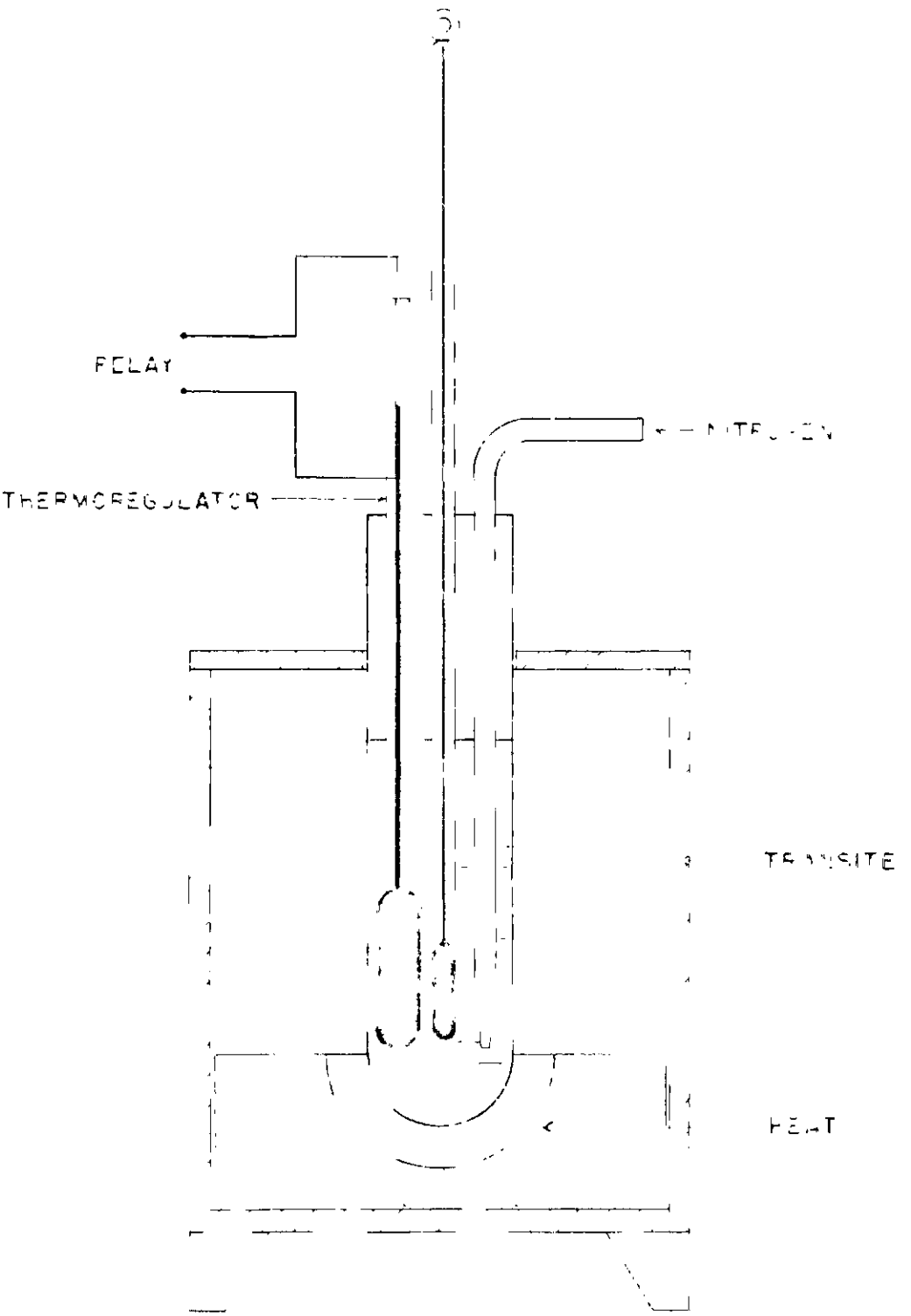


FIG. 6 REARRANGEMENT APPARATUS

air. Nitrogen was first purified by bubbling through two sodium pyrogallate wash towers. Sodium hydroxide 0.05% and glycerol 0.5% by weight were added as catalysts. The mixture was heated at 212°C. ± 1.5° for three hours, and cooled to room temperature. The rearranged oil was refined by the neutral oil procedure outlined in Jamieson (63).

Refining by the neutral oil procedure was found to be preferable to either alkali refining (63) or phosphoric acid refining since a quantitative recovery is possible by this procedure. The iodine value of the sunflower oil decreased by 0.5 units in the rearrangement and refining procedure. The synthetic oil composed of triolein, trilinolein and tripalmitin showed an increase in iodine value of 0.6 units after rearrangement and refining. This was due to a slight loss of tripalmitin, which is solid at room temperature and only slightly soluble in Skellysolve "F" at room temperature. Skellysolve "F" was used as the petroleum ether in the neutral oil refining (63).

Techniques applied to Oil Fractionation

Acetone was selected as the solvent for fractionation of the oils. This solvent has been used by Hilditch (57,58), Riemenschneider et al. (77) and by Bull and Wheeler (22) for fractionation procedures. A solvent ratio of 10:1 was used except where otherwise indicated on the flow sheets

in Figs. 7, 8 and 9.

The crystallization temperatures were selected as those temperatures at which a suitable precipitate appeared. Wherever possible, fractions were crystallized at the same temperatures in different runs in order to have a basis for comparing the effects of various treatments on the oil.

Samples which crystallized at the same temperature were combined in the fractionation procedure. It was necessary to assume that these fractions were of comparable iodine and thiocyanogen values.

Two crystallization procedures were used in fractionating the natural oil. The first (Run 1) was a progressive fractionation from saturated to unsaturated fractions, as indicated on Fig. 7. Fractions were crystallized at -5, -20, -30, and -50°C. These fractions were then recrystallized and combined as shown on the flowsheet. The over-all separation in terms of iodine values was 93.4 units but fractions comprising 51% of the oil separated out at -30°C., and had iodine and thiocyanogen values nearly equal to the original oil (Table VI). The fatty acid analysis corresponded to that of the original oil (Table VI). This indicated an incomplete fractionation.

The second method (Run 2) involved a preliminary separation into relatively saturated and unsaturated fractions at a 3:1 solvent ratio and a temperature of -16°C.

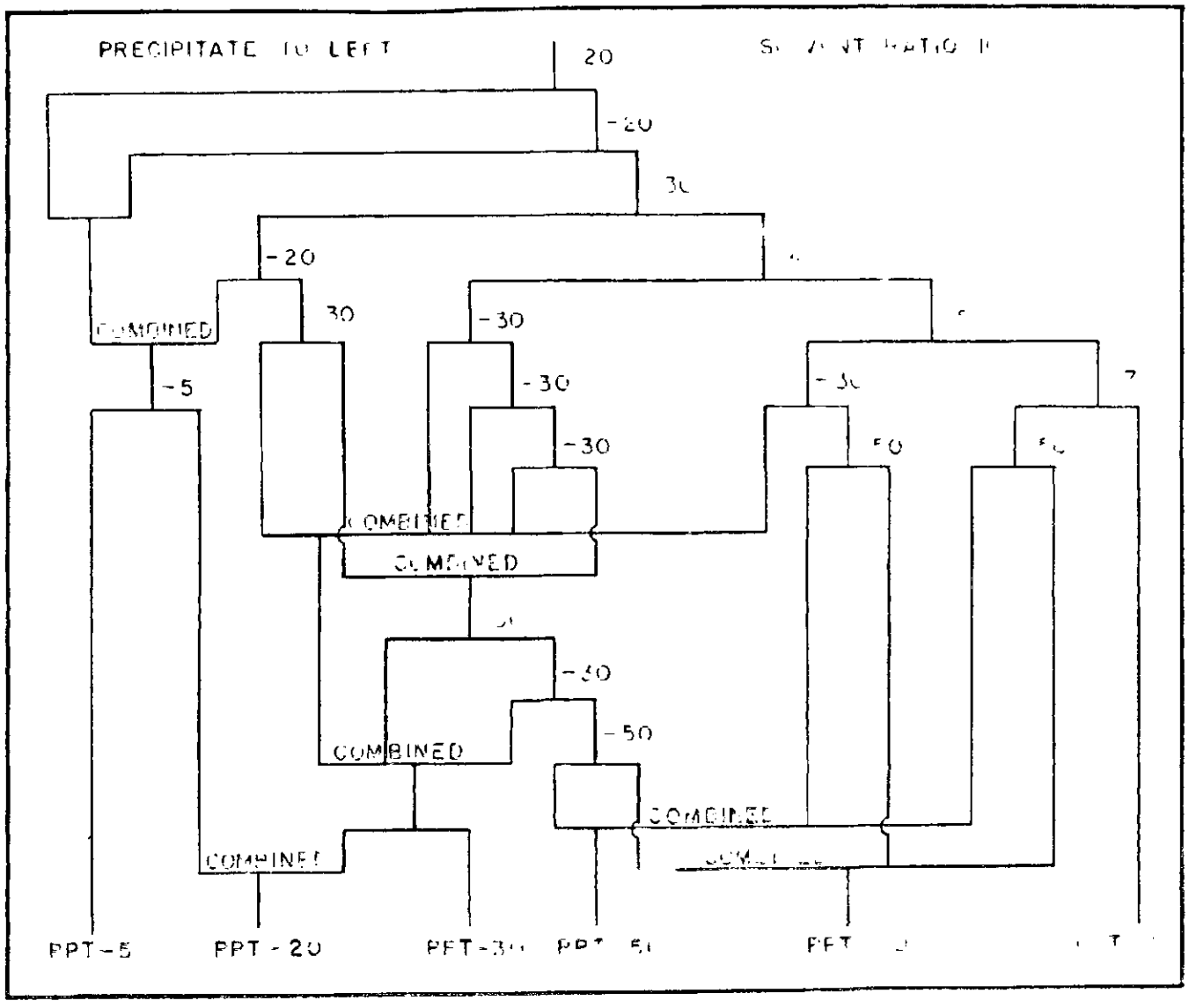


FIG. 7 FLOWSHEET FOR PROJECTIVE

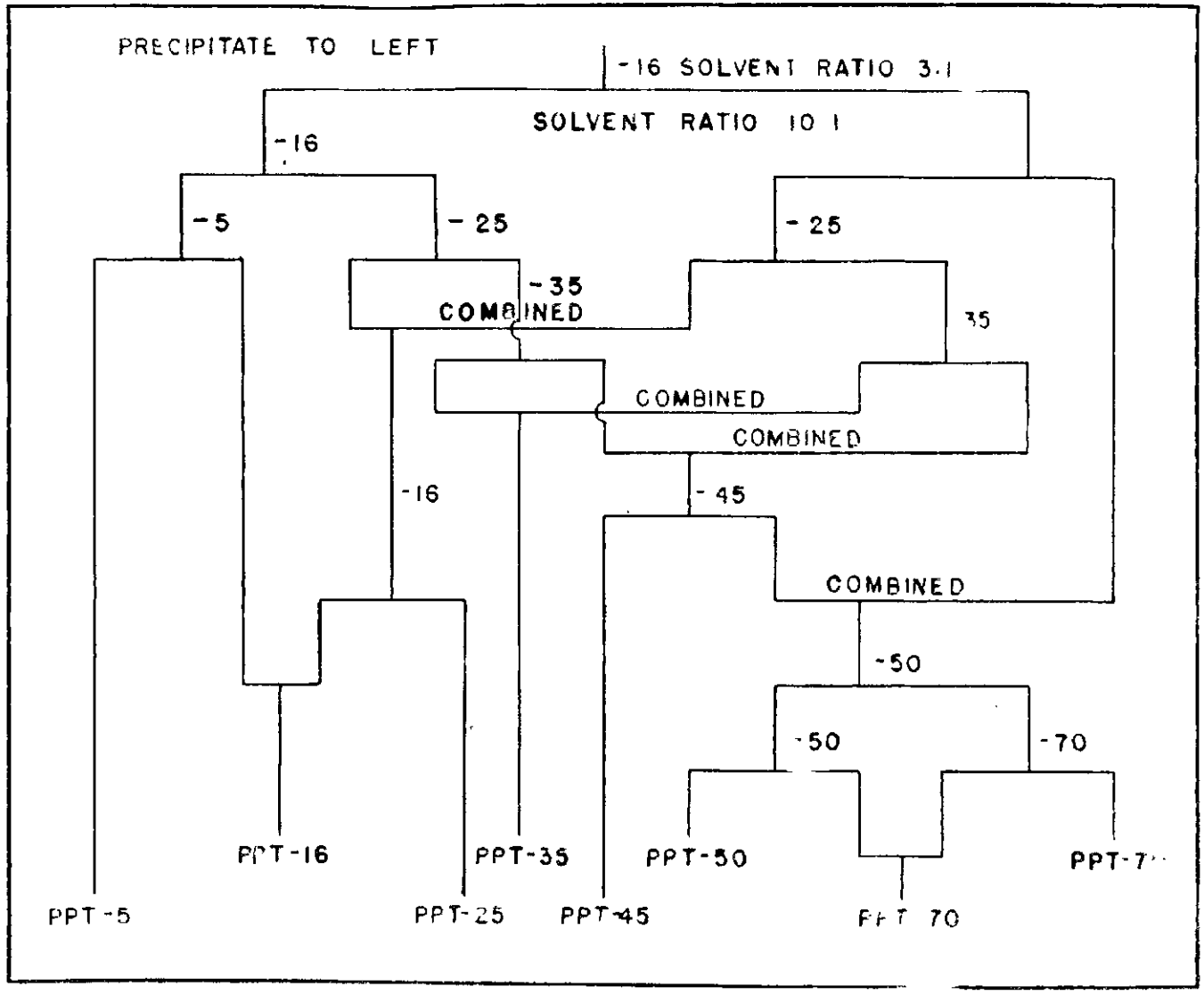


FIG. 8 FLOWSHEET FOR CRYSTALLIZATION

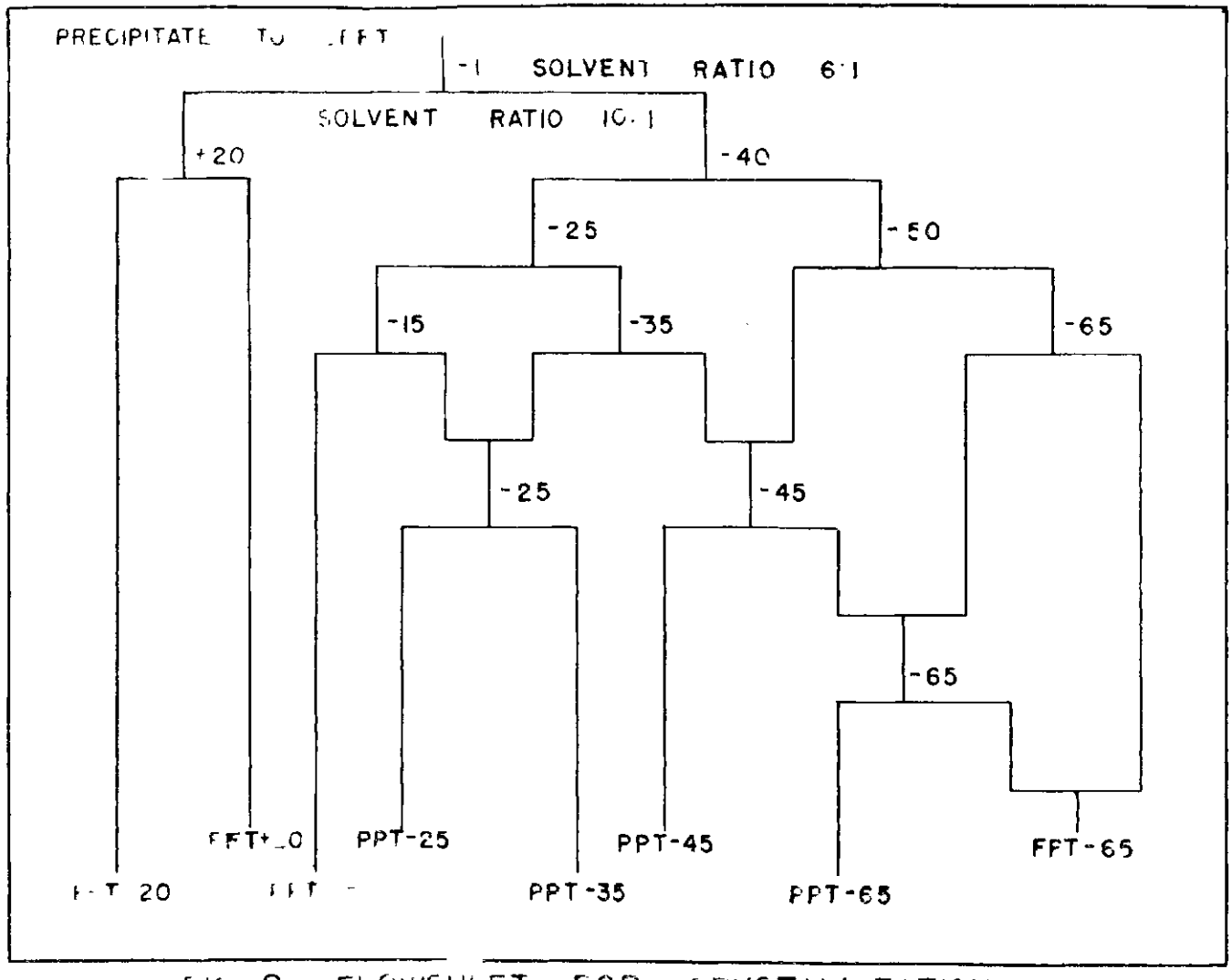


FIG 9 FLOWSHEET FOR CRYSTALLIZATION

for 24 hours. The filtrate was crystallized at 10:1 solvent ratio at -40°C., yielding two fractions. The precipitate was recrystallized at -16°C. at a 10:1 solvent ratio. The four fractions were then crystallized as indicated on Fig. 8. Fractions which crystallized at the same temperatures from the preliminary separations were combined and recrystallized. The over-all separation was 60.5 units in terms of iodine value. Fractions comprising 24% of the oil separated at -35°C. and had iodine and thiocyanogen values comparable to the original oil (Table VII). This fractionation was selected as more efficient since a smaller amount was recovered with iodine and thiocyanogen values comparable to the original oil. This crystallization procedure was employed for subsequent fractionations.

The fractionation procedure was altered for the crystallization of a synthetic oil composed of triolein, trilinolein and tripalmitin in equal proportions by weight. It was found necessary to change the crystallization temperatures of the most saturated fractions due to the presence of a greater quantity of saturated acids as compared to natural oil. The procedure is outlined in the flowsheet in Fig. 9.

The weight of oil in the precipitate and filtrate of the crystallized fractions was determined in the following manner. Solutions of sunflower oil and acetone were made up

in varying concentrations, and placed in a constant temperature bath maintained at 25°C. ± 0.1. Twenty-five ml. aliquots were accurately pipetted into weighed flasks. A graph was constructed (Fig. 10) from the known weight of oil and the weight of 25 ml. of solution. The volume of the unknown solution was measured at room temperature and a representative sample was placed in the constant temperature bath. A 25 ml. aliquot was weighed as before. The weight of oil in the sample was computed from the total volume of solution and the weight of oil in the aliquot. This method was preferable to removing the solvent from the oil and weighing directly since it required less time and there was less chance for the oil to be oxidized in manipulation.

The solvent was removed from the fractions after crystallization by heating for two hours in vacuo. Iodine values (Wijs method, 1 hour) and thiocyanogen values (0.2N reagent, 24 hours, temperature 21°C. ± 0.05) were determined. Single determinations of iodine and thiocyanogen values were made on successive days and the means of triplicates are reported. The standard deviations of the iodine and thiocyanogen values were 0.19 and 0.16 units respectively. The amount of oleic, linoleic and saturated acids were determined in the same manner as the component acids for sunflower oil in the previous section. The equations used were:

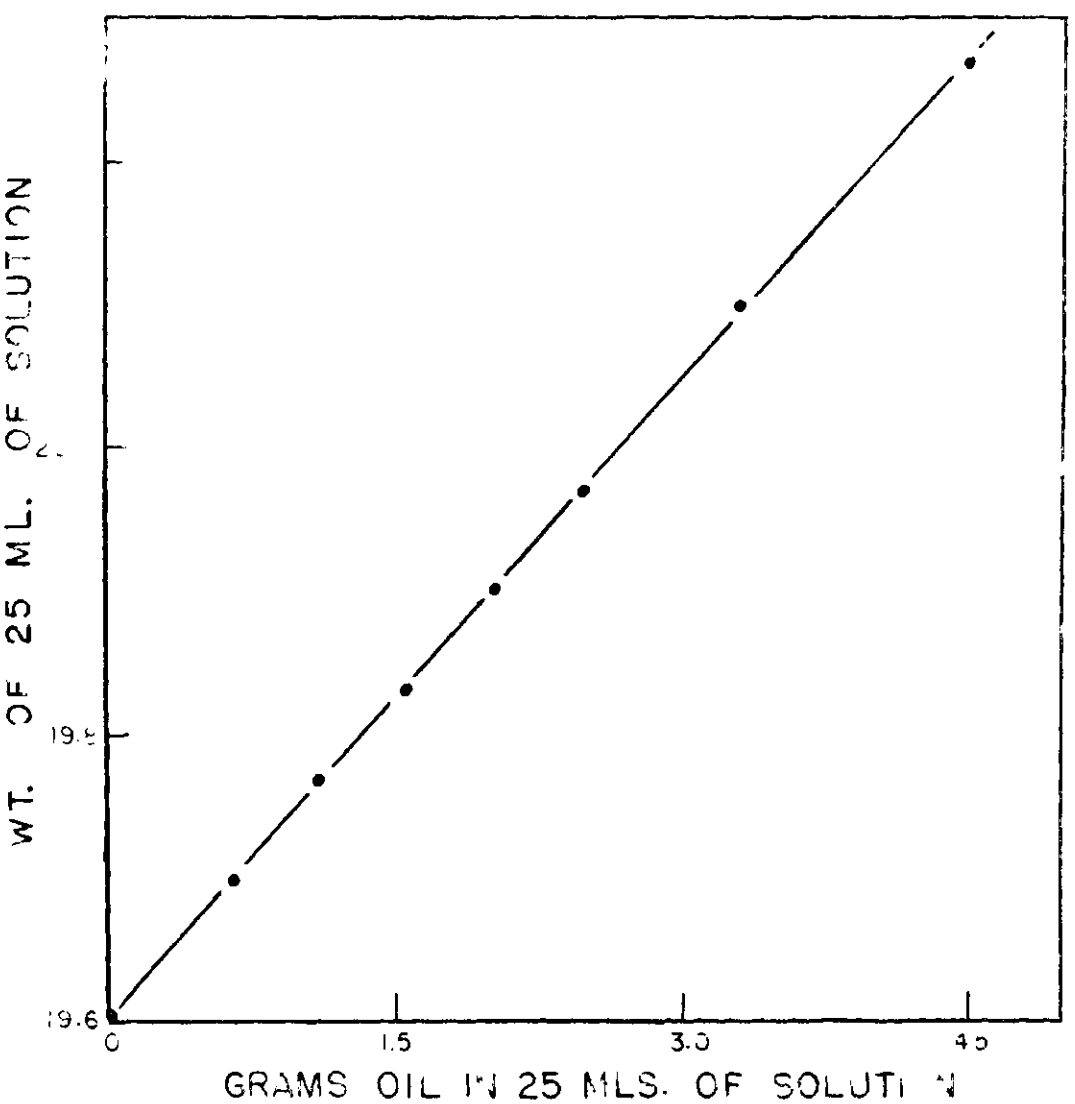


FIG. 10 DETERMINATION OF WEIGHT OF OIL IN SOLUTIONS.

Oleic acid, % = 2.4067 T.V. - 1.2944 I.V.

Linoleic acid, % = 1.1947 (I.V. - T.V.)

Saturated acid, % = 95.5 - (oleic % + linoleic % + unsaponifiable matter %)

where I.V. = iodine value, T.V. = thiocyanogen value.

Saturated acids were determined by the lead-salt-alcohol procedure in fractions of natural sunflower oil. This method yielded an average value of 1.2% higher than that calculated by the iodine-thiocyanogen equations. There was no difference between original oil and crystallized fractions. The calculation of component acids from iodine-thiocyanogen equations was justifiable and was employed for all runs.

Theoretical Triglycerides from the Acids of Sunflower Oil

The iodine and thiocyanogen values of the possible triglycerides of oleic, linoleic, stearic and palmitic acid were calculated. The molecular weights, iodine values and thiocyanogen values of the fatty acids are given by Jamieson (63). These data are tabulated in Table V and included in all figures to facilitate the calculation and interpretation of glyceride structure.

TABLE V

Calculated Iodine and Thiocyanogen Values of Possible Triglycerides from Oleic, Linoleic, Stearic and Palmitic Acids

Triglyceride	General Class	I.V.	T.V.
Trilinolein	Triunsaturated	173.3	92.1
Oleodilinolein	"	144.1	90.1
Linoleodiolein	"	115.0	88.1
Triolein	"	86.0	86.0
Stearodilinolein	Diunsaturated	115.0	61.1
Palmitodilinolein	"	123.0	63.2
Oleopalmitolinolein	"	88.9	61.1
Oleostearolinolein	"	86.0	59.2
Stearodiolein	"	57.2	57.2
Palmitodiolein	"	59.1	59.1
Linoleodistearin	Monounsaturated	57.2	30.4
Linoleodipalmitin	"	61.1	32.5
Oleodistearin	"	28.6	28.6
Oleodipalmitin	"	30.7	30.7
Stearopalmitoolein	"	29.5	29.5
Considering saturated acids in sunflower oil as 1/2 stearic + 1/2 palmitic			
Saturateddilinolein	Diunsaturated	119.0	62.1
Saturateddiolein	"	58.1	58.1
Oleosaturatedlinolein	"	87.4	60.1
Linoleodisaturated	Monounsaturated	59.1	31.4
Oleodisaturated	"	29.6	29.6

Data from the Literature

Data on the fractional crystallization of cottonseed oil from acetone by Hilditch and Maddison (57) and by Riemenschneider, Swift and Sando (77) are presented in Tables IX and X and Fig. 13. Thiocyanogen values of the fractions obtained by Hilditch were computed from the fatty acid composition and iodine values. This necessitated grouping of non C_{18} dienoic and trienoic acids into oleic and linoleic groups. The values obtained cannot be regarded as exactly equivalent to experimental thiocyanogen values, but are sufficiently accurate for illustrative purposes. These data are included to illustrate the efficiency of fractional crystallization of vegetable oils with acetone.

DISCUSSION OF DATA

Fractional Crystallization of Natural Sunflower Oil

Data for fractional crystallization of natural sunflower oil are given in Tables VI and VII. Although Run 2 (Table VII) was selected as giving a more efficient fractionation, the data from Run 1 (Table VI) are included in this section to show that analogous conclusions may be drawn from a greater over-all separation. The iodine and thiocyanogen values of the fractions are illustrated graphically in Fig. 11.

TABLE VI

Run 1. Natural Sunflower Oil crystallized progressively as in Fig. 7

Fraction		% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
	Original Oil*	-	140.4	83.3	18.9	68.1	7.9
A	Ppt. -5°C.	1.9	68.9	43.0	14.2	31.0	49.7
B	Ppt. -20°C.	25.3	130.1	78.1	19.7	62.0	13.2
C	-30°C.	51.0	136.9	82.4	21.1	65.1	8.7
D	-50°C.	16.1	153.5	90.6	19.4	75.1	0.4
E	Residue	5.3	162.3	96.5	22.3	78.6	0.0

* Unsaponifiable Matter 0.58%

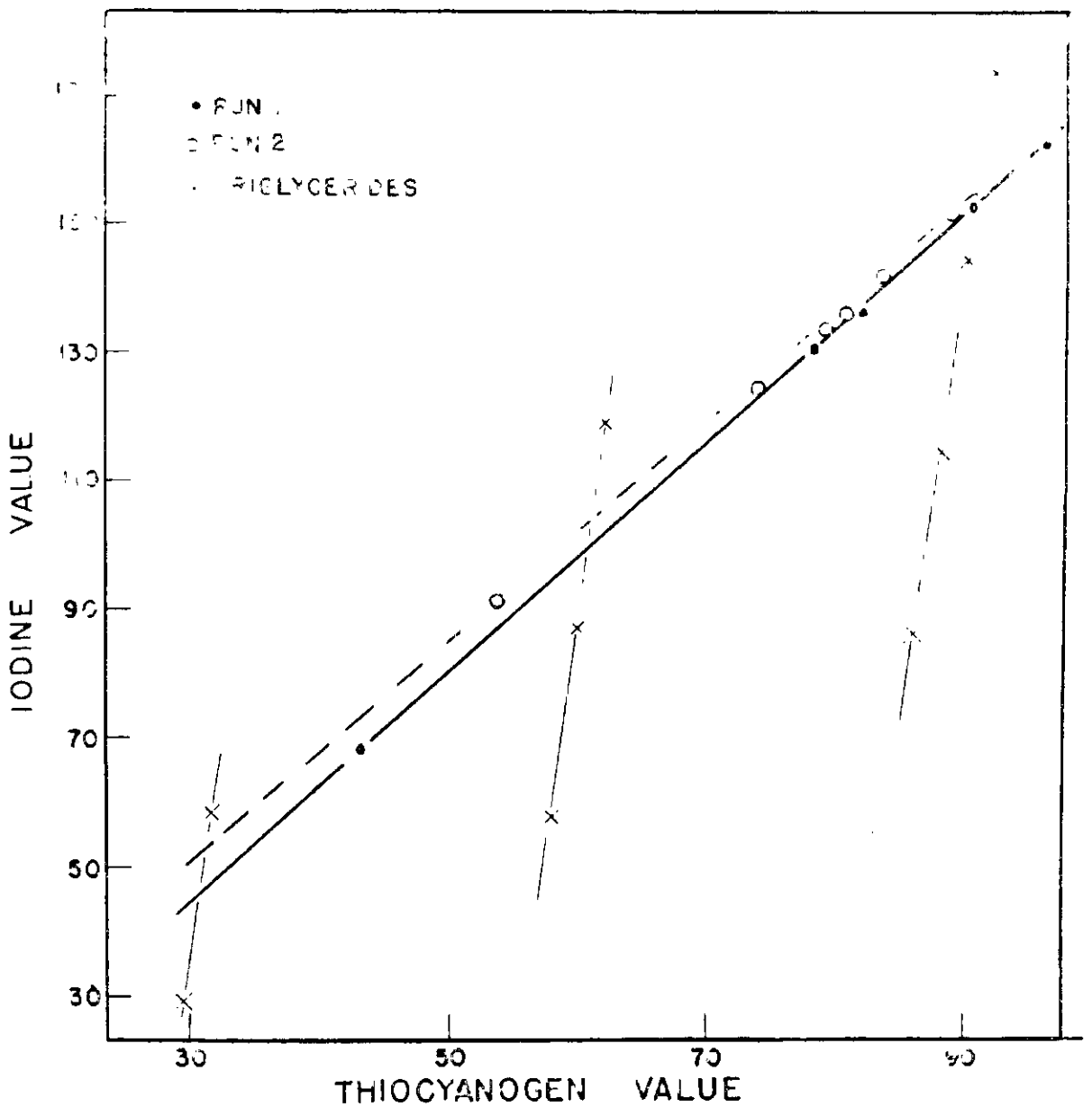


FIG. 11 FRACTIONATION OF NATURAL OIL

TABLE VII

Run 2. Natural Sunflower Oil crystallized as in Fig. 8

Fraction		% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
	Original Oil*	-	138.6	82.2	18.3	67.4	9.2
A	Ppt. -5°C.	3.9	92.1	53.8	10.3	45.8	38.9
B	-16°C.	7.3	124.6	74.0	16.8	60.5	17.7
C	-25°C.	23.5	134.4	79.5	18.4	65.6	11.0
D	-35°C.	24.3	136.2	81.0	18.6	66.0	10.4
E	-45°C.	13.9	142.4	83.8	17.3	70.1	7.6
F	-50°C.	25.3	152.5	88.6	15.9	76.3	2.8
G	-70°C.	0.6	152.6	77.6	-	-	-
H	Residue	0.7	149.4	70.2	-	-	-

* Unsaponifiable Matter 0.58%

In Run 2, Fig. 11, two fractions, G and H, show points which deviate from a straight line joining the remaining points. A similar variation may be found in subsequent Figs. 12, 13, 15. The samples were all kept in 250 ml. glass-stoppered flasks, under nitrogen at -5°C. Determinations of iodine and thiocyanogen values on successive days for the fractions crystallized at -60° to -70°C. showed decreases of one to five units per twenty-four hours. This instability was attributed to a rapid decomposition of

linoleic acid in the absence of antioxidants and the large ratio of surface to volume for these small samples. These fractions comprise only minor amounts, 1-2%, of the oil and, while the analytical values are unreliable, this does not materially affect the conclusions.

Iodine and thiocyanogen values for the theoretically possible triglycerides in sunflower oil given in Table V are plotted in Fig. 11. These glycerides show a scattered relation between iodine and thiocyanogen values, but may be divided into three general classes, monounsaturated, diunsaturated and triunsaturated on the basis of component acids. Almost vertical lines may be drawn joining glycerides of each general class. It is apparent that segregation of an oil into molecular species of glycerides could not result in the observed linear relation between iodine and thiocyanogen values except under two highly improbable conditions. First, if the oil were composed of only three triglycerides whose iodine and thiocyanogen values give a straight line when plotted, e.g., linoleodisaturated, saturateddilinolein and trilinolein, and second, if complete separation into the above general classes were achieved. This second condition also requires that the relative proportions of glycerides in each general class must be such that their iodine and thiocyanogen values fall on a straight line. The points representing the fractions would therefore fall on the vertical

lines for the general classes (Fig. 11).

It is obvious from Fig. 11 that fractionation of sunflower oil does not meet either of the above conditions, since the points representing the fractions lie between the general classes and on a straight line. This indicates that each fraction is composed of triglycerides selected from at least two of the three general classes. The position of the point representing the fraction on the graph is dependent on the relative proportions of glycerides that are present in the fraction. A greater over-all separation, such as was achieved in Run 1, means that the fraction of lowest iodine value contains a larger percentage of monounsaturated triglycerides. Similarly, the fraction of highest iodine value contains relatively more of the triunsaturated glycerides. The intermediate fractions contain the remaining glycerides in proportions related to the iodine values of the fractions. The net effect of a greater over-all separation is to lower the line joining the fractions. Proof for this is shown in Fig. 11. Sunflower oil of iodine value 140.4 was used for Run 1, and the line joining the fractions lies below that for Run 2 in which the oil had an iodine value of 138.6. Fractional crystallization appears to separate the oil into fractions in which the relative proportions of glycerides vary in a regular manner. This does not necessarily mean that each fraction

contains the same triglycerides. The fraction of lowest iodine value may contain glycerides which are not present in the fraction of highest iodine value. From the foregoing discussion it is also apparent that the amount of each fraction will be affected by the amount of each general class of glycerides that is present in the oil.

The crystallization of natural sunflower oil results in fractions whose iodine and thiocyanogen values lie on a straight line. This linear relation indicates that segregation into molecular species has not been achieved. The fractions are composed of mixtures of triglycerides, and the relative proportions vary with the iodine value of the fractions. This statement may be proved from data on the component acids and by reference to Fig. 11. From Tables VI and VII it is seen that linoleic and saturated acid content varies with the iodine value of the fraction. Oleic acid does not vary in such a regular manner but does vary in each fraction. In addition the three acids are present in all fractions. In Fig. 11 the points representing the fractions lie between the general classes of triglycerides, and must therefore be composed of a mixture of glycerides selected from at least two of the general classes.

The conception of glyceride structure proposed by Hilditch assumes an "even" distribution of fatty acids among the glycerol molecules. This structure usually provides for

the formation of a maximum amount of mixed triglycerides. If sunflower oil has an "even" structure, molecular rearrangement to a random structure should be possible on the assumption that the component acids are equal in chemical activity. A completely random structure would contain the simple triglycerides in greater proportion than the natural oil. Successful rearrangement should be indicated by an increase in the proportion of trilinolein. Bailey et al. (7) found that molecular rearrangement of peanut oil broke up the glycerides which contributed to difficult filtration in crystallization procedures. Accordingly, a sample of sunflower oil (Run 3) was rearranged as outlined in the previous section.

The rearranged oil was crystallized by the same procedure as the natural oil and the resulting data are shown in Table VIII and Fig. 12. Data for Run 2 are included in Fig. 12 for comparison.

A linear relation was again obtained and by the previous argument segregation into molecular species was not achieved. The points representing the fractions lie between the general classes and therefore these fractions must be composed of a mixture of glycerides. The relative proportions of glycerides vary with the iodine value of the fraction. Comparison of the two runs in Fig. 12 reveals a grouping of the fractions in the rearranged oil.

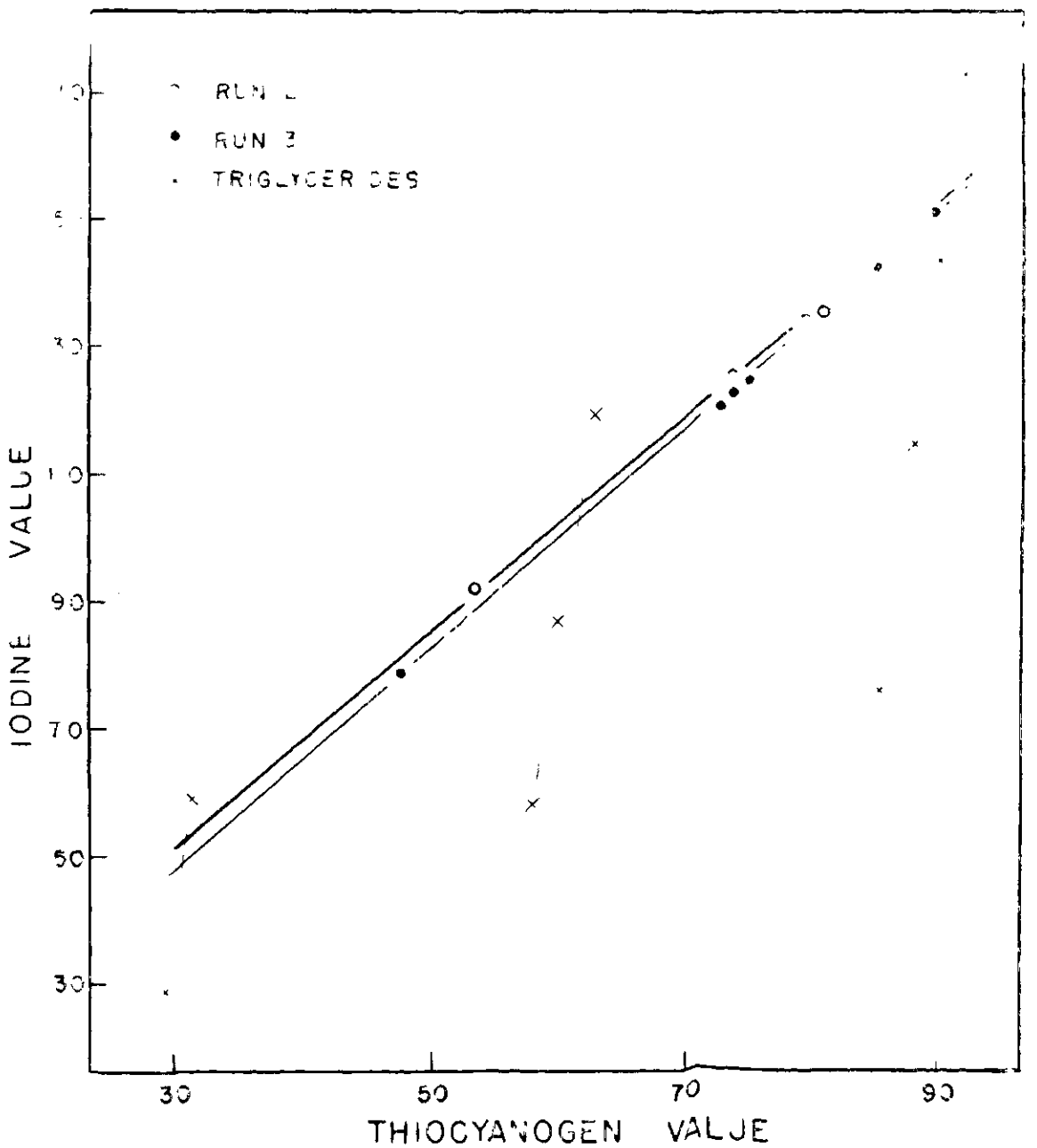


FIG. 12 FRACTIONATION OF NATURAL AND REARRANGED OILS.

TABLE VIII

Run 3. Natural Sunflower Oil subjected to Molecular Rearrangement and crystallized as in Fig. 8

Fraction		% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
	Original Oil	-	138.3	82.6	19.6	66.7	8.7
	Rearranged Oil*	-	137.8	81.9	18.7	66.8	9.5
A	Ppt. -5°C.	5.3	79.4	47.6	11.7	38.0	45.2
B	Ppt. -16°C.	6.3	121.2	72.6	17.8	58.1	19.0
C	-25°C.	6.3	123.4	73.7	17.7	59.3	17.9
D	-35°C.	18.1	123.9	74.9	19.7	58.6	16.5
E	-45°C.	35.4	142.9	85.3	20.4	68.7	5.8
F	-50°C.	23.9	151.9	89.3	18.4	74.7	1.8
G	-70°C.	4.4	146.6	86.8	19.1	71.5	4.4
H	Residue	1.9	150.2	89.2	20.4	72.8	1.8

* Unsaponifiable Matter 0.58%

Since the crystallization procedures were identical, the grouping appears to result from the rearrangement procedure.

If the data in Tables VII and VIII are compared it is seen that the most saturated fraction (A) obtained in the rearranged oil is 12.7 units lower in iodine value and only 0.6% lower in weight than the corresponding fraction from the natural oil. In addition fractions comprising 65.6% of the total were recovered from the rearranged oil with iodine

values greater than 142. The corresponding fractions from natural oil comprise only 40.5%. It would appear that rearrangement may have increased the proportion of triunsaturated glycerides. This does not necessarily follow since the natural oil fractions crystallized at -25° C. (I.V. 134.4, yield 23.5%) and -35° (I.V. 136.2, yield 24.3%) may contain 30.5% of material with iodine value 142 on the basis of a material balance. The remaining 7.3% would consist of two fractions having iodine values of 123 and 124. Such a division could be interpreted to mean that no essential difference exists between the glyceride structure of the natural and the rearranged oils. Thus, as long as the points representing the crystallized fractions lie on a straight line and between the general classes of triglycerides, the fractions must be composed of mixtures of triglycerides. Furthermore, because of the straight line relation between iodine and thiocyanogen values, the relative proportions of individual glycerides in each fraction must vary in a regular manner with the iodine value of the fraction.

Data from the Literature

This linear relation is not an intrinsic property of sunflower oil. The data for cottonseed oil given in Tables IX and X and presented graphically in Fig. 13 are included to illustrate this point. The cottonseed oils were

crystallized from acetone by Hilditch (57) and by Riemenschneider et al. (77). Points representing the fractions are seen to lie on straight lines and between the general classes of triglycerides. The points for the fractions in Table X do not give as strict a linear relation. The deviations may be due to the fact that the thiocyanogen values were calculated for these fractions from the iodine values and fatty acid composition.

TABLE IX

Crystallization of Cottonseed Oil by Riemenschneider, Swift and Sando

Fraction	Weight	% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
A	149 g.	15.3	52.5	30.8	10.6	25.0	64.4
B	11	1.1	58.2	33.5	10.2	28.5	61.3
C	18	1.8	66.3	38.3	12.0	32.3	55.7
D	451	46.2	107.1	62.1	19.9	51.9	28.2
E	154	15.8	133.4	74.8	18.8	67.6	13.6
F	145	14.9	140.6	78.8	19.7	71.4	8.9
G	48	4.9	148.3	79.9	13.3	79.0	7.7

Fractional Crystallization of Sunflower Oil with added Trilinolein

Since sunflower oil (cf. Table VII) contains 67.4% linoleic acid and 18.3% oleic acid, it will be composed

TABLE X

Crystallization of Cottonseed Oil
by Hilditch et al.

Fraction	Weight	% Weight	I.V.	Calculated T.V.	% Oleic*	% Linoleic	% Saturated
A	4.9g.	0.8	38.3	28.2	19.2	12.2	67.9
B	86.1	14.1	57.0	37.7	18.3	22.0	59.7
C	197.4	32.3	97.0	63.3	26.1	42.4	31.5
D	62.4	10.2	107.9	71.8	29.3	48.6	22.1
E	192.9	31.6	124.7	78.2	26.8	59.8	13.4
F	66.9	11.0	134.0	83.5	26.7	65.0	8.3

* Includes tetradecenoic and hexadecanoic acids.

predominately of unsaturated glycerides, and should contain a considerable proportion of trilinolein. Hilditch (44) states that simple triglycerides do not occur in appreciable amount unless 60% or more of a single acid is present. On the basis of random distribution there should be 30.6% of trilinolein. However, no trilinolein could be isolated as such in any of the three crystallizations of natural oil. This failure to segregate the glycerides appears to be caused by associations between unsaturated glycerides.

To test the existence of such associations 18.5 g. of synthetic trilinolein (I.V. 167.1) was mixed with 81.5 g.

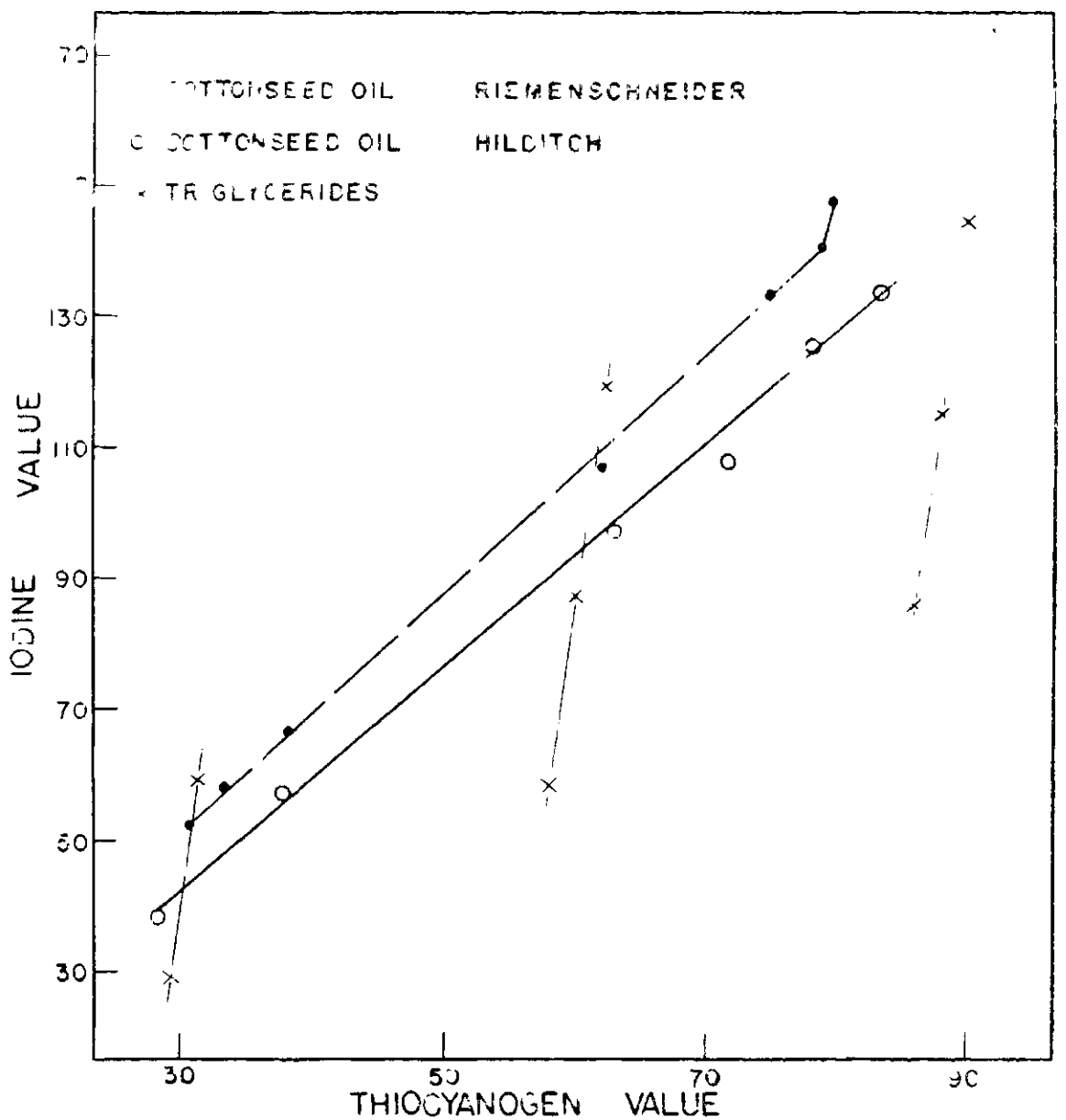


FIG. 13 FRACTIONATION OF COTTONSEED OIL

of sunflower oil. The mixture was fractionally crystallized by the same procedure as Run 2 (Fig. 8) and the data are given in Table XI and presented graphically in Fig. 14. The maximum spread between the fractions was 53.4 units in iodine value as compared to 60.5 units for the natural oil. The

TABLE XI

Run 4. 81.5 g. Sunflower Oil mixed with 18.5 g. Synthetic Trilinolein and crystallized as in Fig. 8

Fraction		% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
	Original Oil	-	137.7	82.3	19.8	66.2	9.0
	Mixed Oil*	-	143.1	84.4	17.9	70.1	2.0
A	Ppt. -5°C.	3.1	103.3	61.4	14.0	50.1	30.9
B	-16°C.	5.0	120.8	72.0	17.0	58.2	19.8
C	-25°C.	6.5	132.5	79.2	19.0	63.8	12.3
D	-35°C.	22.7	133.7	79.8	18.9	64.5	11.7
E	-45°C.	41.5	147.8	86.8	17.6	72.9	4.5
F	-50°C.	17.1	156.7	91.0	16.3	78.4	0.3
G	-70°C.	1.9	153.8	86.2	8.3	80.8	5.9
H	Residue	1.4	143.9	83.3	14.2	72.4	8.4

* Unsaponifiable Matter 0.47%

two fractions, G and H, comprise 2.1% more material than the same fractions for Run 2, and showed a similar decrease in iodine and thiocyanogen values with time.

The line in Fig. 14 shows a slight curvature, with a definite change in slope at iodine value 138. The four fractions, A, B, C and D, at which point the slope of the graph changes, contained 21.7% less material than the corresponding fractions for Run 2. The two remaining fractions, E and F, contained 19.5% more material than similar fractions for Run 2. There has evidently been a preferential separation of the added trilinolein into the two upper fractions. However, fraction A, iodine value 103.3, is 11.2 units higher in iodine value and 0.8% lower in material than the corresponding fraction in Run 2. The similar yield of material and higher iodine value indicate a shifting of more unsaturated glycerides into this fraction. The most unsaturated fraction, F, (17.1%) had an iodine value of 156.7, only 4.3 units higher than a similar fraction recovered from natural oil. This fact is rather surprising in view of the addition of 18.5% of synthetic trilinolein of I.V. 167.1.

A specific attempt to recover the added trilinolein was made by recrystallizing the four fractions (C, D, E and F) occurring at -25°, -35°, -45° and -50°. The data for the seven fractions are given in Table XII and included in Fig. 14. Four fractions, C, D, F and G, comprising 46.6% of the oil, occurred in the triunsaturated region, indicating a shift of unsaturated material. A straight line may be

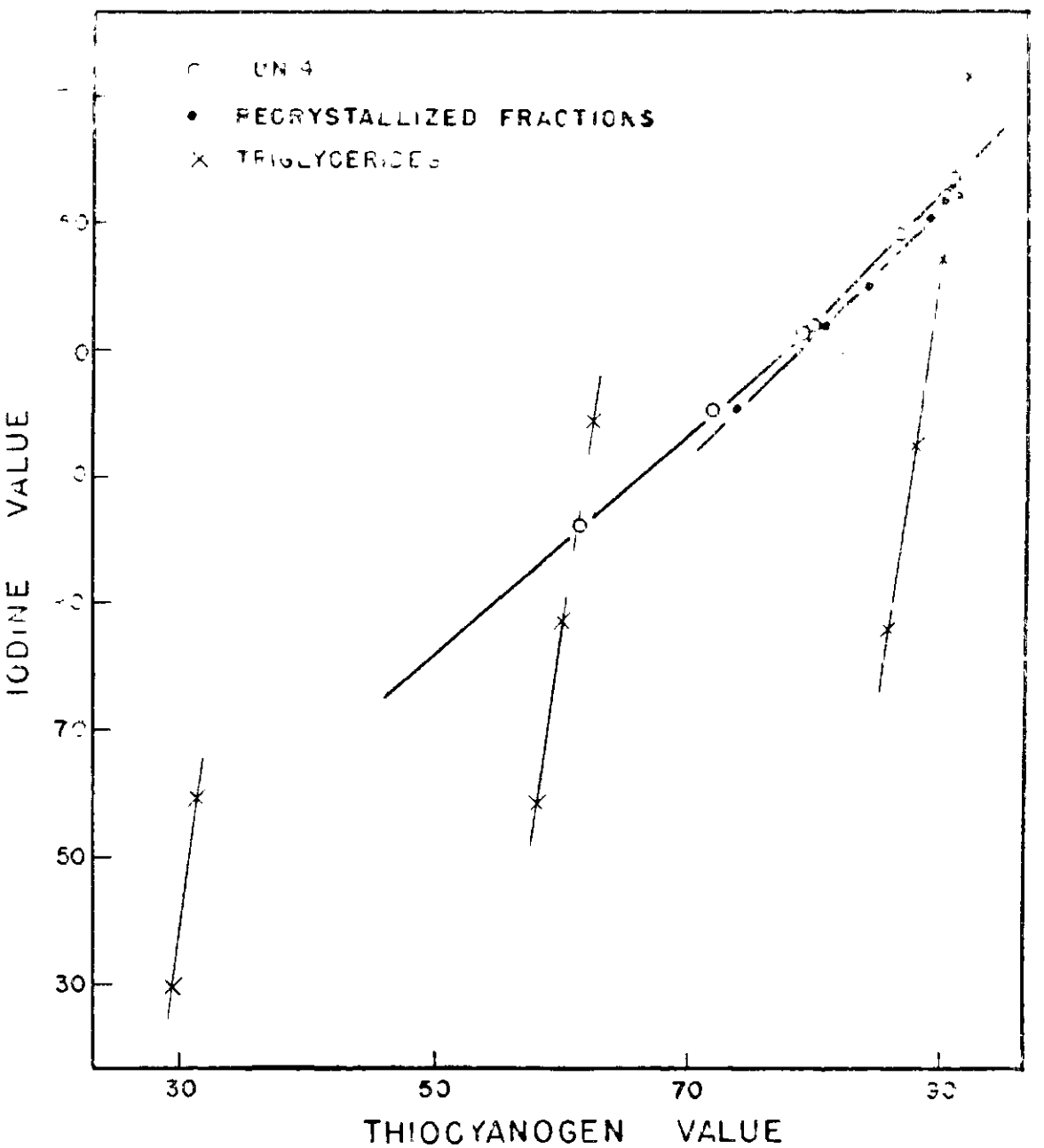


FIG. 14 FRACTIONATION OF MIXED OIL

TABLE XII

Recrystallization of Fractions C, D, E and F in Table VIII

Fraction			Solvent Ratio	% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
A	Ppt.	-35°	(10:1)	11.9	119.2	73.9	13.6	54.1	27.4
B	Fft.	-35°	(10:1)	10.9	140.6	84.5	21.3	67.1	6.7
C		-45°	(10:1)	15.1	153.9	90.5	18.7	75.7	0.7
D		-45°	(27.5:1)	14.4	150.6	89.8	21.1	72.7	1.3
E	Ppt.	-45°	(27.5:1)	12.0	134.6	81.1	21.0	63.9	10.2
F		-40°	(10:1)	15.0	155.1	90.6	17.4	77.0	0.7
G	Fft.	-40°	(10:1)	2.1	153.7	91.6	21.5	74.2	0.0

drawn representing the fractions, which indicates that the proportions of glycerides still vary in a regular manner. However, these data must be treated as approximate, since the iodine and thiocyanogen values of the seven fractions decreased with time. The data does illustrate that the previous fractions were further separated, with a major proportion shifted into the triunsaturated region, but that segregation into molecular species has not been achieved. The added trilinolein was not recovered as such, but caused a shift in the relative proportions of glycerides in the fractions as compared to the same fractions obtained from natural oil.

Fractional Crystallization of Synthetic Oil

It has been shown that trilinolein added to a natural oil is distributed over the relatively unsaturated fractions and cannot be recovered by crystallization. The unsaturated fractions of natural oil contain mixed glycerides of oleic and linoleic acid, which do not differ markedly from trilinolein. Accordingly, a synthetic oil was made up of triolein, trilinolein and tripalmitin in equal proportions by weight, to test the method of crystallization on simple triglycerides. Since tripalmitin is relatively insoluble in acetone at room temperature, the crystallization was modified as indicated in Fig. 9.

Data are given in Table XIII and shown graphically in Fig. 15. Fraction B was not recombined in further crystallizations (Fig. 9) and appears out of line with the remaining fractions. The fractions, G and H, will not be considered in this discussion since the iodine and thiocyanogen values decreased with time, due to decomposition of linoleic acid. These two fractions make up 9.8% of the original oil.

A fraction comprising 30.6% of the oil (A) was recovered at $+20^{\circ}$C. with iodine and thiocyanogen values of 1.0 and 1.4 respectively. Chemical analysis reveals no linoleic acid and 2.1% of oleic acid in this sample. This represents a very high recovery of the original tripalmitin.

TABLE XIII

Run 5. Synthetic Oil (20.2 g. Triolein, 20.3 g. Trilinolein, 20.4 g. Tripalmitin) crystallized as in Fig. 9

Fraction		% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
	Original Oil	-	84.3	58.8	32.3	30.5	32.7
A	Ppt. +20°C.	30.6	1.0	1.4	2.1	0.0	93.4
B	Fft. +20°C.	6.3	96.7	68.2	39.0	34.0	22.5
C	Ppt. -15°C.	18.5	80.4	79.7	87.7	0.9	6.9
D	-25°C.	7.6	108.6	84.0	61.5	29.4	4.6
E	-35°C.	11.6	137.5	89.0	36.2	58.0	1.3
F	-50°C.	15.6	156.4	91.8	18.5	77.2	0.0
G	-60°C.	3.8	146.8	87.7	21.2	70.6	3.8
H	Residue	5.9	129.6	77.4	18.6	62.3	14.6

The fraction of iodine value 80.4 (C) lies near the point for triolein in Fig. 15. Chemical analysis of this sample shows 0.9% linoleic acid and 6.9% of palmitic acid. Fraction F of iodine value 156.4 contains only oleic and linoleic acids. However, these fractions comprise only 18.5 and 15.6% of the oil respectively. The two fractions intermediate in iodine value contain 19.2% of the oil. The recovery of triolein and trilinolein has not been as quantitative as for tripalmitin. It is notable that the four

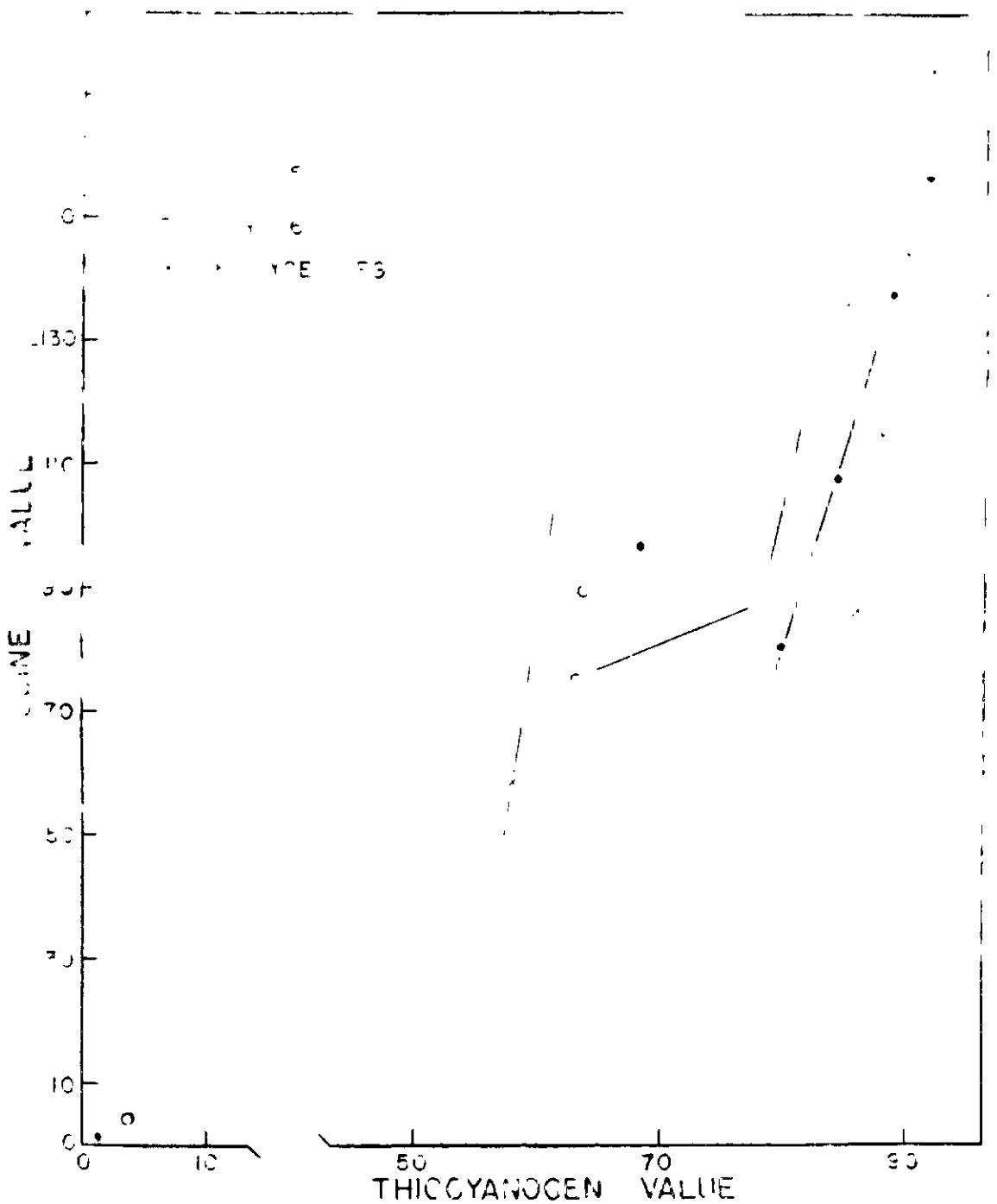


FIG. 15 FRACTIONATION OF SYNTHETIC OIL

fractions, C, D, E and F, lie on a straight line, indicating that the fractions are composed of the glycerides in proportions relative to the iodine value. Segregation into molecular species has not been achieved even in the oil composed of three simple triglycerides.

The unsaturated fractions contain the major proportions of mixed glycerides. It has previously been suggested that there is a molecular association in unsaturated glycerides which makes difficult the separation into molecular species. The fact that four of the fractions give a straight line relationship appears to confirm this view. The net effect of fractional crystallization is to separate the oil into fractions in which the glycerides occur in a regular pattern relative to the iodine value.

To test the effectiveness of the rearrangement procedure, the synthetic oil was rearranged and fractionally crystallized. The results are tabulated in Table XIV and included in Fig. 15.

The three fractions, B, G and H, are omitted in the following discussion for the same reasons as in the synthetic oil above. A fraction comprising 26.8% of the oil was obtained with iodine and thiocyanogen values of 3.1 and 2.7 respectively. This represents 3.8% less material than for the synthetic oil and a lower recovery of palmitic acid. Fig. 15 reveals that the remaining four fractions, C, D, E

and F, have been shifted away from the triunsaturated region. This indicates the presence of diunsaturated and monounsaturated glycerides such as would be formed by rearrangement. Thus, the rearrangement procedure was effective in producing a change in the glyceride structure of the synthetic oil.

TABLE XIV

Run 6. Synthetic Oil (20.1 g. Triolein, 19.7 g. Trilinolein, 20.4 g. Tripalmitin) subjected to Molecular Rearrangement and crystallized as in Fig. 8

Fraction		% Weight	I.V.	T.V.	Oleic %	Linoleic %	Sat'd. %
	Original Oil	-	82.9	58.1	32.6	29.6	33.3
	Rearranged Oil	-	83.5	58.9	33.7	29.4	32.4
A	Ppt. +20°C.	26.8	3.1	2.7	2.6	0.4	92.5
B	Fft. +20°C.	8.8	89.9	64.0	37.6	31.0	26.9
C	Ppt. -15°C.	6.9	75.2	63.3	55.1	9.9	30.6
D	-25°C.	22.6	87.8	78.2	74.5	11.5	9.5
E	-35°C.	7.5	125.9	82.4	35.4	52.0	8.1
F	-50°C.	15.7	147.9	88.3	21.1	71.2	3.2
G	-60°C.	8.2	142.5	86.3	23.2	67.1	5.1
H	Residue	3.5	128.5	78.8	23.4	59.3	12.8

GLYCERIDE COMPOSITION OF SUNFLOWER OIL

Two general hypotheses have been postulated to explain glyceride structure. The first, "even distribution", was proposed by Hilditch (63) working on the assumption that the fatty acids are evenly distributed over the glycerol molecules in natural fats and oils. He found that fractional crystallization failed to isolate pure simple triglycerides and concluded that the fatty acids are so arranged that maximum formation of mixed glycerides occurs. Obviously, the glycerides present and the amounts of each type will depend on the order in which the fatty acids are selected for distribution. The second hypothesis is "random distribution", based on the assumption that the fatty acids are all equivalent and are distributed according to the theory of probability. This conception implies that all the theoretically possible glycerides are present in natural fats and the amounts of each are dependent on the relative concentration of the component fatty acids.

For the calculations which follow, stearic and palmitic acid are combined and the term saturated acids applied for the computation of glyceride structures. This reduces the number of acids to three, saturated (S), oleic (O), and linoleic (L). A similar procedure was employed by Hilditch (57, 58) to simplify the calculations for "even distribution" and the net effect is that glycerides containing saturated acids

are not specifically designated as stearo- or palmito-glycerides.

The following glyceride structures are possible on the basis of random distribution of fatty acids: OLS; OOS; OOL; OLL; SLL; SSL; SSO; SSS; LLL; and OOO. The glycerides are designated by the component acids, disregarding configuration. The simple triglycerides will occur as the cube of the percentage of fatty acid. The probability of selecting two acids the same and one different, e.g. OSS, will be 3(O,% x S,% x S,%). This is apparent from numbering the positions on the glycerol molecule. Representing glycerol as 1. CH_2OH,

2. CHOH

3. CH_2OH,

the acids have three choices for position and may occur as OSS, SSO and SOS. In a similar manner OLS may occur in six
123 123 123
ways and the probability will be 6 times the product of the acid percentages or 6(O,% x L,% x S,%). The determination of random distribution consists of performing the above computations using the acid percentages in each fraction. The percentage of each glyceride is multiplied by the weight percent of each fraction and results are added to give the total glyceride composition.

The calculation of "even distribution" follows the procedure outlined by Hilditch and Meara (45). The procedure consists of dividing one acid between the other two and

computing the glycerides and percentages of each. A specific example will illustrate the method. If the following component acids and percentages are assumed: oleic 20%, linoleic 70% and saturated acids 10%; and linoleic acid is divided between oleic and saturated acids, the calculations are as follows:

Oleic (O) 20.0 Linoleic (L) 70.0 Stearic (S) 10.0

Dividing linoleic in proportion to oleic and saturated acids gives

Oleic 20.0 Saturated 10.0

Linoleic = 20/30 x 70 = 46.6 Linoleic = 10/30 x 70 = 23.4

Combining these quantities to form triglycerides gives,

Oleodilinolein 60.0% Saturated dilinolein 30.0%

Trilinolein 6.6% Trilinolein 3.4%

Total glycerides obtained are oleodilinolein, 60.0%; saturated dilinolein, 30.0%; and, trilinolein, 10.0%. This structure may be confirmed as "even" distribution by a simple illustration. The acids are taken in the ratio oleic 6, linoleic 21, and saturated 3, which is equivalent to the percentages given above. These are distributed evenly over 10 glycerol molecules, denoted by the symbol G, using linoleic first, as follows:

(L	(L	(L	(L	(L
G(L	G(L	G(L	G(L	G(L
(L	(S	(S	(S	(O
(L	(L	(L	(L	(L
G(L	G(L	G(L	G(L	G(L
(O	(O	(O	(O	(O

This gives 1 molecule of trilinolein (10%), 3 molecules of saturated dilinolein (30%) and 6 molecules of oleodilinolein (60%) identical with the calculated structure.

In a similar manner oleic acid may be divided between saturated and linoleic acids, or saturated acids between oleic and linoleic acids. The procedure may also be modified to give a maximum amount of a selected glyceride. Thus it is possible to obtain several glyceride compositions which may differ in component glycerides and the relative proportions of each.

Glyceride structures were calculated for natural sunflower oil and rearranged sunflower oil by even and random distribution of the fatty acids. The data are presented in Tables XV, XVI and XVII. A random structure based on the acid composition of the whole oil is included in Tables XVI and XVII. The glyceride compositions are given on a mole percent basis.

The data in Table XV are included to show that similar glyceride structures were obtained from different fractionation procedures. This is especially true of the amount of each general class of glycerides. The discussion of glyceride structure will therefore be confined to the data given in Tables XVI and XVII since the crystallization procedures were identical and separated the oil more efficiently into the respective fractions. Table XVI contains four calculated structures based on the oil fractions. The structures all

TABLE XV

Calculated glyceride structures of sunflower oil from data in Table VI.

Triglyceride	Even Distribution			Random Distribution
	Divide Oleic	Divide Linoleic	Divide Saturated	Fractionated Oil
Trisaturated (SSS)	-	-	-	0.4
Oleodisaturated (OSS)	0.1	0.1	0.4	0.9
Linoleodisaturated (LSS)	0.9	1.0	0.8	2.5
Oleolinoleosaturated (OLS)	14.6	1.8	12.9	7.7
Saturated diolein (SOO)	-	-	-	1.2
Saturated dilinolein (SLL)	11.9	24.8	13.8	12.0
Triolein (OOO)	-	-	-	1.0
Oleodilinolein (OLL)	48.8	61.6	50.6	30.9
Linoleodiolein (LOO)	-	-	-	9.4
Trilinolein (LLL)	23.5	10.7	22.0	34.1
Totals on Glyceride Classes				
Class				
Trisaturated	-	-	-	0.4
Monounsaturated	1.0	1.1	1.2	3.4
Diunsaturated	26.5	26.6	26.7	20.9
Triunsaturated	72.3	72.3	72.3	75.4

TABLE XVI

Calculated glyceride structures of sunflower oil from data in Table VII

Triglyceride	Even Distribution			Random Distribution	
	Divide Oleic	Divide Linoleic	Divide Saturated	Fractionated Oil	Whole Oil
Trisaturated (SSS)	-	-	-	0.6	0.2
Oleodisaturated (OSS)	-	-	0.2	0.8	0.6
Linoleodisaturated (LSS)	-	0.9	0.8	3.2	2.8
Oleolinoleosaturated (OLS)	13.6	1.6	12.5	7.5	8.7
Saturated diolein (SOO)	-	-	-	1.0	1.1
Saturated dilinolein (SLL)	14.7	28.2	17.5	14.4	17.3
Triolein (OOO)	-	-	-	0.6	0.6
Oleodilinolein (OLL)	41.8	52.2	41.1	27.6	26.7
Linoleodiolein (LOO)	1.1	-	-	6.9	6.7
Trilinolein (LLL)	28.8	17.1	27.9	37.4	35.3
Class					
Trisaturated	-	-	-	0.6	0.2
Monounsaturated	-	0.9	1.0	4.0	3.4
Diunsaturated	28.3	29.8	30.0	22.9	27.1
Triunsaturated	71.7	69.3	69.0	72.5	69.3

TABLE XVII

Calculated Glyceride Structure of Rearranged Sunflower Oil in Run 3 from Data in Table VIII

Triglyceride	Even Distribution			Random Distribution	
	Divide Oleic	Divide Lino-leic	Divide Satu-rated	Frac-ticna-ted Oil	Whole Oil
Trisaturated (SSS)	-	-	-	0.6	0.1
Oleodisaturated (OSS)	0.7	-	0.8	1.0	0.6
Linoleodisaturated (LSS)	1.4	2.8	0.7	3.3	2.2
Oleolinoleosaturated (OLS)	13.3	3.4	12.7	7.4	8.5
Saturated diolein (SOO)	-	-	-	1.1	1.2
Saturated dilinolein (SLL)	13.6	22.1	15.3	11.9	15.2
Triolein (OOO)	-	-	-	0.8	0.8
Oleodilinolein (OLL)	45.9	53.9	46.4	30.0	28.8
Linoleodiolein (LOO)	-	1.3	-	8.5	8.0
Trilinolein (LLL)	25.1	16.5	24.1	35.4	34.6
Class					
Trisaturated	-	-	-	0.6	0.1
Monounsaturated	2.1	2.8	1.5	4.3	2.8
Diunsaturated	26.9	25.5	28.0	20.4	24.9
Triunsaturated	70.6	71.7	70.5	74.7	72.2

vary in component glycerides and relative percentages of each. It was therefore necessary to devise some method to test the validity of these calculated structures. The following method is proposed.

In the previous section on fractional crystallization it was shown that when the iodine and thiocyanogen values of the fractions are plotted, a straight line results. Moreover, if the iodine and thiocyanogen values of the theoretically possible triglycerides are superimposed on the same figure, the straight line representing the fractions intercepts the vertical lines for each class of triglycerides. These intercepts must represent the proportions of the glycerides occurring in the oil that belong in each class. Furthermore, it was indicated that each fraction must be composed of triglycerides selected from at least two of the three classes. Accordingly, the glycerides of the calculated structure are grouped into three classes, monounsaturated, diunsaturated and triunsaturated, depending on the component acids. Each class will contain from one to four glycerides. The proportion of each glyceride in a class multiplied by the iodine and thiocyanogen values gives an iodine and thiocyanogen value for the class. If these values are plotted on the graph for the iodine and thiocyanogen values of the fractions, a point will be obtained which is representative of the calculated component glycerides in each class. Since the intercepts of the straight line representing the frac-

tions and the vertical line for the glyceride classes are determined by the relative proportions of the various glycerides in each class, it follows that the calculated values for each class must fall on the intercept, if a correct structure has been determined.

The computed iodine and thiocyanogen values for each class of glycerides are summarized in Table XVIII, together with iodine and thiocyanogen values for the intercepts shown in Fig. 11. The structures in Table XVI were tested by means of these data. Some of the structures are obviously incorrect. Division of oleic acid between saturated and linoleic acids shows monounsaturated glycerides to be absent. From Fig. 11, this is obviously incorrect since the point representing fraction A lies between the vertical lines for mono and diunsaturated glycerides.

Division of linoleic acid produced glycerides in each general class. However, reference to Table XVIII and Fig. 11 shows that the points representing the iodine and thiocyanogen values of monounsaturated and diunsaturated glycerides would fall above those for the intercepts. The point for triunsaturated glycerides would fall below the intercept. This means that some oleodisaturated glyceride must be present, and that the percentages of saturated dilinolein and oleodilinolein are too high.

Division of saturated acids gives a structure in which the points approximate the intercepts. However, the propor-

TABLE XVIII

Iodine and thiocyanogen values of glyceride classes in Table XVI, and of intercepts in Fig. 11.

Glyceride Class	Even Distribution: Dividing Oleic I.V.	Dividing Oleic T.V.	Dividing Linoleic I.V.	Dividing Linoleic T.V.	Dividing Saturated I.V.	Dividing Saturated T.V.	Random Distribution I.V.	Random Distribution T.V.	Intercept from Graph I.V.	Intercept from Graph T.V.
Monounsaturated	-	-	59.2	31.5	53.4	31.2	53.4	31.2	53.2	31.3
Diunsaturated	103.8	61.2	117.3	62.0	105.9	61.3	106.0	61.3	103.9	61.3
Triunsaturated	155.3	90.9	151.3	90.6	155.8	90.9	155.9	90.9	154.0	90.8

TABLE XIX

Iodine and thiocyanogen values of glyceride classes in Table XVII, and of intercepts in Fig. 12.

Glyceride Class	Even Distribution: Dividing Oleic I.V.	Dividing Oleic T.V.	Dividing Linolenic I.V.	Dividing Linolenic T.V.	Dividing Saturated I.V.	Dividing Saturated T.V.	Random Distribution I.V.	Random Distribution T.V.	Intercept from Graph I.V.	Intercept from Graph T.V.
Monounsaturated	49.4	31.1	59.2	31.5	43.8	30.9	52.5	30.9	50.2	31.2
Diunsaturated	103.4	61.2	114.8	61.9	104.7	61.2	104.3	61.2	101.8	61.2
Triunsaturated	154.4	90.8	150.3	90.5	154.1	90.8	154.0	90.8	153.2	90.7

tions of linoleo disaturated, saturated dilinolein and trilinolein are slightly high relative to oleo disaturated, saturated oleolinolein, and oleo dilinolein. If the component glycerides represented by this calculation are correct, and the percentage of each general class is correct, it is possible to calculate the amount of each which will give a point coinciding exactly with the intercept.

Using 1.0% monounsaturated, 30.0% diunsaturated and 68.9% triunsaturated, the following glyceride structure was computed:

Oleodisaturated	0.3%	Saturated dilinolein	14.1%
Linoleodisaturated	0.7%	Oleodilinolein	42.4%
Oleolinoleosaturated	15.9%	Trilinolein	26.5%

Hilditch states (45) that an error of two to three units percent is possible in a calculated glyceride structure. The most marked difference of the above "corrected" structure from that secured by dividing saturated acids is 3.4 units % in saturated dilinolein and oleolinoleo saturated glycerides. The remainder are well within the error suggested by Hilditch.

The data for random structures were tested in the same manner and were found to give points as close to the intercepts as those obtained for the "even" division of saturated acids. On this basis, the structure by random distribution fits the experimental data as well as the best even distribution. A comparison of the general classes for random distribution and division of saturated acids given in Table XVI

shows the random data to be similar in monounsaturated and triunsaturated glycerides and lower in diunsaturated glycerides. Examination of the individual triglycerides in the random data shows the presence of small quantities (1.5 and less) of trisaturated, triolein, and saturated diolein which are not given by "even" distribution. An appreciable quantity of linoleo diolein is also indicated for random distribution. With the exception of oleodilinolein and trilinolein the remaining glycerides are in the same order of magnitude as for "even" distribution. From these data it is concluded that the glyceride composition of sunflower oil does not differ markedly from that predicted on a random distribution of fatty acids.

It has been proposed that if the fatty acids of sunflower oil are "evenly" distributed, molecular rearrangement should effect a more random distribution. This change would be indicated by an increased quantity of simple triglycerides. Glyceride structures were calculated for the rearranged oil in the same manner as for the natural oil and are presented in Table XVII. The structures were each tested in the proposed manner.

Table XIX shows a summary of the iodine and thiocyanogen values for each class of calculated glycerides and for the intercepts obtained from Fig. 12. The "even" structure given by division of linoleic acid is incorrect since the points

deviate markedly from the intercepts. The structures obtained by division of oleic and division of saturated acids show a marked similarity for the diunsaturated and triunsaturated classes. However, the structure given by division of oleic acid gives a closer agreement with the intercept for the monounsaturated glycerides. Therefore this is considered the most nearly correct "even" structure. The structure obtained by random distribution gives an equally close agreement when tested by the proposed procedure. This indicates that the rearrangement was ineffective or that sunflower oil has a random structure.

It has been shown that molecular rearrangement effected a change in the component glycerides of a simple synthetic oil. Hence it is concluded that sunflower oil has a structure which approximates a random arrangement so closely that any deviations cannot be detected by present methods.

If the random structure in Table XV is compared with the corresponding structure in Table XVI, a very striking agreement is noted. Furthermore, from Tables XVI and XVII the random structures calculated from the fatty acid composition of the whole oil are seen to agree with the random structures calculated from the fractions. It is thus evident that fractionation of the oil provides no information on glyceride structure that can not be derived more simply from analyses of the whole oil.

The results of this study indicate that the conception of "even distribution" proposed by Hilditch contributes nothing of value for elucidation of the structure of an unsaturated oil such as that obtained from sunflower seed. The hypothesis was originally developed from work done on the more saturated solid seed fats and later extended to studies of peanut, cottonseed and other liquid seed fats. In a recent publication Jackson and Longenecker (62) from work on the solid seed fat of Babassu nuts conclude that the glyceride structure of this material can not be distinguished from that predicted on a random distribution of the fatty acids.

SUMMARY

A study of sunflower oil was undertaken to determine:

(1) The effect of varietal and environmental factors on the iodine value of the oil.

(2) The relation of iodine value to fatty acid composition of the oil.

(3) The relations of component acids determined from equations involving iodine values - thiocyanogen values and iodine values - unsaturated acids.

(4) The extent of glyceride separation that is possible by low temperature fractional crystallization of the oil.

(5) The glyceride structure of sunflower oil.

(6) The effect of molecular rearrangement on the glyceride structure of sunflower oil.

Seventy-two samples of sunflower seed comprising 12 varieties, grown at 6 different stations, were selected for the study. Chemical analyses for iodine values, thiocyanogen values, saturated acids and unsaponifiable matter were made by standard procedures on each sample.

The most reliable method to date of computing the amount of unsaturated C_{18} acids in an oil consists of set-

ting up simultaneous equations involving iodine values, thiocyanogen values and unsaturated acids. The following equations were secured from the most reliable data in the literature:

$$89.9\ x + 181.1\ y + 273.1\ z = 100\ \text{I.V.}$$

$$89.9\ x + 97.0\ y + 166.7\ z = 100\ \text{T.V.}$$

$$x + y + z = u$$

where, x = oleic acid, %; y = Linoleic acid, %; z = Linolenic acid, %; I.V. = iodine value; T.V. = thiocyanogen value; and, u = unsaturated acids, %.

The mean iodine value, thiocyanogen value and unsaturated acids for the 72 samples were substituted in the above equations. Solution of the equations for component acid percentages revealed 0.1% linolenic acid which was considered negligible in view of the analytical errors. The equations were therefore modified to include only the unsaturated acids, oleic and linoleic. This permitted computation of component acids by two sets of equations, one involving iodine and thiocyanogen values, and the other iodine values and unsaturated acids. This procedure permitted a check on the empirical thiocyanogen value of linoleic acid in the glycerides of the oil. To secure agreement between the component acid percentages as calculated by both methods, a value of 97.4 units must be used as the empirical thiocyanogen value of linoleic acid.

Statistical analyses of the data revealed significant differences in station and varietal means for the oil properties. Mennonite samples gave higher iodine values than Sunrise or Sunrise hybrids. The iodine values of the samples increased for the more northerly stations. Significant correlation coefficients were secured between iodine values and thiocyanogen values, oleic, linoleic and saturated acids. Prediction equations were computed for the calculation of oil properties from iodine values. No significant differences were found in component acid percentages calculated from iodine-thiocyanogen value equations and iodine value-unsaturated acid equations. Accordingly, the equations employing iodine and thiocyanogen values were used in the calculation of component acids in subsequent data on fractionation of sunflower oil.

Natural sunflower oil was fractionally crystallized by two procedures. It was found that a more efficient separation resulted from a preliminary separation of the oil into four fractions, followed by recrystallization of each fraction, than by a progressive fractionation from saturated to unsaturated fractions. A graph was constructed of iodine and thiocyanogen values of the fractions, and the points representing the fractions gave a linear relation. Points representing the possible theoretical triglycerides from the acids of sunflower oil gave a scattered relationship on

the same graph. The theoretical triglycerides were divided into three general classes, monounsaturated, diunsaturated and triunsaturated, on the basis of component acids. The nearly vertical lines joining each class of glycerides were intercepted by the line representing the fractions. The fractionation of sunflower oil resulted in a separation into fractions each composed of a mixture of glycerides. The proportions of glycerides in each fraction varied in a regular manner with the iodine value of the fraction.

Assuming "even" distribution of the fatty acids among the glycerol molecules, as proposed by Hilditch, molecular rearrangement of the oil should effect a more random distribution. This would be indicated by an increase in the percentage of trilinolein. A sample of sunflower oil was subjected to molecular rearrangement and fractionally crystallized. The iodine and thiocyanogen values of the fractions gave a linear relation, indicating that segregation into molecular species had not been achieved. In spite of a difference in the grouping of the points representing the fractions, the calculated glyceride structure was unchanged by the rearrangement procedure. No trilinolein was recovered as such in spite of the presence of 67.4% linoleic acid in the original oil. By a random distribution of the fatty acids 30.6% trilinolein should be present. Hilditch suggests that considerable proportions of simple glycerides are

present only when a single acid exceeds 60% of the total acids present.

To test the effectiveness of crystallization, trilinolein was mixed with sunflower oil and the mixture fractionally crystallized. Points representing the iodine and thiocyanogen values of the fractions showed a linear relation, and no trilinolein was recovered as such. The added trilinolein was distributed over all the fractions. A specific attempt to recover the trilinolein by recrystallization of the more unsaturated fractions was unsuccessful. A linear relation between iodine and thiocyanogen values of the fractions was again obtained. It appears that association of the unsaturated glycerides occurs and prevents segregation into molecular species.

A synthetic oil composed of simple triglycerides, triolein, trilinolein and tripalmitin was fractionally crystallized. A good recovery of tripalmitin was secured, but the remaining fractions contained triolein and trilinolein in proportions which varied directly with the iodine values of the fractions. Points representing the unsaturated fractions gave a linear relation similar to that noted for natural oil.

The effectiveness of the molecular rearrangement method was tested by treating a sample of the synthetic oil and fractionally crystallizing the product. The results

showed that the structure of the simple glycerides was altered with the production of mono- and diunsaturated glycerides, as would be expected in molecular rearrangement.

Glyceride structures were calculated for natural and rearranged sunflower oil on the basis of both "even" and "random" distributions of the fatty acids. The validity of each structure was tested by dividing the calculated component glycerides into monounsaturated, diunsaturated and triunsaturated classes. The most nearly correct structure was taken as that for which the iodine and thiocyanogen values of the glyceride classes gave points which coincided with the intercepts of the straight line representing the fractions and the nearly vertical lines representing the classes of glycerides.

Random distribution of the fatty acids yielded a structure which was equally satisfactory with the best structure calculated by "even" distribution. No obvious difference was detected between the glyceride structures of natural and rearranged sunflower oil, which is interpreted as evidence that the glyceride structure of sunflower oil closely approaches that predicted from a random distribution of the fatty acids.

This study of sunflower oil indicates that:

(1) Iodine value of the oil is affected by varietal and environmental factors.

(2) a close correlation between iodine value and oil composition exists. Linoleic acid increases with iodine value, whereas oleic and saturated acids decrease.

(3) The use of the value 97.4 as the empirical thiocyanogen value for linoleic acid yields reliable data on the component acids. In addition equations involving only iodine value, thiocyanogen value and unsaponifiable matter may be used to compute the component acids.

(4) Fractional crystallization does not segregate sunflower oil into molecular species of glycerides, but separates the oil into fractions in which the glycerides occur in regular proportions which vary directly with the iodine value of the fractions.

(5) The glyceride structure of sunflower oil closely approximates that calculated from a random distribution of the fatty acids. Furthermore, the random structure calculated from the component acids of the whole oil shows good agreement with the random structure calculated from the component acids of the fractionated oil.

(6) Molecular rearrangement effects no change in the

glyceride structure within the limits detectable by the methods employed in this study.

APPENDIX A

TABLE I

Description of Varieties

Sask. Hybrid No. 1 - (Sunrise x S-488)

Sask. Hybrid No. 2 - (S-37-$\overline{402}$ x S-37-$\overline{388}$)

Sask. Hybrid No. 3 - (Sunrise x S-37-$\overline{388}$)

Sask. Hybrid No. 4 - (S-37-$\overline{388}$ x S-37-$\overline{49}$)

Sask. Hybrid No. 5 - (Sunrise x S-37-$\overline{49}$)

Mennonite (Rosthern) - strain of Mennonite from Rosthern, Sask.

Mennonite (Manitoba) - strain of Mennonite from Manitoba.

Mennonite (Winkler) - strain of Mennonite from Winkler, Manitoba.

Sunrise - Sunrise variety

Scott Selection No. 1)
Scott Selection No. 2) - selections from Mennonite grown at Scott Experimental Station.
Scott Selection No. 3)

TABLE II

Iodine Values of Sunflower Samples

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	140.5	140.4	139.1	140.9	136.7	140.0
Sask. Hybrid No. 2	143.1	141.6	140.2	144.2	141.3	140.7
Sask. Hybrid No. 3	141.4	140.6	138.8	141.9	138.8	137.9
Sask. Hybrid No. 4	143.4	143.4	140.6	144.4	141.7	140.1
Sask. Hybrid No. 5	143.8	143.2	141.1	144.3	139.6	138.8
Mennonite (Rosthern)	141.6	141.7	138.7	143.6	139.1	139.9
Mennonite (Manitoba)	141.9	143.0	139.9	143.8	139.5	138.5
Mennonite (Winkler)	141.4	140.9	139.6	141.7	138.7	139.2
Sunrise	139.9	138.3	137.3	138.9	136.2	136.2
Scott Selection No. 1	142.1	142.4	138.4	142.5	138.9	139.7
Scott Selection No. 2	141.6	142.1	139.1	143.0	138.5	139.6
Scott Selection No. 3	142.1	142.3	140.1	142.9	138.9	139.5

TABLE III

Thiocyanogen Values of Sunflower Samples

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	83.3	81.8	82.8	83.4	81.1	80.9
Sask. Hybrid No. 2	83.1	83.3	83.1	83.4	83.5	82.5
Sask. Hybrid No. 3	82.9	82.0	82.8	82.8	82.5	81.3
Sask. Hybrid No. 4	83.6	83.4	83.2	84.1	82.8	82.1
Sask. Hybrid No. 5	83.8	83.1	83.3	84.0	82.4	81.5
Mennonite (Rosthern)	83.4	83.4	83.3	83.9	82.4	82.6
Mennonite (Manitoba)	83.6	83.4	83.6	84.3	82.6	81.4
Mennonite (Winkler)	83.5	83.1	83.8	83.5	82.2	82.5
Sunrise	82.7	80.5	82.6	81.6	80.6	80.4
Scott Selection No. 1	83.4	83.8	83.5	84.1	82.8	81.9
Scott Selection No. 2	83.6	83.6	83.6	84.3	82.6	83.1
Scott Selection No. 3	83.8	83.9	83.8	84.2	82.7	82.0

TABLE IV

Unsaponifiable Matter in Sunflower Samples

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	.62	.66	.56	.71	.62	.51
Sask. Hybrid No. 2	.67	.69	.54	.69	.59	.54
Sask. Hybrid No. 3	.53	.63	.53	.68	.56	.50
Sask. Hybrid No. 4	.60	.63	.55	.66	.48	.52
Sask. Hybrid No. 5	.69	.51	.57	.70	.53	.52
Mennonite (Rosthern)	.43	.55	.58	.61	.57	.50
Mennonite (Manitoba)	.45	.60	.59	.62	.63	.59
Mennonite (Winkler)	.42	.46	.47	.65	.63	.51
Sunrise	.68	.72	.60	.76	.70	.57
Scott Selection No. 1	.59	.47	.56	.63	.63	.59
Scott Selection No. 2	.39	.54	.50	.64	.56	.54
Scott Selection No. 3	.33	.50	.57	.57	.54	.54

TABLE V

Experimental Saturated Acids in Sunflower Samples

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	8.5	9.7	8.9	9.1	10.0	10.6
Sask. Hybrid No. 2	8.4	8.3	7.9	7.4	8.0	8.7
Sask. Hybrid No. 3	8.7	9.4	8.2	8.5	8.8	9.6
Sask. Hybrid No. 4	8.4	8.1	8.0	8.3	8.0	9.1
Sask. Hybrid No. 5	7.8	8.5	8.0	7.8	8.2	9.8
Mennonite (Rosthern)	7.8	8.4	8.0	7.6	8.5	8.8
Mennonite (Manitoba)	7.6	8.2	7.7	7.3	8.1	9.4
Mennonite (Winkler)	7.8	9.0	7.9	8.0	8.6	8.9
Sunrise	9.4	10.6	9.0	9.6	10.3	10.4
Scott Selection No. 1	8.4	8.1	8.0	7.2	8.2	9.1
Scott Selection No. 2	7.6	8.1	7.9	7.0	8.6	8.7
Scott Selection No. 3	7.7	8.0	7.9	7.2	8.6	9.1

TABLE VI

Calculated Saturated Acids in Sunflower Samples

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	7.9	9.6	8.5	9.7	10.2	9.9
Sask. Hybrid No. 2	8.3	8.0	8.3	7.1	7.8	9.0
Sask. Hybrid No. 3	8.7	9.6	8.4	8.5	8.6	10.2
Sask. Hybrid No. 4	7.8	8.0	8.1	7.4	8.7	9.5
Sask. Hybrid No. 5	7.5	8.6	8.0	7.4	9.0	10.1
Mennonite (Rosthern)	8.0	8.1	7.7	7.6	8.9	8.8
Mennonite (Manitoba)	7.8	8.0	7.4	7.1	8.7	10.1
Mennonite (Winkler)	7.9	8.3	7.4	7.8	9.0	8.9
Sunrise	9.5	10.9	8.5	9.8	10.7	11.1
Scott Selection No. 1	8.0	7.7	7.4	7.1	8.4	9.5
Scott Selection No. 2	8.0	7.9	7.5	7.1	8.6	8.2
Scott Selection No. 3	8.2	7.5	7.3	7.1	8.7	9.5

TABLE VII

Unsaturated Acids in Sunflower Samples

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	86.4	85.1	86.0	85.6	84.9	84.3
Sask. Hybrid No. 2	86.4	86.5	87.0	87.4	86.9	86.2
Sask. Hybrid No. 3	86.2	85.4	86.8	86.4	86.2	85.4
Sask. Hybrid No. 4	86.5	86.8	87.0	87.6	87.0	85.9
Sask. Hybrid No. 5	87.0	86.5	87.0	87.0	86.7	85.2
Mennonite (Rosthern)	87.3	86.5	86.9	87.3	86.5	86.2
Mennonite (Manitoba)	87.4	86.7	87.2	87.6	86.8	85.5
Mennonite (Winkler)	87.2	86.1	87.1	86.8	86.3	86.0
Sunrise	85.4	84.2	85.9	85.2	84.5	84.5
Scott Selection No. 1	86.5	86.9	86.9	87.7	86.7	85.8
Scott Selection No. 2	87.5	86.8	87.1	87.8	86.3	86.3
Scott Selection No. 3	87.5	87.0	87.0	87.7	86.4	85.9

TABLE VIII

Oleic Acid % = 2.4067 T.V. - 1.2944 I.V.

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	18.7	15.3	19.1	16.5	18.4	13.6
Sask. Hybrid No. 2	14.8	17.2	18.5	15.1	18.0	16.6
Sask. Hybrid No. 3	16.3	15.3	19.7	15.7	19.0	17.1
Sask. Hybrid No. 4	15.6	15.2	18.2	15.3	16.0	16.2
Sask. Hybrid No. 5	15.7	14.7	17.8	15.3	17.7	16.4
Mennonite (Rosthern)	17.6	17.1	21.0	15.9	18.3	17.8
Mennonite (Manitoba)	17.6	15.7	20.3	16.7	18.3	16.6
Mennonite (Winkler)	18.0	17.7	20.9	17.5	18.4	18.4
Sunrise	17.0	14.8	21.1	16.5	17.7	17.1
Scott Selection No. 1	16.8	17.3	21.8	18.1	19.4	16.3
Scott Selection No. 2	17.9	17.1	21.1	17.6	19.5	19.4
Scott Selection No. 3	17.7	17.7	20.3	17.7	19.1	16.8

TABLE IX

Oleic Acid % = 1.9857 u - 1.0964 I.V.

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	17.5	15.1	18.4	15.7	16.6	14.0
Sask. Hybrid No. 2	14.7	16.5	19.1	15.5	17.7	17.0
Sask. Hybrid No. 3	16.2	15.4	20.1	15.9	18.9	18.3
Sask. Hybrid No. 4	14.6	15.1	18.5	15.5	17.5	17.0
Sask. Hybrid No. 5	15.2	14.8	18.0	14.5	19.1	16.9
Mennonite (Rosthern)	18.1	16.4	20.6	16.0	19.2	17.9
Mennonite (Manitoba)	17.9	15.3	19.7	16.2	19.3	18.0
Mennonite (Winkler)	18.2	16.5	19.9	17.0	19.4	18.2
Sunrise	16.2	15.4	20.2	16.9	18.5	18.5
Scott Selection No. 1	16.0	16.5	20.9	17.9	19.9	17.2
Scott Selection No. 2	18.5	16.6	20.5	17.6	19.5	18.3
Scott Selection No. 3	17.9	16.7	19.3	17.5	19.2	17.6

TABLE X

Linoleic Acid % = 1.1947 (I.V.-S.C.N.)

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	68.3	69.9	67.3	68.6	66.3	71.5
Sask. Hybrid No. 2	71.7	69.6	68.2	72.6	69.1	69.4
Sask. Hybrid No. 3	70.0	70.0	66.9	70.6	67.3	67.7
Sask. Hybrid No. 4	71.5	71.7	68.6	72.1	70.3	69.3
Sask. Hybrid No. 5	71.6	71.7	69.1	72.1	68.3	68.5
Mennonite (Rosthern)	69.5	69.7	66.2	71.4	67.7	68.4
Mennonite (Manitoba)	69.6	71.2	67.2	71.1	67.9	68.2
Mennonite (Winkler)	69.2	69.0	66.7	69.5	67.5	67.7
Sunrise	68.3	69.1	65.3	68.4	66.4	66.7
Scott Selection No. 1	70.1	70.0	65.7	69.7	67.1	66.1
Scott Selection No. 2	69.3	70.0	66.4	70.2	66.8	67.4
Scott Selection No. 3	69.6	69.8	67.3	70.1	67.2	68.7

TABLE XI

Linoleic Acid % = 1.0964 I.V. - 0.9857 u

Varieties	Stations					
	Univ. of Sask.	Indian Head	Morden	Melfort	Swift Current	Lethbridge
Sask. Hybrid No. 1	68.9	70.0	67.7	70.0	68.3	70.3
Sask. Hybrid No. 2	71.7	70.0	67.9	71.9	69.2	69.2
Sask. Hybrid No. 3	70.1	70.0	66.6	71.5	67.3	67.1
Sask. Hybrid No. 4	72.0	71.7	68.5	72.1	69.5	69.9
Sask. Hybrid No. 5	71.8	71.7	69.0	72.5	67.6	68.2
Mennonite (Rosthern)	69.2	70.1	66.3	71.3	67.3	68.4
Mennonite (Manitoba)	69.5	71.4	67.5	71.4	67.5	67.6
Mennonite (Winkler)	69.0	69.5	67.2	69.8	66.9	67.8
Sunrise	69.2	68.7	65.8	69.8	66.1	66.0
Scott Selection No. 1	70.5	70.4	66.1	68.3	66.8	68.6
Scott Selection No. 2	69.0	70.2	66.6	70.2	66.8	68.0
Scott Selection No. 3	69.6	70.2	67.8	70.2	67.4	68.3

TABLE XII

Analyses of Variance for Oil Properties: Mean Squares

Oil Property	Variance due to Varieties	Stations	Interaction
Iodine Value	8.5295**[1]	31.5501**	.4957
Thiocyanogen Values	2.54485**	4.81967**	.18442
Exp. Saturated Acids %	2.2567**	3.55189**	.10474
Calc. Saturated Acids %	2.5780**	3.59781**	.22787
Unsaponifiable Matter %	.01284**	.02687**	.00379
Unsaturated Acids %	2.52449**	3.29389**	.11965
Oleic Acid % (I.V.-T.V.)	4.63904**	24.1859**	.75771
Oleic Acid % (I.V.-u)	3.86545**	25.21100**	.61936
Linoleic Acid % (I.V.-T.V.)	5.12949**	24.5535**	.51998
Linoleic Acid % (I.V.-u)	4.41367**	27.0489**	.50879
Degrees of Freedom	11	5	55

[1] Denotes that the 1% level of significance has been attained.

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