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A rapid method for stereospecific glyceride analysis and its application to soybean and oat varieties

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**A RAPID METHOD FOR STEREOSPECIFIC GLYCERIDE ANALYSIS AND
ITS APPLICATION TO SOYBEAN AND OAT VARIETIES**

Iowa State University

PH.D. 1981

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**A rapid method for stereospecific glyceride analysis and
its application to soybean and
oat varieties**

by

Wenchi Peter Pan

**A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY**

Major: Food Technology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Signature was redacted for privacy.

For the Graduate College

**Iowa State University
Ames, Iowa**

1981

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INTRODUCTION

The glyceride structure and fatty acid composition may affect the physical properties and performance of fats and oils. The specific location of fatty acids within triglyceride molecules is of particular importance. This influences the frequency of combinations and melting properties (2). Raghuveer and Hammond (49) have shown that glyceride structure affects the rate of autoxidation of fats and oils. Recent studies (40, 43) have suggested that the glyceride structure of different peanut oils might determine their tendency to cause arterial disease when fed to rats.

Only a limited number of soybean varieties (18) and no oat varieties have been analyzed for glyceride structure by either lipase hydrolysis or stereospecific analysis. Previous methods of lipase hydrolysis or stereospecific analysis were very lengthy and tedious and required three to five days to complete. In this study, a rapid method was developed to determine the variation of glyceride structure. This method was applied to a large number of soybean and oat varieties as well as a palm oil sample.

LITERATURE REVIEW

Importance of Glyceride Structure

Importance of glyceride structure to autoxidation

The glyceride structure of fats and oils may affect their rates of autoxidation. The specific location of a fatty acid on the triglyceride molecule particularly may affect the susceptibility to oxidation (52).

Raghuveer and Hammond (49) found that the rate of autoxidation of mixtures of 1.5-2.0% of trilinolin and trilinolenin in tridecanoin decreased after randomization. This was thought to be caused by a more complete dispersal of the unsaturated fatty acids in the mixture. There was a decrease in stability on randomization for most fats. To account for this Raghuveer and Hammond (49) proposed a hypothesis based on the hexagonal packing of glyceride acyl chains in the molten state. They suggested that the acyl chains at sn-1- and sn-3-positions should oxidize faster than those at sn-2-position. Thus, the placement of the unsaturated fatty acids on the sn-2-position of the triglyceride molecule should stabilize a fat toward autoxidation. Zalewski and Gaddis (60) found that lard oxidized more quickly after randomization, but they disagreed with the explanation of Raghuveer and Hammond. They claimed that the change in stability of lard was

caused by loss of antioxidant during randomization in open vessels at 60 C. However, Raghuv eer and Hammond had done their randomization under vacuum.

Hoffmann et al. (26) studied the oxidative stability of synthetic triglycerides containing palmitate, stearate, oleate and linoleate and found that the stability of various triglycerides appeared not to be determined solely by the total unsaturation. Triglycerides with the same fatty acids on the sn-1- and sn-3-positions were reported to be more stable against oxidation than the 1,2-position isomers. The reason is unknown. They further pointed out that palm oil was more stable than lard. When both lipids were randomized under a nitrogen atmosphere, the oxidative stability of palm oil decreased even below that of the unrandomized lard, whereas the stability of randomized lard had scarcely changed. They hypothesized the cause might be due to the presence of 1-oleo-2,3-distearin. Randomization of palm oil causes a significant increase (7 to 20%) in this triglyceride, whereas randomization of lard causes only a slight change in this particular triglyceride. Hammond¹ synthesized 1-oleo-2,3-distearin and 1,3-distearo-2-olein and reported that the former molecule oxidized 2.5 times faster than the latter.

¹Hammond, E. G., Department of Food Technology, Iowa State University, Ames, Iowa, 1968.

Catalano et al. (9) carried out autoxidation tests on mono-unsaturated triglycerides isolated from the natural fats of pig, palm, cocoa butter and the interesterified fats of pig and palm. They noted that a greater oxidation resistance of the samples composed predominantly of triglycerides of 1,3-distearo-2-olein as compared with those containing a high percentage of 1-oleo-2,3-distearin. They found this difference disappeared when the triglycerides were randomized.

Fatemi and Hammond (19) reported that the proportions of oleate, linoleate, and linolenate hydroperoxides formed during the oxidation of soybean and olive oils were similar before and after randomization. They suggested that glyceride structure did not greatly affect the peroxide types in the autoxidation of natural oils.

Lau (37) studied the effect of randomization on the oxidation products of corn oil. She found that randomized corn oil oxidized 3 to 4 times faster than natural oil, whereas corn oil methyl esters oxidized approximately as fast as randomized oil. She suggested that the natural glyceride structure of corn oil might affect its oxidation rate.

Importance of glyceride structure on nutrition

Extensive dietary trials have suggested that the triglyceride structure of a fat, as well as its fatty acid composition, determine its atherogenic potential (35).

Myher et al. (43), using chromatography and stereospecific analysis techniques, investigated the triglyceride structure of native, simulated, and interesterified peanut oils, which had previously been reported to have a great difference in their atherogenic potential. They found that the native oil, which contained a greater proportion of triglycerides with linoleic acid in sn-2-position and arachidic, behenic, and lignoceric acids in sn-3-position, were more atherogenic than interesterified oils. They suggested that the atherogenicity might arise from a relative metabolic unavailability of linoleic acid from the native peanut oil caused by the long chain saturated fatty acids concentrated on the sn-3-position. During digestion, pancreatic lipase acts slowly on such long chain fatty glyceride esters.

Sanders (53) stereospecifically analyzed 6 peanut varieties and found that the distribution of the fatty acids among the sn-1-, sn-2-, and sn-3-positions were nonrandom. The percentages of palmitic and stearic acids were very small at the sn-2-position and were greater at the sn-1- than the sn-3-position. Long chain fatty

acids were preferred at the sn-3-position. The sn-2-position of all varieties was high in unsaturated fatty acids. Van Pee et al. (55) used stereospecific analysis to determine the glyceride structure of eight African peanut varieties. Confirming the results of Sanders (53), they found that arachidic acid occurred almost exclusively at the sn-1- and sn-3-positions. Linoleic acid was preferred at the sn-2-position, whereas oleic acid was equally distributed among the three positions. They also concluded that the amounts of palmitic, stearic, oleic and linoleic acids in the sn-1-, sn-2-, and sn-3-positions, were linearly related to their respective content in the total diglycerides. Hokes and Worthington (27) reported the pancreatic lipase hydrolysis results of 8 peanut varieties. They found that the ratios of linoleic acid vs. oleic acid ranged from 0.2 to 1.1 for triglycerides, 0.2 to 2.6 for sn-2-monoglycerides. These analyses (27, 53, 55), however, involved only positional analyses of the fatty acids without identifying any molecular species. Recently, Manganaro et al. (40) made a detailed investigation of the glyceride structure of three varieties of peanut oils of varying atherogenic activity. On the basis of their analysis of the molecular species of the sn-1,2-, sn-2,3- and sn-1,3-diglycerides, they concluded that the fatty acids of the glycerol molecule were combined with each other

based on their relative molar concentrations at each of the three positions, the so-called 1-random-2-random-3-random hypothesis of glyceride structure. The differences in the composition of the molecular species of the triglycerides among the three peanut oils were related to their atherogenic activity. The South American oil, which possessed the greatest relative proportion of the total linoleic acid in either the sn-2-position of the triglyceride or in combinations with long chain saturated acids, were considered to possess the greatest atherogenic potential and this was borne out by biological testing.

The actual mechanism by which a dietary fat exerts its atherogenic potential remains only partially understood. However, the identification of specific atherogenic triglycerides should provide useful insights into the potential atherogenicity of natural fats and lead to practical methods for the elimination or suppression of this undesirable property.

Several studies pointed out erucic acid as the toxic factor responsible for the cardiopathogenicity of rapeseed oils (1, 3, 4). Kramer et al. (34) reported that rapeseed oils which were low in erucic acid caused myocardial lesions when fed to weanling rats. They found that the cardiopathogenicity appeared to be associated with the triglyceride structure of the oil, and not to non-glyceride

components present in fully-refined rapeseed oils. Ohlson et al. (46) conducted the stereospecific analysis of some cruciferae species and found that the saturated acids and acids with more than eighteen carbon atoms preferred either of the outer positions of glycerol. Erucic acid had a clear preference for the sn-3-position. When in the outer positions, unsaturated fatty acids with eighteen carbon atoms preferred the sn-1- to the sn-3- position. Hulan et al. (29) performed feeding tests of rapeseed oils with rats and found that the myocardial lesions associated with feeding 20% rapeseed oil diets were not related to the content of erucic acid per se. They suggested that an asymmetric distribution of linolenic acid in the triglyceride (8), and a triglyceride imbalance in the oil might play a role in causing these lesions in rats.

Importance of glyceride structure on texture

Glyceride structure has a great effect on the physical properties of a fat, especially its texture. The melting characteristics of a fat reflect the properties of its constituent fatty acids, but their arrangement into glycerides can also affect the melting behavior (2). For example, consider a fat which is about 66% palmitic acid + stearic acid and 33% oleic acid. In the case of cocoa butter, which has a fatty acid composition

approximating this, these acids are arranged with one oleic acid in the sn-2-position of each triglyceride (38). The fat is essentially all saturated-oleic-saturated. This gives it a very short melting range around 37 C. If the saturated fatty acids were collected into trisaturated glycerides, they would comprise 66% of the fat. The result would be a very hard fat melting at a much higher temperature and over a longer range (2).

Alteration of Oil Composition by Breeding

Rapeseed oil is characterized by the presence of large amounts of erucic acid and smaller amounts of eicosenoic acid. Rapeseed oil of the variety Brassica campestris may have over 40% of erucic acid, whereas Brassica napus oil usually has 25% erucic acid (12). Nutritional research in Europe and Canada (1, 4, 51) prompted attempts to change rapeseed oil composition to those with low levels of erucic acid. A plant breeding and selection program led to the introduction of Oro, which has almost no detectable erucic acid.

Dutton et al. (15) carried out organoleptic studies on stored soybean oil, stored cottonseed oil, and a cottonseed oil into whose glyceride structure linolenic acid had been introduced with an interesterification

catalyst. They concluded that linolenic acid is responsible for the "fishy-painty-grassy-melony" flavors in oxidized soybean oil. This led to attempts to breed soybean varieties with low linolenic acid content.

The linolenic acid in commercial soybean oil usually ranges between 7 and 8%. Experience with hydrogenated soybean oil suggests that the flavor stability is markedly improved by reducing the linolenic acid to 3%. It would be even more desirable if the linolenic acid content could be reduced to less than 1% (10, 47).

Hammond and Fehr (24) used plant breeding to reduce the linolenic acid content of soybean oil and improve oil stability. By crossing strains with the lowest linolenic acid content available, they found it was possible to produce offspring with linolenic acid content 1 to 1.5% lower than the best parental strain. Using mutagenic treatments, such as x-rays and ethyl methanesulfonate (EMS), they attempted to introduce more variability for fatty acid composition into the population. So far the lowest linolenic acid content Hammond and Fehr¹ have obtained is 3.7%. This has been accompanied by major

¹Hammond, E. G. and W. R. Fehr, Department of Food Technology and Agronomy, Iowa State University, Ames, Iowa, 1981.

shifts in linoleic acid from 53 to 38% and oleic acid from 25 to 44%.

Wilson et al. (59) attempted to improve soybean oil by altering fatty acid composition through plant breeding. Their results showed that the linolenic acid concentration in soybean oil was reduced by selection for high levels of oleic acid. They also found that the glyceride structure types were changed and the combined level of triolein, monooleyl-dilinolein, and dioleyl-monolinolein in seed from one of the selected lines was doubled compared with the levels in the highest parental cultivar.

Rapeseed oil suffers from a high content of linolenic acid as well as erucic acid. Attempts to reduce the linolenic acid content of rapeseed oil from its usual values of 10% by mutation breeding have resulted in varieties as low as 3.2% (50).

Safflower oil with a high oleic acid (78.8% as opposed to the usual value of 13.9%) was discovered in one plant introduction (32, 58). This characteristic can be bred into other varieties (33).

Frey and Hammond (21) explored the economic feasibility of oats as an oil crop in Iowa. Based on the survey of 445 oat cultivars, they found oil percentages ranging from 2.0 to 11.0%. The fatty acid composition of 64 cultivars gave the following variation: palmitic acid, 14 to 23%;

stearic acid, <1 to 4%; oleic acid, 29 to 53%; linoleic acid, 24 to 48%; linolenic acid, <1 to 5%. They also concluded that the oil percentage was positively correlated with oleic acid and negatively correlated with linoleic and linolenic acids. Most of their samples had less than 2% linolenic acid and this might contribute to the flavor stability of oat oil. Kalbasi-Ashtari and Hammond (31) compared the stability of refined oat oil with soybean oil at 25 and 55 C by peroxide values and organoleptic tests. They found that the stability of oat oil was increased by the addition of citric acid and was significantly greater than that of soybean oil, especially at 25 C.

De la Roche et al. (11a) examined 9 strains of common oats (Avena sativa) and concluded that high-lipid strains contained a greater proportion of triglyceride and a lower proportion of phospholipid. They also found the oil content correlated positively with oleic acid percentage and suggested the oleic acid content might be used as an indicator to select oats with higher oil content, and at the same time selection could be made to keep linolenic acid at less than 2%.

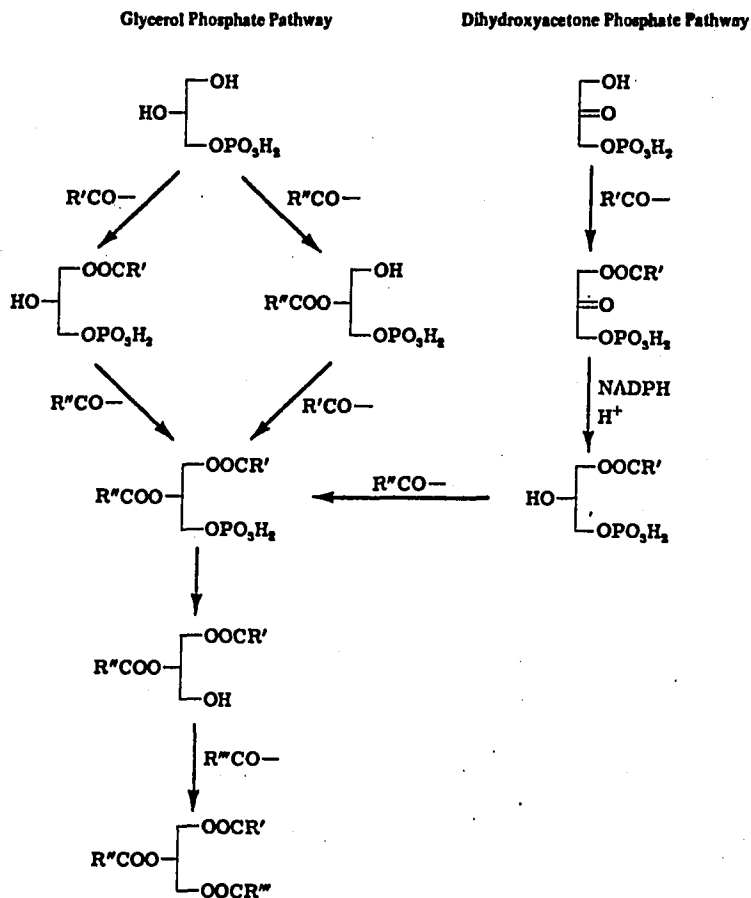
Glyceride Biosynthesis and Theories of Glyceride Distribution

Glyceride biosynthesis

Three routes for glyceride biosynthesis have been defined.

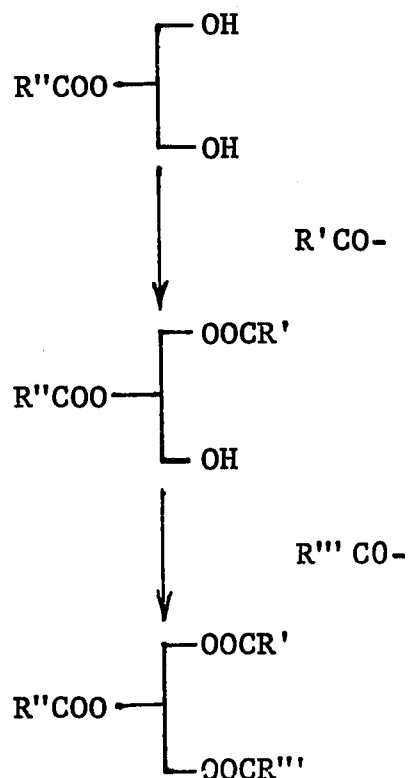
(a) Glycerol phosphate and dihydroxyacetone phosphate pathways for the de novo biosynthesis of triglycerides.

These two pathways are diagrammed as follows:



(b) Monoglyceride pathway.

Monoglyceride pathway



This is chiefly a mechanism for rebuilding glycerides which have been partially hydrolyzed by digestive lipases. However, it was shown (41, 42) that in fat-producing plant tissues triglyceride biosynthesis occurred via both the glycerol phosphate pathway and monoglyceride pathway.

Theories of glyceride distribution

Several hypotheses have been proposed to account for the observed distribution of fatty acids in triglycerides

(38). Some of these theories include mathematical predictions of the particular triglyceride distributions that occur. These theories always assume random combinations occur except for the specific restraints imposed by the specific theory.

At present, the most widely accepted theory is the 1-random-2-random-3-random theory proposed by Tsuda in 1962, as cited by Litchfield (38). In this theory, it was assumed that three different pools of fatty acids were separately distributed to the sn-1-, sn-2-, and sn-3-positions of all glycerol molecules in a fat. Within its respective position, each pool of acids was distributed at random. The following general equation can be used to calculate the amount of each component triglyceride:

$$\% \text{ sn-XYZ} = \left[\begin{array}{c} \text{mole \%X at} \\ \text{sn-1-position} \end{array} \right] \left[\begin{array}{c} \text{mole \%Y at} \\ \text{sn-2-position} \end{array} \right] \left[\begin{array}{c} \text{mole \% Z at} \\ \text{sn-3-position} \end{array} \right] (10^{-4})$$

Attempts have been made to predict the distribution of particular acyl groups at various positions. Based on lipolysis results, Gunstone et al. (23) indicated that among the eighteen-carboned unsaturated acids, oleic and linolenic acids were more likely to be found in the sn-1-, and sn-3-positions, while linoleic acid was more frequently found in the sn-2-position. This was taken into account by

Evans et al. (16) who proposed three rules to calculate the glyceride composition of a typical vegetable oil.

- (a) Saturated acids and those with chain lengths greater than 18 carbons are first distributed equally at the 1,3-positions.
- (b) Oleic and linolenic acids are then distributed equally and randomly on the unfilled 1-, 2-, and 3-positions, with excess from the 1- and 3-positions being added to the 2-position.
- (c) All remaining positions are filled by linoleic acid.

Measuring Glyceride Structure

When a glycerol molecule is esterified with a mixture of n different fatty acids, the number of possible triglycerides that can be formed will be n^3 . For instance, the triglyceride mixtures found in most plant seeds contain only five to ten different fatty acids, which can generate 125 to 1,000 possible molecular species of triglycerides. This complexity, together with the very similar chemical and physical properties of the various molecules, makes the complete analysis of natural triglycerides extremely difficult.

Complete positional analysis of triglycerides became possible when Brockerhoff (5, 7) developed the procedures

of stereospecific analysis. In the first method, referred to as the sn-1,3-diglyceride method, deacylation of triglycerides was carried out with Grignard reagent (ethyl magnesium bromide). The sn-1,2(2,3)-diglycerides were separated from the sn-1,3-diglycerides by preparative thin layer chromatography (TLC). The sn-1,3-diglycerides were converted to sn-1,3-diacyl-2-phosphatidylphenol using phenyl dichlorophosphate. Subsequent treatment with phospholipase A₂ would specifically hydrolyze the sn-1-acyl group from an sn-2-phosphatide, leaving a lysophosphatide containing the sn-3-acyl chain. In the second method, referred to as the sn-1,2(2,3)-diglyceride method, triglycerides were incubated with pancreatic lipase to give sn-2-monoglycerides. Simultaneously, the triglycerides were deacylated to representative sn-1,2(2,3)-diglycerides, which could be isolated by preparative TLC. Those sn-1,2(2,3)-diglycerides were reacted with phenyl dichlorophosphate to produce a mixture of sn-1,2-diacyl-3-phosphatidylphenol and sn-2,3-diacyl-1-phosphatidylphenol. Subsequent treatment with phospholipase A₂ liberated the fatty acids from the 2-position of the sn-3-phosphatide but left the sn-1-phosphatide unhydrolyzed. Separation and fatty acid analysis of the various reaction products allowed the determination of the glyceride structure.

Lands et al. (36) used a different approach to the glyceride structure analysis, based on the stereospecificity of diglyceride kinase. The initial step was the preparation of sn-1,2(2,3)-diglycerides by pancreatic lipase and then incubation of the diglycerides with diglyceride kinase prepared from Escherichia coli. This converted the sn-1,2-diglyceride to sn-1,2-diacyl-3-phosphatide while the sn-2,3-diglycerides remained unphosphorylated. Subsequent separation by TLC and gas liquid chromatography (GLC) analysis of the methyl esters of the sn-2-monoglyceride and the sn-1,2-diacyl-3-phosphatide determined the glyceride structure of a lipid.

The stereospecific analysis of maize oil triglycerides from different strains by De la Roche et al. (11b) and Weber et al. (57) indicated significant differences in the fatty acid distribution at the 3 positions in some strains and they concluded that both concentration of fatty acids in the total triglyceride and the positional specificity might account for the difference in glyceride structure. Subsequent studies by Weber and Alexander (56) showed that the oil content, fatty acid composition, and fatty acid placement within the triglyceride could be subject to genetic modification in corn.

Fatemi and Hammond (18) applied Brokerhoff's second method to stereospecifically analyze 17 different varieties

of soybean samples and indicated that there was little palmitic or stearic acids on the sn-2-position, and the sn-1-position was consistently richer in palmitic, stearic, and linolenic acids than the sn-3-position. They also found that the sn-3-position was enriched in oleic acid and the sn-2-position with linoleic acid. Plotting the percentage of fatty acids on the glycerol positions versus the percentage in the whole oil gave straight lines. They found one soybean variety that had a deviant glyceride distribution and considered it might be under genetic control.

Both Brockerhoff's and Lands' methods require 2 to 3 days to perform. A method which can be completed within 8 hr is necessary if the dozens of stereospecific analyses required for a complete analysis of molecular species are to be performed on a routine basis.

Recently, Dutta et al. (14) presented an on-plate lipase hydrolysis method, in which the enzymatic reaction, extraction of the reaction products and their resolution were performed on the same TLC plate without solubilizer or calcium chloride. This method considerably shortened the required time and minimized the loss of products due to transfer. Meanwhile, Dutta and Das (13) published a method on the phospholipase A₂ hydrolysis of phosphatidylcholine and the separation of the products on a single

plate. With certain appropriate modifications, a procedure, which requires smaller sample size and less time (ca. 8 hr) to perform, has been developed to stereospecifically analyze the glyceride structure of a number of strains of soybeans, oats and a sample of palm oil.

MATERIALS AND METHODS

Materials

Soybeans

One-hundred-forty-seven soybean samples were obtained from the Department of Agronomy, Iowa State University. These included 47 samples of Glycine soya, 40 samples of Steel EMS (ethyl methanesulfonate)-treated, 12 samples of PI (plant introduction) 68-788, 12 samples of Amsoy and 36 samples of PI68-788 and Amsoy crosses.

The mutagen EMS treatment procedure for Steel soybeans was as follows:

- (1) Beans were soaked for 16 hr in distilled water with bubbled air at room temperature.
- (2) EMS solution was prepared just prior to treating the beans. The EMS was dissolved in a 0.1 M phosphate buffer (pH 7.0) and adjusted to 0.025 M.
- (3) Presoaked beans were treated for 16 hr in an ample amount of 0.025 M EMS solution (i.e., approximately 2 X volume of soaked beans).
- (4) Treated beans were washed thoroughly in running tap water, blotted, and planted wet. As soon as planting was completed, the field was irrigated to prevent seed desiccation.

The Steel was treated with EMS in May, 1976. It was grown one generation in Ames, Iowa, two generations in Puerto Rico, and grown in Ames again in 1977. Individual plants were harvested.

For the crosses, PI68-788 was crossed with Amsoy in Ames, Iowa, 1977. The seeds were grown once in Puerto Rico and advanced to another generation in Ames in 1978. Individual plants were harvested.

Forty-eight soybean samples of Glycine max were obtained from U.S. Regional Soybean Laboratory, Urbana, Illinois.

Oats

One-hundred-three oat samples were obtained from the Department of Agronomy, Iowa State University. These included 60 samples of common oats, Avena sativa, and 43 samples of wild oats, Avena sterilis.

Palm

One sample of palm fruit oil and one sample of palm kernel oil extracted from pejebaye fruit, Bactris gasipaes, were supplied by Dr. George Mora, University of Costa Rica, San Jose, Costa Rica.

Solvents

Except for hexane and diethyl ether, which were dried and distilled before use, the rest of solvents were of analytical-reagent grade and were used directly.

Reference lipids

1,2-diolein concentrate and GLC reference standard #64 were obtained from Nu-Chek-Prep, Inc. (Elysian, MN). Phosphatidic acid (PA), phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) were purchased from Sigma Chemical Co. (St. Louis, MO).

Escherichia coli B

A culture was obtained from Dr. A. Atherly, Department of Genetics, Iowa State University. The culture was maintained on agar slants of trypticase soy media. The rate of growth of the bacteria in liquid cultures was determined by measuring turbidity at 675 nm (48).

Reagents

Lipase solution Seventy milligrams of porcine pancreatic lipase (EC 3.1.1.3) from Sigma Chemical Co., was dissolved in 0.4 ml of 1 M tris buffer, pH 8.2. The mixture was centrifuged at 167 x g for 10 min and the supernatant was used (14).

Phospholipase A₂ (EC 3.1.1.4) Lyophilized snake venom Ophiophagus hannah (King Cobra) was purchased from Sigma Chemical Co. Two and one-half milligrams of venom were dissolved in 0.25 ml of 0.005 M calcium chloride solution (containing 0.5 M tris, and adjusted to pH 7.5).

The venom was dissolved completely in the calcium chloride solution and was used immediately.

Plate preparation

Glass plates (20 cm x 20 cm) were coated with 0.5-mm layers of Silica gel G (Analabs, Inc., North Haven, CT), dried in a cool place, activated at 110°C for 1 hr and stored in a desiccator.

Methods

Lipid extraction

For each sample, 5 gm of soybeans or 2 gm of oats were pressed in a hydraulic press (Pasadena Hydraulic Inc., CA) in plastic cups fitted with stainless steel pistons. The lipids were extracted from the crushed seeds with 12 ml hexane for 6 hr, the hexane was decanted and the meal was washed twice with 8 ml of hexane. The solvent was evaporated under a stream of nitrogen.

Triglyceride isolation

More than 95% of the soybean oils are triglycerides (58), so they were used directly as triglyceride sources. The triglyceride content of oat oils ranges from 61.5% to 79.5% according to Kalbasi-Ashtari and Hammond (31), so oat oils required isolation of the triglycerides from other lipids.

About 35 mg of oat oil in a 1:1 mixture with hexane was applied to a Silica gel G plate with a sample streaker (Applied Science Inc., State College, PA). The plate was developed in hexane-diethyl ether-acetic acid (50:50:0.5, V/V/V). After spraying the plate with 2',7'-dichlorofluorescein in 95% alcohol (0.2% W/V), the triglyceride band was visualized under UV light. The triglyceride band was scraped off the plate and extracted with 20 ml ether containing 10% methanol. The extract was separated from the silica gel by filtration through a fritted glass filter using a vacuum. The solvent was evaporated under a stream of nitrogen. For the determination of fatty acid composition, a portion of soybean oil, palm oil, or oat triglyceride was converted to methyl esters by transesterification in 1 N methanolic sodium methoxide (2.2 gm sodium in 100 ml methanol) for 1 hr at room temperature. The fatty acid composition of the methyl esters was determined by GLC. A Beckman GC5 gas chromatograph equipped with a flame ionization detector (FID) was used under the following conditions:

Column -- 1.8 m x 3.3 mm

Packing -- 15% EGSS-X on 100/120 mesh Chromatosorb
P (Applied Science, State College, PA).

Nitrogen flow rate -- 50 ml/min

Hydrogen flow rate -- 40 ml/min

Air flow rate -- 300 ml/min

Column temperature -- 180 C (for the palm kernel oil methyl esters, the temperature was programmed from 50 to 185 C).

Peak areas were measured with a computerized electronic voltmeter (Commodore PET 2001 series Professional Computer, Commodore Business Machines, Santa Clara, CA). The accuracy of the GLC analysis was established with a standard mixture of methyl esters. When the peak areas deviated more than 0.5% from the correct values, correction factors were applied to the areas.

Lipase hydrolysis

The procedures described by Dutta et al. (14) were adopted with certain modifications.

One quarter milliliter of lipase solution containing ca. 25 mg porcine pancreatic lipase was applied as a band on a Silica gel G plate. The triglyceride sample (around 25 mg) in hexane (1:1 V/V) was overlaid as evenly as possible on the enzyme band. The plate was placed immediately on top of a hot plate (temperature stabilized at 40 C). After 3 min, the reaction was stopped by exposing the plate to hydrogen chloride vapor for 1 min in a closed TLC chamber containing some concentrated hydrochloric acid in three 50-ml beakers. The acid fumes were removed from the

plate by placing it under a stream of air for 1 min. The products of lipolysis and the unreacted triglycerides were removed from the reaction zone by developing the plate 3 times with diethyl ether in a saturated chamber up to 2 cm from the line of application. The ether was removed from the plate by a stream of air. The plate was then developed to the top with hexane-diethyl ether-acetic acid (50:50:0.5 V/V/V) in a saturated chamber. After removal of the solvents from the chromatogram by a stream of air, the different bands were identified by spraying with 2',7'-dichlorofluorescein. The R_f values for 2-monoglyceride and 1,2(2,3)-diglyceride were 0.12 and 0.27 respectively. These 2 bands were scraped off the plate and eluted from the silica gel with diethyl ether. The 2-monoglyceride was transesterified to methyl esters as described previously and the fatty acid composition was determined by GLC.

Preparation of diglyceride kinase

Escherichia coli B cells were grown to the middle of the logarithmic phase in 0.2% glucose and a mixture of inorganic ions as described by Garen and Levinthal (22). For a large-scaled cell preparation, a 10-L Microferm fermentor (New Brunswick Scientific Co., Inc., New Brunswick, N.J.) was used. The harvested bacteria were washed and disrupted in a solution containing 0.1% cysteine

hydrochloride and 0.01 M dibasic sodium phosphate (final pH 7.0) according to Pieringer and Kunnes (48). A French press (courtesy of Dr. F. Williams, Department of Bacteriology, Iowa State University) was employed to break the bacteria. The crude homogenate was centrifuged at 30,000 x g at 4 C for 30 min in a Sorvall RC2-B refrigerated centrifuge (Ivan Sorvall Inc., Newtown, Conn.). The pellet was washed by resuspension in cysteine-phosphate followed by centrifugation again at 30,000 x g for 30 min. To partially purify this particulate-bound enzyme, this pellet was resuspended in 20 ml of cysteine-phosphate and lyophilized for 15 hr according to Horvath and Pieringer (28). The lyophilized material was mixed for 10 min at room temperature with a 1% aqueous solution of Triton-X-100 (Supelco, Inc., Bellefonte, PA), in a ratio of 1 ml of solution to 10 mg of lyophilized preparation. This mixture was centrifuged at 30,000 x g for 30 min at 4 C. The pellet from this centrifugation was resuspended in 1% Triton-X-100 in a volume equal to the volume of the suspension before centrifuging. The supernatant as well as the pellet was tested for diglyceride kinase activity.

Modified Lands' method

The deacylation of triglyceride samples was done by lipase hydrolysis as before except that the lipids

were visualized by spraying with 1% iodine in methanol. It was said that dichlorofluorescein might be carried along with the diglyceride and inhibit the diglyceride kinase activity (36).

In addition to the original Lands' method (36), a simplified on-plate procedure was attempted.

The solvent was evaporated from the 1,2(2,3)-diglyceride band, and a mixture of the following reagents was applied over the diglyceride band as evenly as possible: 10 μ l of 20% (W/V) of mixed bile salts (Difco Lab., Detroit, MI); 0.10 ml of 0.05 M ATP (Sigma Chemical Co., St. Louis, MO); 0.05 ml of 1.0 M $MgCl_2$; 0.05 ml of 0.50 M sodium phosphate buffer, pH 7.95; 0.10 ml of diglyceride kinase preparation, ca. 10 mg/ml. After clasping the reaction zone with a piece of glass to prevent its drying out, the plate was incubated in a moisture-saturated oven at 37 C. At the end of various times (1, 1.5, and 2 hr), the reaction was stopped by exposing the plate to hydrogen chloride vapor. After removal of the acid fumes, the plate was developed 3 times with chloroform-methanol (2:1 V/V) in a saturated chamber up to 1 cm from the line of application. The solvents were removed from the plate by a stream of air and the plate was then developed to 14 cm with chloroform-methanol-water (65:36:8 V/V/V). The bands were visualized with 2',7'-dichlorofluorescein. The phosphatidic acid

band was scraped off and eluted with 10% methanol in ethanol. After evaporation of the solvent, the phosphatidic acid was transesterified to methyl esters as described previously, and the fatty acid composition was determined by GLC.

Stereospecific analysis by phospholipase A₂ on a single plate

The procedure of Dutta and Das (13) was adopted with some modifications.

Phosphorylation of diglycerides To synthesize the substrate of phospholipase A₂--phosphatides, the procedure of Brockerhoff (6) was used on a reduced scale.

The sn-1,2(2,3)-diglycerides (ca. 6-7 mg), obtained by on-plate lipase hydrolysis, were dissolved in 0.2 ml of diethyl ether and added dropwise with constant shaking to a mixture of 0.2 ml of pyridine, 0.2 ml of diethyl ether, and 0.1 ml of phenyl dichlorophosphate. After 60 min at room temperature, 1 ml of pyridine, 0.6 ml of diethyl ether, and several drops of distilled water were added with cooling. The reaction mixture was mixed with 6 ml of methanol, 5 ml of distilled water, 6 ml of chloroform, and 0.2 ml of triethylamine in a separatory funnel. After a brief shaking, the lower, chloroform layer was recovered and evaporated to obtain the phosphatides.

Phospholipase A₂ hydrolysis of phosphatides

From

the procedures of Dutta and Das (13), and Brockerhoff (7), a modified procedure was developed. A phospholipase A₂ (EC 3.1.1.4) solution (0.25 ml) containing 2.5 mg of Ophiophagus hannah venom was applied with the sample streaker on a 0.5-mm TLC plate. The synthesized phosphatide in freshly distilled diethyl ether containing 5% methanol was applied as evenly as possible over the enzyme band. The plate was immediately placed in a TLC chamber saturated with diethyl ether vapor (four 25-ml beakers containing the solvent were placed in the chamber for this purpose) and kept at room temperature. After 40 min, the plate was transferred into another saturated TLC chamber and developed to the top with chloroform-methanol-14 M ammonia (85:15:2 V/V/V). The phospholipid bands were detected by spraying the plate with aqueous Rhodamine 6G (0.1% W/V), and identified by comparing the R_f values with that of phosphatidylcholine and lysophosphatidylcholine. The approximate R_f values for lysophospholipid and unhydrolyzed phospholipid were 0.15 and 0.39 respectively. The lysophosphatidylphenol band was recovered from the plate and eluted with chloroform-methanol (2:1 V/V). The fatty acid composition of the lysophospholipid was determined as previously.

Calculation of the composition

In the Lands' method, the glyceride structure was calculated as following:

$$\text{sn-1} = 2(\text{phosphatidate}) - (\text{monoglyceride})$$

$$\text{sn-2} = \text{monoglyceride from pancreatic lipase hydrolysis}$$

$$\text{sn-3} = 3(\text{original triglyceride}) - 2(\text{phosphatidate})$$

In the on-plate modification of Brokerhoff's procedures, the following computations were carried out:

$$\text{sn-1} = \text{lysophosphatide from phospholipase A}_2 \text{ hydrolysis}$$

$$\text{sn-2} = \text{monoglyceride from pancreatic lipase hydrolysis}$$

$$\text{sn-3} = 3 (\text{original triglyceride}) - (\text{lysophosphatide}) - (\text{monoglyceride})$$

RESULTS AND DISCUSSION

Method Development

Dutta's lipase method

In the traditional procedure of pancreatic lipase hydrolysis of triglycerides as described by Luddy et al. (39), the addition of emulsifiers (such as bile salt) and lipase cofactor (calcium ions), as well as a vigorous shaking are required for the satisfactory progress of the reaction. The reaction products are isolated by diethyl ether extraction and resolved by preparative TLC.

The Dutta's on-plate lipase method performed here does not require the addition of bile salts or CaCl_2 . Besides, the shaking and extraction of reaction mixtures can be omitted. As a result, the loss of products due to transfer can be minimized and the entire operation does not take more than one hour.

Dutta et al. (14) used iodine vapor to visualize the different bands. But iodine vapors add to the double bonds of unsaturated fatty acids and interfere with subsequent GLC analysis. The non-destructive fluorescent dye 2',7'-dichlorofluorescein was used instead to avoid this problem.

Attempts to modify stereospecific analysis by Lands' method

Lands' method (36) is based on the stereospecificity of Escherichia coli diglyceride kinase. Because this method is very tedious, a procedure which can be completed on a single plate was attempted.

The E. coli B cells (weight 6.10 gm) were broken by three passages through a French press and partially purified as described in the methods section. The pellet and the supernatant of the cell homogenates partially purified with Triton-X-100 were used as enzyme sources. When the original Lands' method (36) was tested on soybean oils, the result agreed fairly well with those obtained with Brockerhoff's method (7), but the diglyceride kinase activity was low. When the partially purified diglyceride kinase (both pellet and supernatant fractions) were used to replace the original crude homogenate preparation (48), the enzyme activity was significantly improved. But even with the improved activity of the partially purified kinase, when Lands' method was attempted on a single TLC plate similar to those used by Dutta et al. (14), no phosphatidate band could be detected.

Possibly certain ingredient(s) in the coating material, Silica gel G, might inactivate the bacterial diglyceride kinase. This possibility was explored by adding 0.1 gm of Silica gel G or Silica gel H (without CaSO₄ binder)

respectively to the kinase reaction mixture, performed according to Lands' original method. The results were compared with controls (without silica gel). Tubes with silica gel added showed no phosphorylation. This indicated that the silica gel inactivated the kinase. The CaSO_4 binder obviously made no difference. The development of an on-plate modified Lands' method seems impossible at present, unless other TLC media can be used which do not inhibit the enzyme. But I discontinued this approach and adapted the on-plate phospholipase A_2 method of Dutta and Das (13) for a rapid stereospecific analysis.

Modified Dutta's phospholipase A_2 method

As outlined in the methods section, rapid stereospecific analysis was achieved by preparing mono- and diglycerides by the lipase method of Dutta et al. (14). The diglyceride was phosphorylated with phenyl dichlorophosphate according to Brokerhoff (6) and the phenyl phospholipids were hydrolyzed with phospholipase A_2 using a method similar to Dutta and Das (13). The sn-2-position was obtained from the monoglyceride and the sn-1 from the lysophosphatide. As recommended by Nutter and Privett (45), the snake venom from Ophiophagus hannah (King Cobra) was chosen to carry out the stereospecific hydrolysis of synthesized phospho-

lipids, because it does not preferentially hydrolyze any of the individual fatty acids.

Dutta and Das (13) used a substrate to enzyme ratio of 4:1 and a reaction time 15 min at 25 C, and obtained ca. 75% hydrolysis of phosphatidylcholine (PC). To assure the hydrolysis reaction was carried to completion, several different substrate to enzyme ratios and reaction times were compared. Tests with a 3:1 substrate to enzyme ratio and 40 min incubation at room temperature gave around 95% hydrolysis of PC, which was very similar to those obtained by Brockerhoff's original over-night shaking method (7). A comparison of these methods is given in Table 1.

Table 1. Comparison of sn-1 fatty acid composition of a soybean oil sample by over-night shaking and on-plate method

Method	Fatty acid composition (mole %)				
	16:0	18:0	18:1	18:2	18:3
Over-night shaking	14.10±0.56 ^a	7.17±0.30	40.99±1.64	32.40±1.30	5.33±0.24
On-plate	14.07±0.58	7.20±0.32	41.23±1.46	32.32±1.21	5.17±0.22

^aAverage of three trials.

The on-plate method, starting from the phospholipid synthesis and proceeding through the phospholipase A₂ on-plate hydrolysis to the isolation of lysophosphatidylphenol (sn-1-position), usually could be completed in 3 hr. Six to eight samples could be analyzed stereospecifically daily. Possibly the rate of analysis could be increased to ten samples per day.

The method was considered suitably convenient and rapid for routine application and was used for numerous oilseed analyses.

Pancreatic Lipase Hydrolysis of Soybean Samples

Before the development of the stereospecific analysis method, the modified pancreatic lipase method of Dutta et al. (14) was applied to assess the variation in soybean glyceride structure. The composition of the sn-2-position is compared with the triglyceride composition for these samples in Figures 1-8. The results for the sn-2-position and the total glyceride composition also are given in the appendix. The results are generally in close agreement with those previously reported by Fatemi and Hammond (18). There is very little saturated fatty acids (palmitic and stearic acids) on the sn-2-position. The data were fitted by

Figure 1. Percentage of palmitic acid on the sn-2-position of glycerol vs. the percentage of palmitic acid in the triglyceride

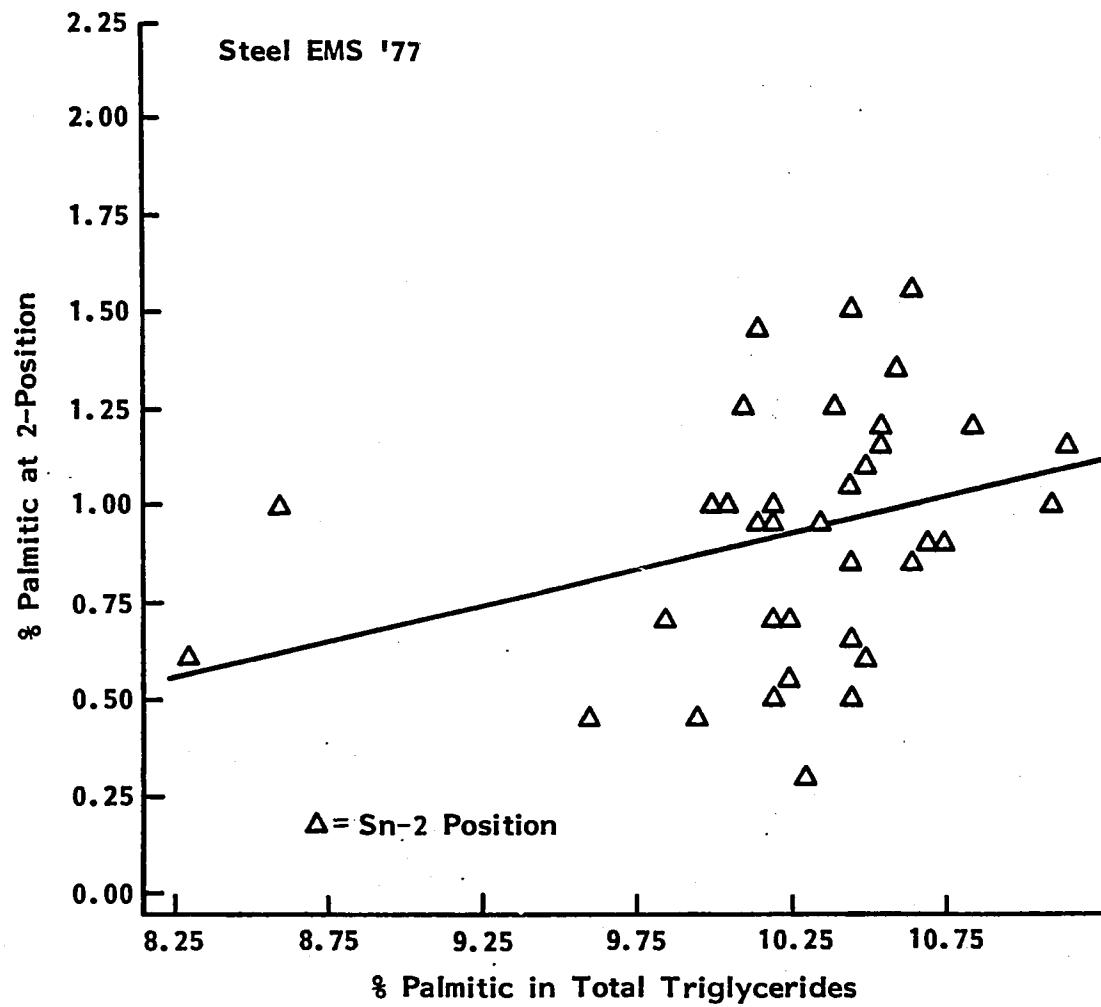


Figure 2. Percentage of oleic acid on the sn-2-position of glycerol vs. the percentage of oleic acid in the triglyceride

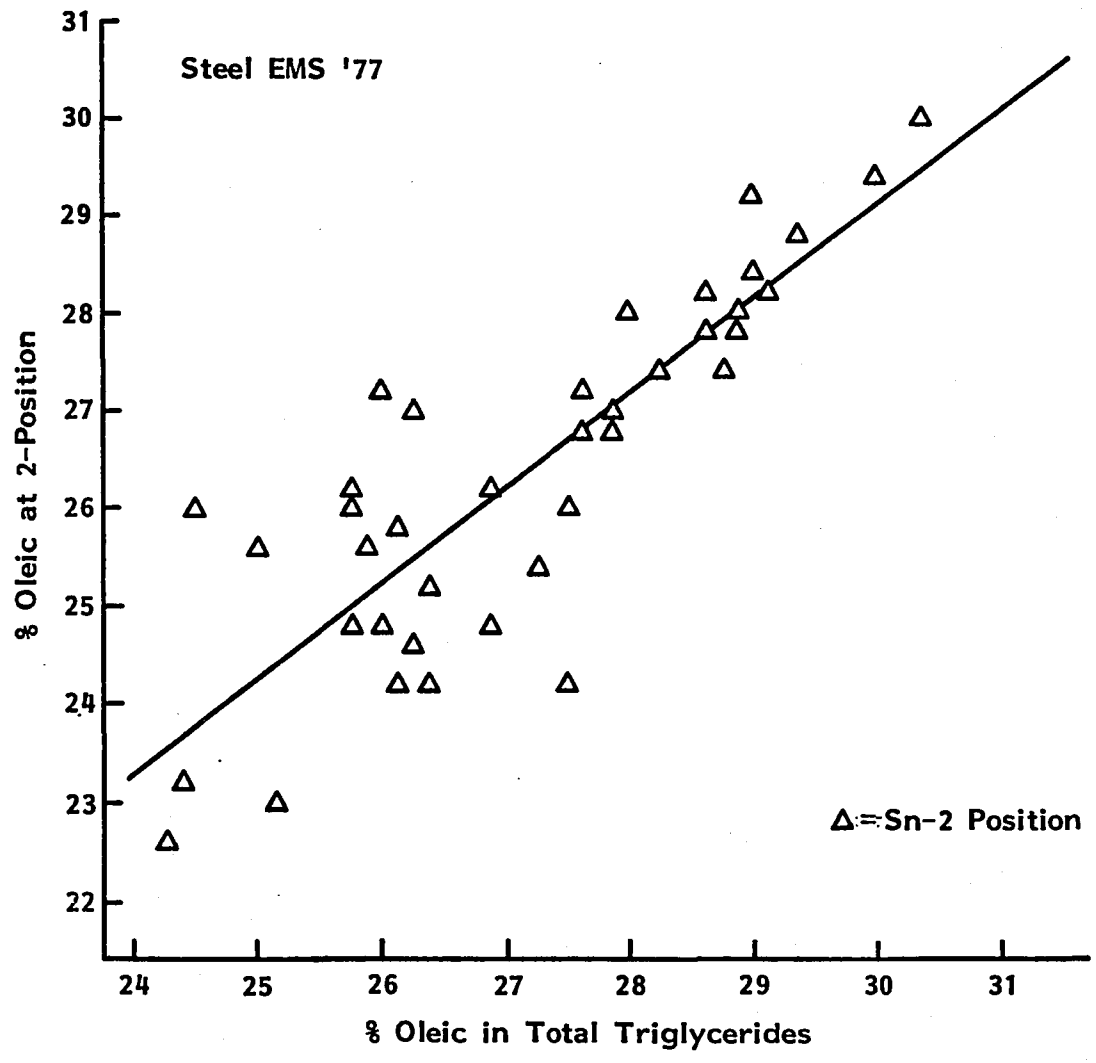


Figure 3. Percentage of linoleic acid on the sn-2-position of glycerol vs. the percentage of linoleic acid in the triglyceride

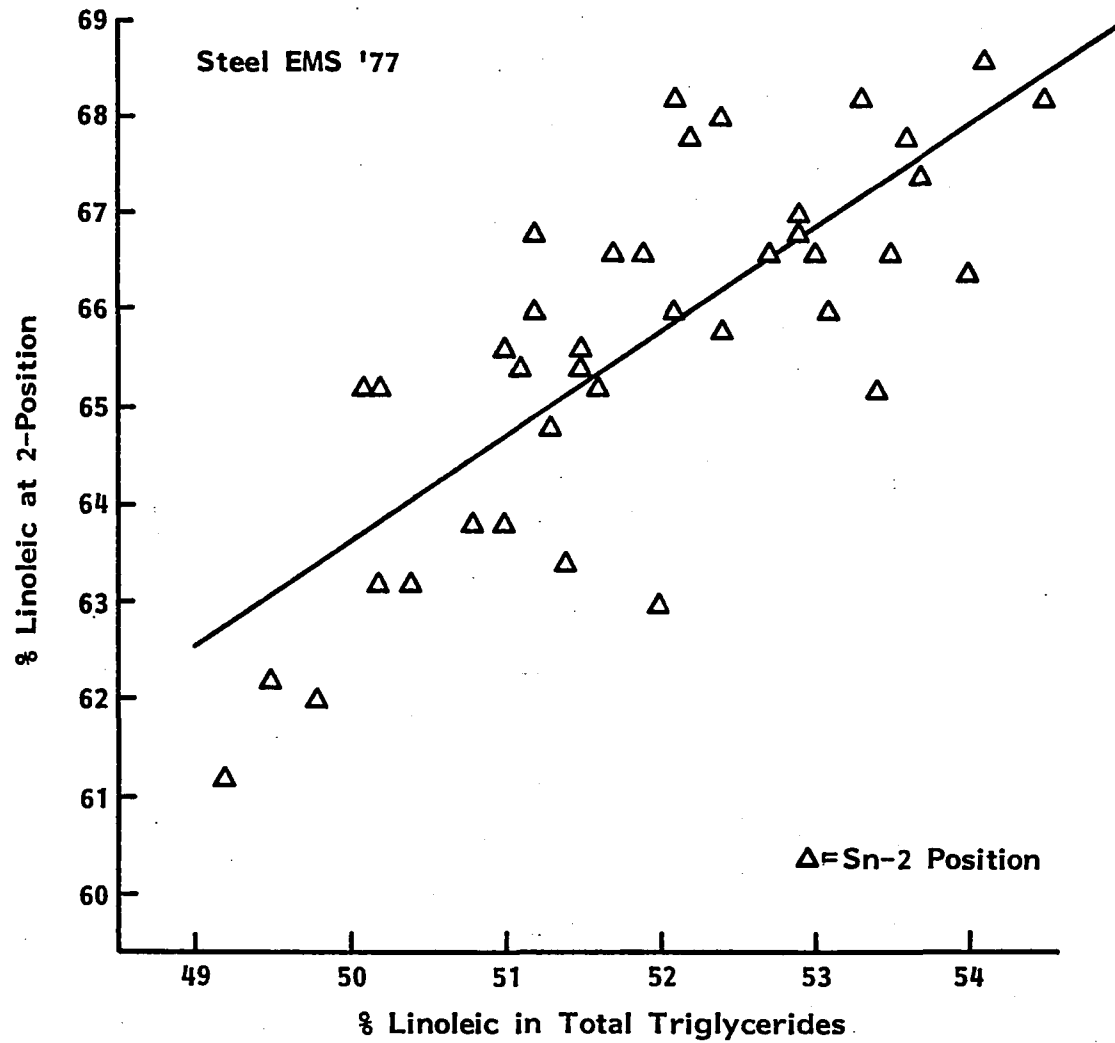


Figure 4. Percentage of linolenic acid on the sn-2-position of glycerol vs. the percentage of linolenic acid in the triglyceride

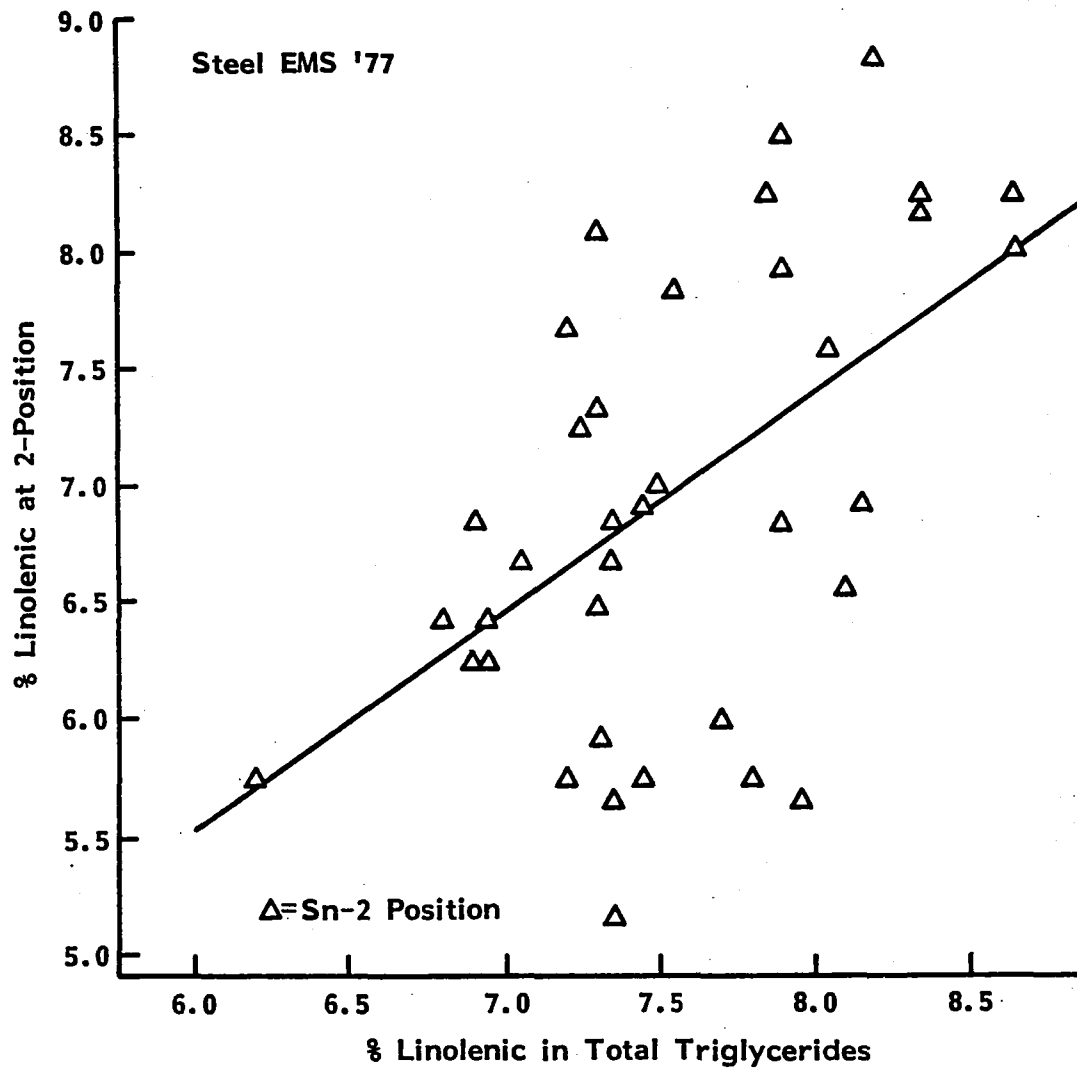


Figure 5. Percentage of palmitic acid on the sn-2-position of glycerol vs. the percentage of palmitic acid in the triglyceride

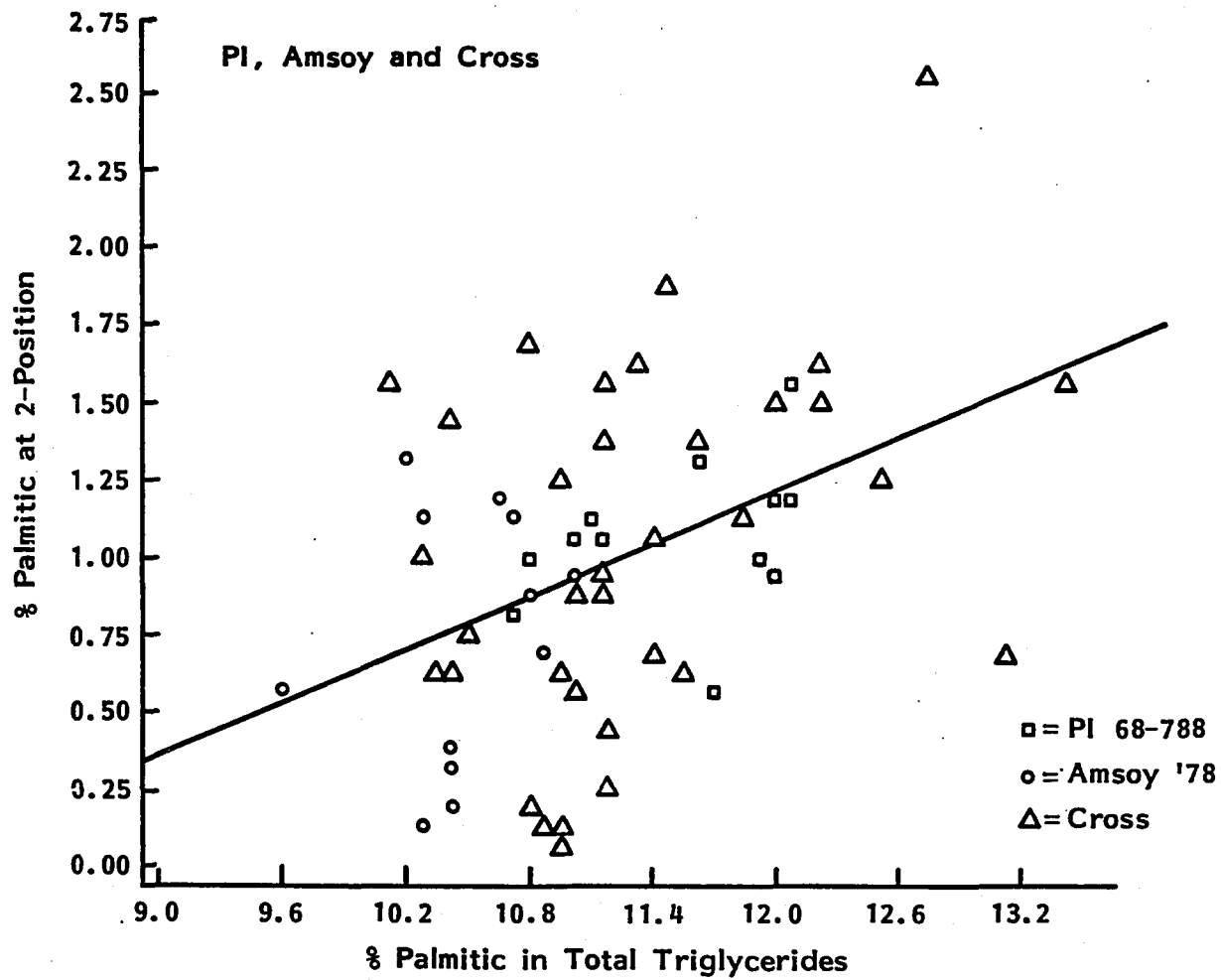


Figure 6. Percentage of oleic acid on the sn-2-position of glycerol vs. the percentage of oleic acid in the triglyceride

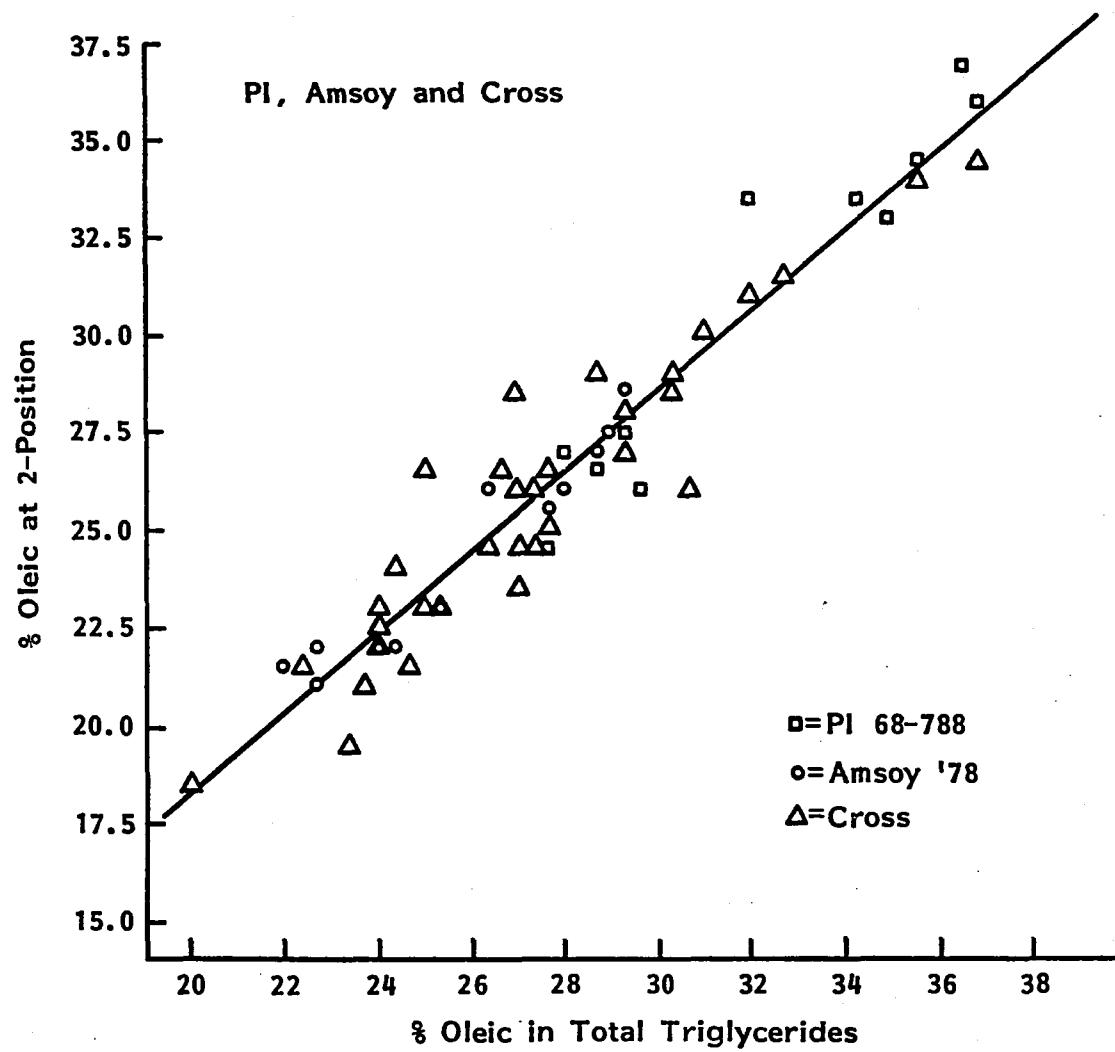


Figure 7. Percentage of linoleic acid on the sn-2-position of glycerol vs. the percentage of linoleic acid in the triglyceride

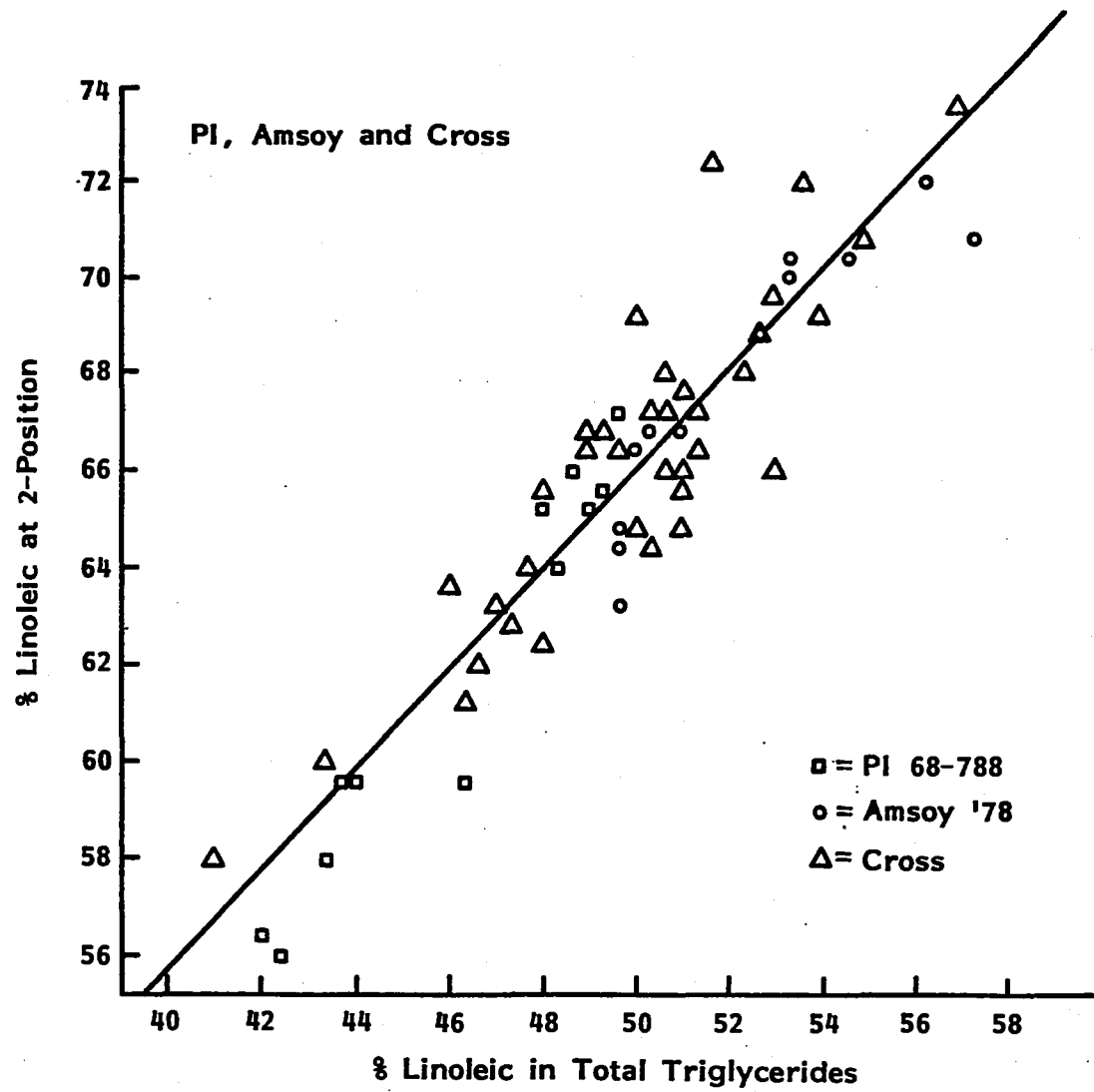


Figure 8. Percentage of linolenic acid on the sn-2-position of glycerol vs. the percentage of linolenic acid in the triglyceride

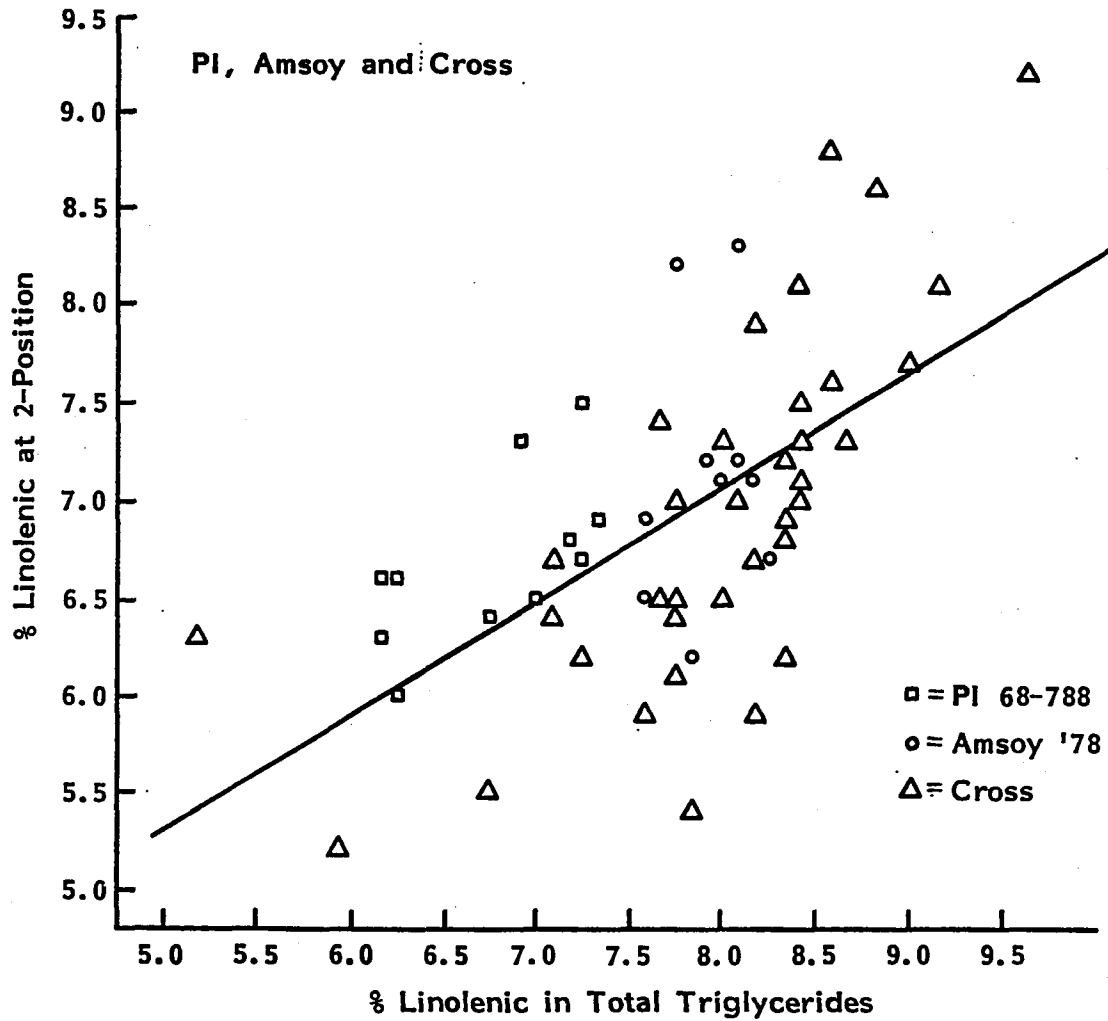


Table 2. Linear regression of the fatty acid compositions on the sn-2-position of triglycerides vs. the fatty acid composition of the whole fat for the soybean varieties Steel EMS-treated

Fatty acid	Position	Slope	Probability slope $\neq 0$	Intercept	Probability intercept $\neq 0$	R ²	Slope intercept 0	Number of deviants
16:0	2	0.19±0.12	0.13	-0.97±1.25	0.44	0.06	0.09±0.01	1 ^a
18:0	2	--	--	--	--	--	--	--
18:1	2	0.96±0.10	0.0001	0.18±2.69	0.95	0.72	0.97±0.01	3
18:2	2	1.08±0.14	0.0001	9.78±7.28	0.19	0.61	1.27±0.03	1
18:3	2	0.93±0.25	0.0006	-0.06±1.89	0.98	0.27	0.93±0.02	1

^aBased on Proc Univariate (25). Those deviated two standard deviations from the regression lines were counted as deviants.

Table 3. The coefficient of variation (CV) of the deviations for the soybean varieties Steel EMS-treated

Position	Fatty acid				
	16:0	18:0	18:1	18:2	18:3
2	41.94	--	3.67	1.75	11.75

Table 4. Linear regression of the fatty acid compositions on the sn-2-position of triglycerides vs. the fatty acid composition of the whole fat for the soybean varieties Amsoy, PI68-788 and their crosses

Fatty acid	Position	Slope	Probability slope $\neq 0$	Intercept	Probability intercept $\neq 0$	R ²	Slope intercept 0	Number of deviants
16:0	2	0.28±0.08	0.0008	-2.17±0.89	0.02	0.18	0.09±0.01	1 ^a
18:0	2	--	--	--	--	--	--	--
18:1	2	1.02±0.04	0.0001	-2.16±1.10	0.06	0.92	0.95±0.01	4
18:2	2	1.03±0.05	0.0001	14.49±2.58	0.0001	0.87	1.32±0.01	3
18:3	2	0.59±0.09	0.0001	2.38±0.73	0.0018	0.40	0.89±0.01	2

^aBased on Proc Univariate (25). Those deviated two standard deviations from the regression lines were counted as deviants.

Table 5. The coefficient of variation (CV) of the deviations for the soybean varieties Amsoy, PI68-788 and their crosses

Position	Fatty acid				
	16:0	18:0	18:1	18:2	18:3
2	45.92	--	4.43	2.15	8.99

linear regression, and the slopes, intercepts and R^2 are given in Tables 2 and 4.

Slopes less than 1 mean the placement of a fatty acid on a position is not favored. Slopes of the best line (intercept allowed) in Tables 2 and 4 are ≤ 1 . The slopes for palmitic acid are particularly low in accordance with its low concentration on the sn-2-position.

The intercepts of the best line in Table 4 are significantly different from zero, especially in the case of linoleic acid. The intercept for linoleic acid is large but not significant in the case of Table 2. While the slopes can be considered a measure of the tendency of an enzyme to place a fatty acid on a particular position, the meaning of intercepts is less clear. Possibly it could reflect the formation of pools of fatty acids that would be used to acylate a glycerol position. A positive intercept would indicate a greater concentration of a fatty acid than chance in the pool and a negative intercept a lesser concentration than chance. But it is possible that the intercepts are an artifact of the linear regression. It may be the intercepts are really zero and that the best fit indicates an intercept because the data fall within a limited range, and a small quadratic component necessary for fit over the full range is not perceived. For this reason, slopes also have been calculated

with the intercept forced to zero. These reveal that linoleic acid is favored in sn-2-position while oleic and linolenic acids are slightly discriminated against.

The R^2 values indicate how well the best-line data (with intercept) fit the linear regression. Linear regression accounts for little of the variation in palmitic acid and only a small amount in linolenic acid. The regression fits fairly well for oleic and linoleic acids.

The coefficient of variation (CV) given in Tables 3 and 5 is the standard deviation of the deviations of the data from the regression line divided by the sample mean and multiplied by 100. It is a measure of the dispersion of the data around the regression lines. The magnitude of the coefficient is inversely proportional to the amount of each fatty acid on the sn-2-position. This suggests that a considerable amount of the variation is experimental error in analysis. The CV for palmitic acid was much reduced in subsequent experiments and that for linolenic acid was reduced to some extent suggesting that the error in measuring the small amount of palmitic and linolenic acids on sn-2-position was reduced with practice. The CV for the replicated data in Table 1 is ~ 4 which gives an estimate of the size of analytical error. Presumably CVs > 4 may indicate biological variation. The Steel-EMS samples were analyzed in the hope that the ethyl methanesulfonate treatment had induced variation in the glyceride structure of these plants, however, in comparison with the rest of the data on

soybeans their variation from the linear regression as a group or instances of wide deviation by individual plants was not remarkable.

The data for the PI68-788 in Table 4 are for a strain considered a glyceride structure genetic deviant by Fatemi and Hammond (18). Figures 5-8 show that the PI68-788, Amsoy and the progeny of their cross fall on the same lines except possibly for linolenic acid in Figure 8. These lines are not very different from those for Steel-EMS in Figures 1-4. These results are not surprising because the deviation that caused PI68-788 to be singled out was mostly in the sn-1- and sn-3-positions rather than the sn-2-position.

The standard deviation of the deviations of the fatty acid values from the linear regression was calculated. Those values that differed two standard deviations (SD) from the linear plots were considered outliers and were counted possible deviants. In these data the most frequently deviating fatty acid on sn-2-position was oleic acid. It will be necessary to advance such plants another generation and test the progeny to verify that these are truly deviants from the general pattern. It seemed from the data that great variation from the general pattern was rare. Since deviants previously found in corns (57) and soybeans (18) differed primarily on the sn-1- and sn-3-positions, it was decided to concentrate on developing a method for rapid stereospecific analysis.

Stereospecific Analysis of Soybeans:

Glycine max and Glycine soya

Analyses were made on 48 plant introductions of Glycine max and 47 of Glycine soya. G. soya is closely related to G. max and can be crossed with it. It seemed possible that G. soya which had not been selected for desirable properties by humans, might demonstrate greater and more frequent variations in fat composition.

The percentage of specific fatty acids at three positions of the glycerol is plotted vs. the percentage of that fatty acid in the whole triglyceride and given in Figures 9-18. The results of stereospecific analysis are shown in the appendix and the statistical analyses are summarized in Tables 6 and 8. The coefficients of variation (CV) are listed in Tables 7 and 9.

The results are generally similar to those of Fatemi and Hammond (18). There is very little saturated fatty acids on the sn-2-position, and the sn-1-position is consistently richer in palmitic and linolenic acids than is the sn-3-position. The sn-3-position is richer in oleic acid.

The algebraic sum of the intercepts is very close to zero and the average of the slopes for each fatty acid positions is nearly one. This indicates a good fit over the range examined as do the R^2 values which range from 0.68 to 0.97. If one goes by the best-fit lines (intercept $\neq 0$), the slopes indicate oleic and linoleic acids showed a preference on sn-3-position, but these trends are off-set by large intercepts.

Figure 9. Percentage of palmitic acid on the sn-1- and sn-3-positions of glycerol vs. the percentage of palmitic acid in the triglyceride

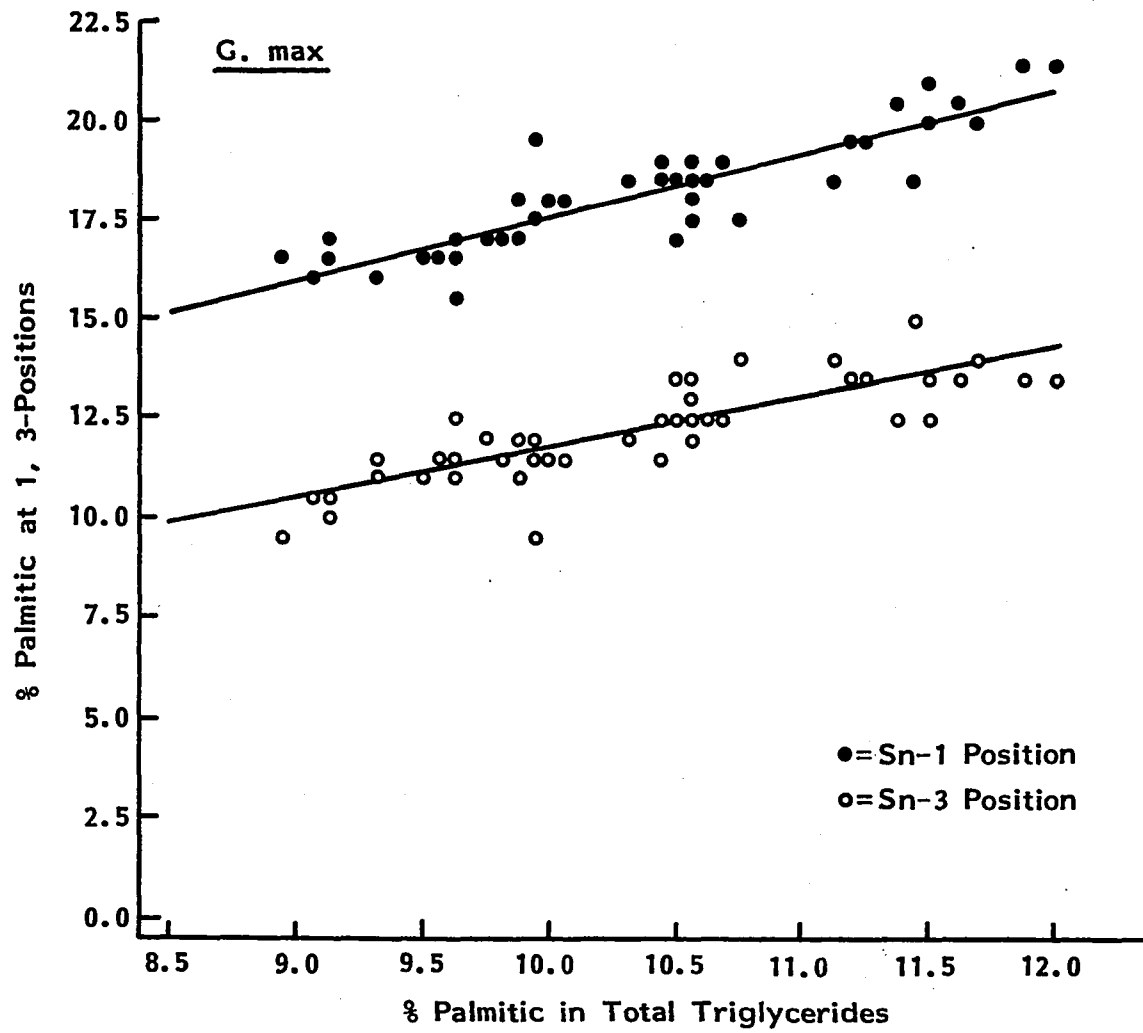


Figure 10. Percentage of stearic acid on the sn-1- and sn-3-positions of glycerol vs. the percentage of stearic acid in the triglyceride.

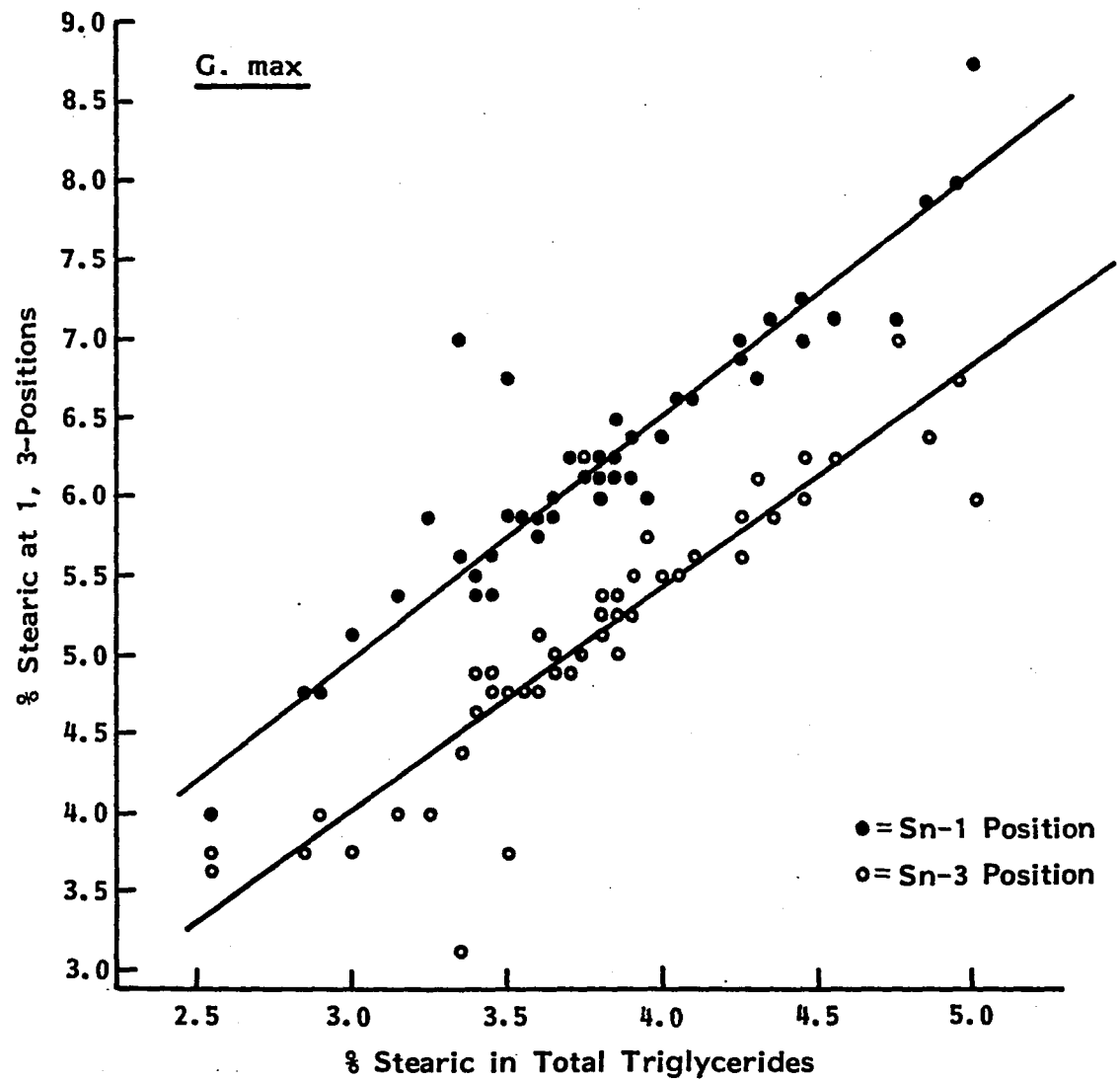


Figure 11. Percentage of oleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of oleic acid in the triglyceride

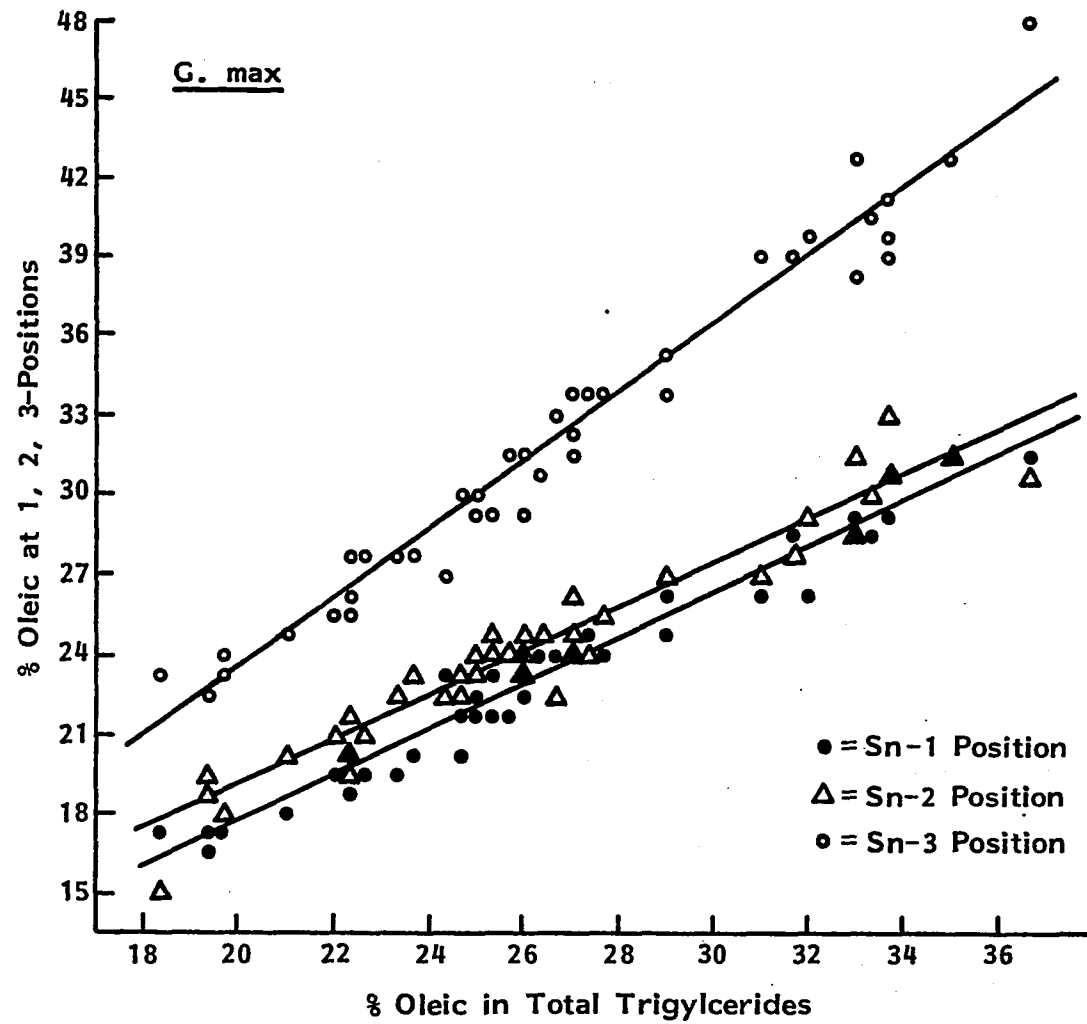


Figure 12. Percentage of linoleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linoleic acid in the triglyceride

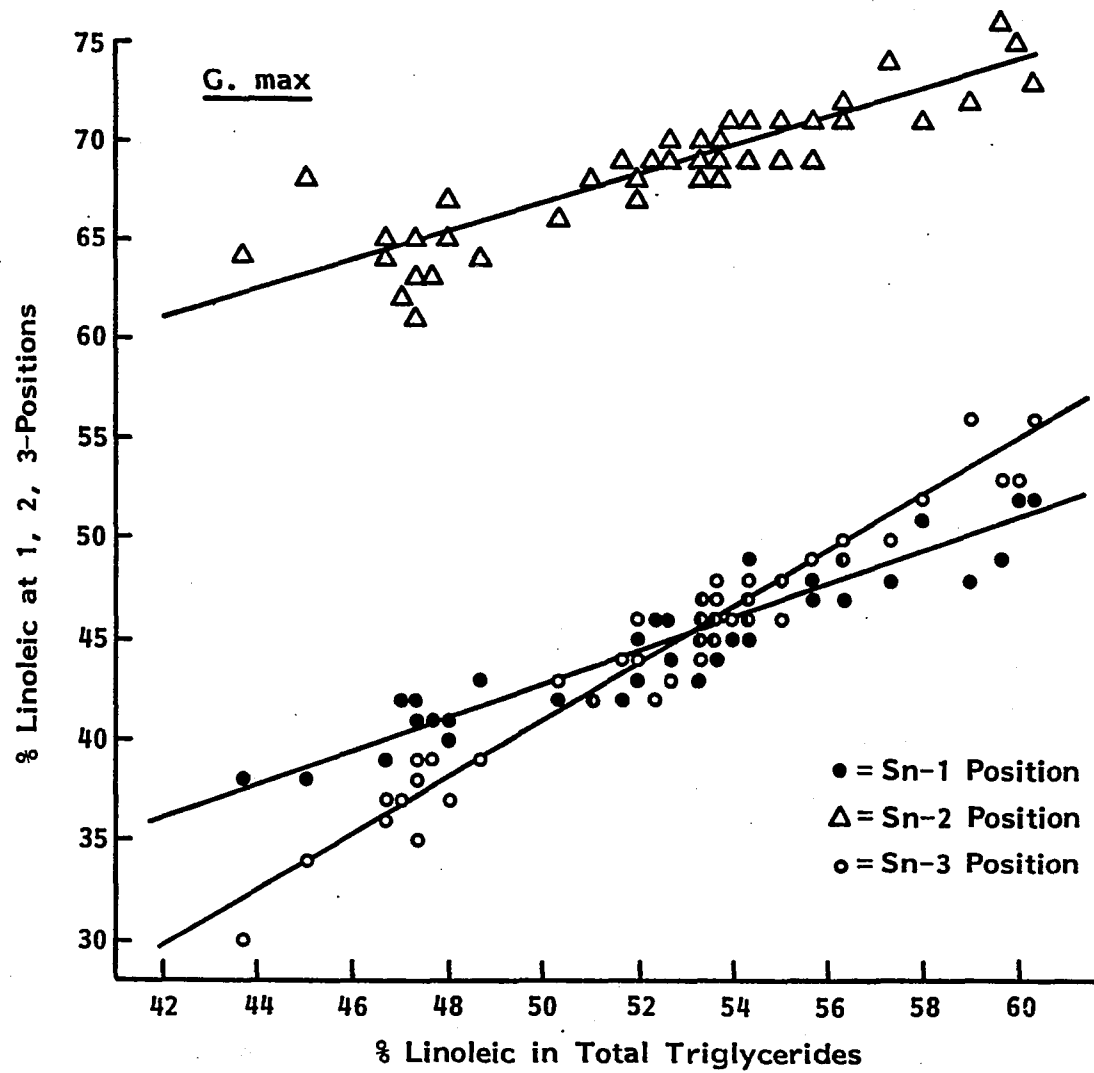


Figure 13. Percentage of linolenic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linolenic acid in the triglyceride

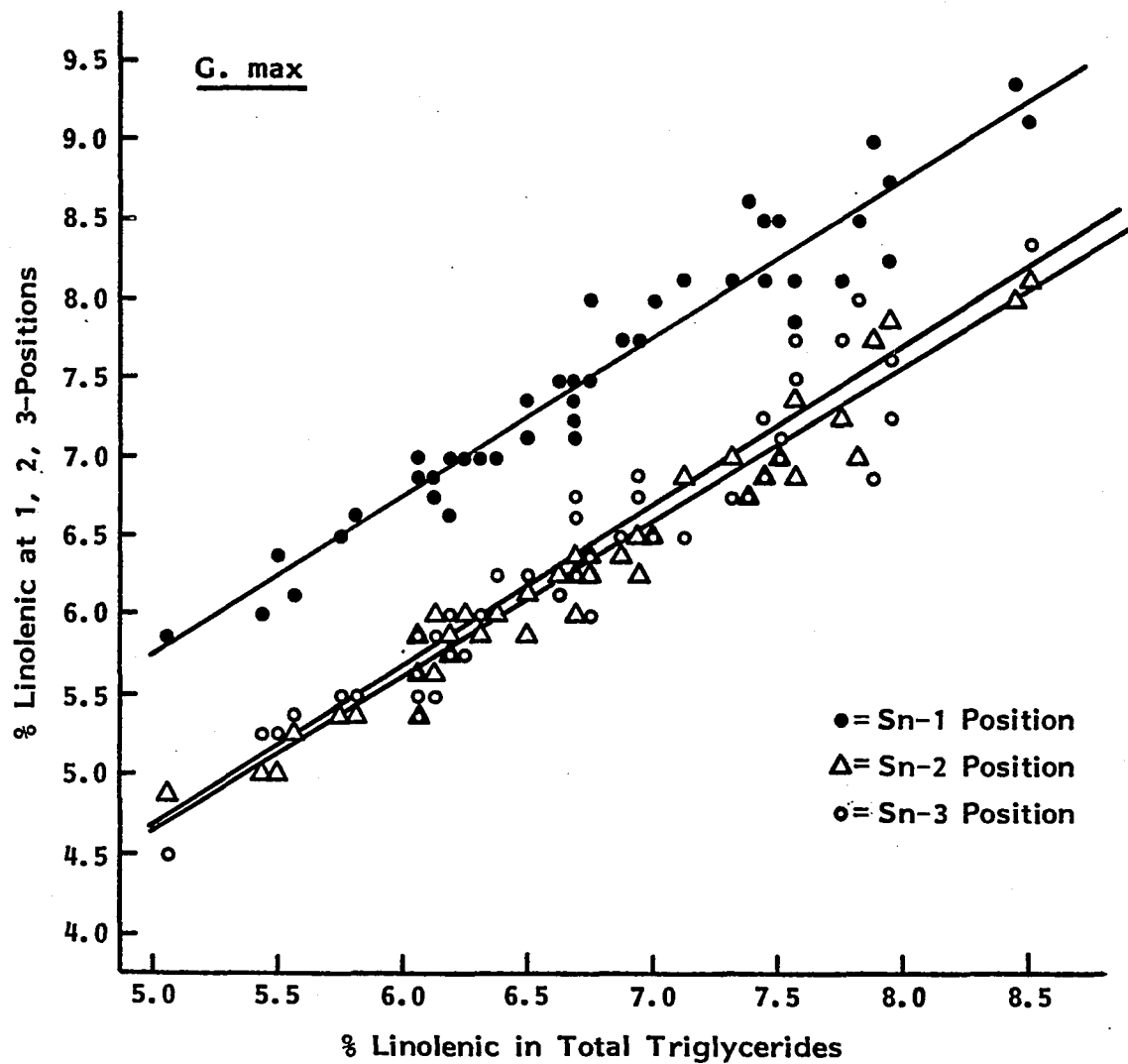


Figure 14. Percentage of palmitic acid on the sn-1- and sn-3-positions of glycerol vs. the percentage of palmitic acid in the triglyceride

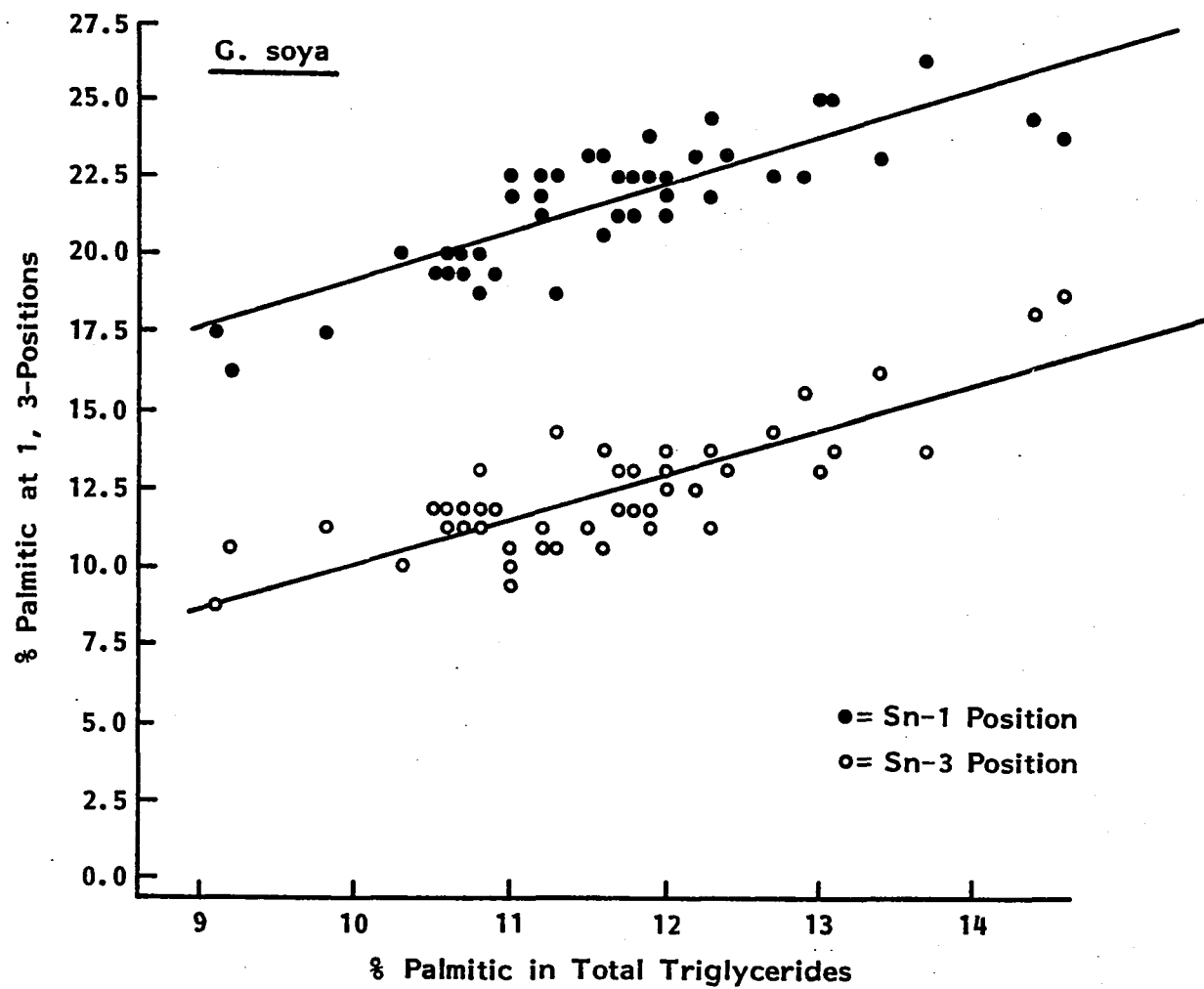


Figure 15. Percentage of stearic acid on the sn-1- and sn-3-positions of glycerol vs. the percentage of stearic acid in the triglyceride

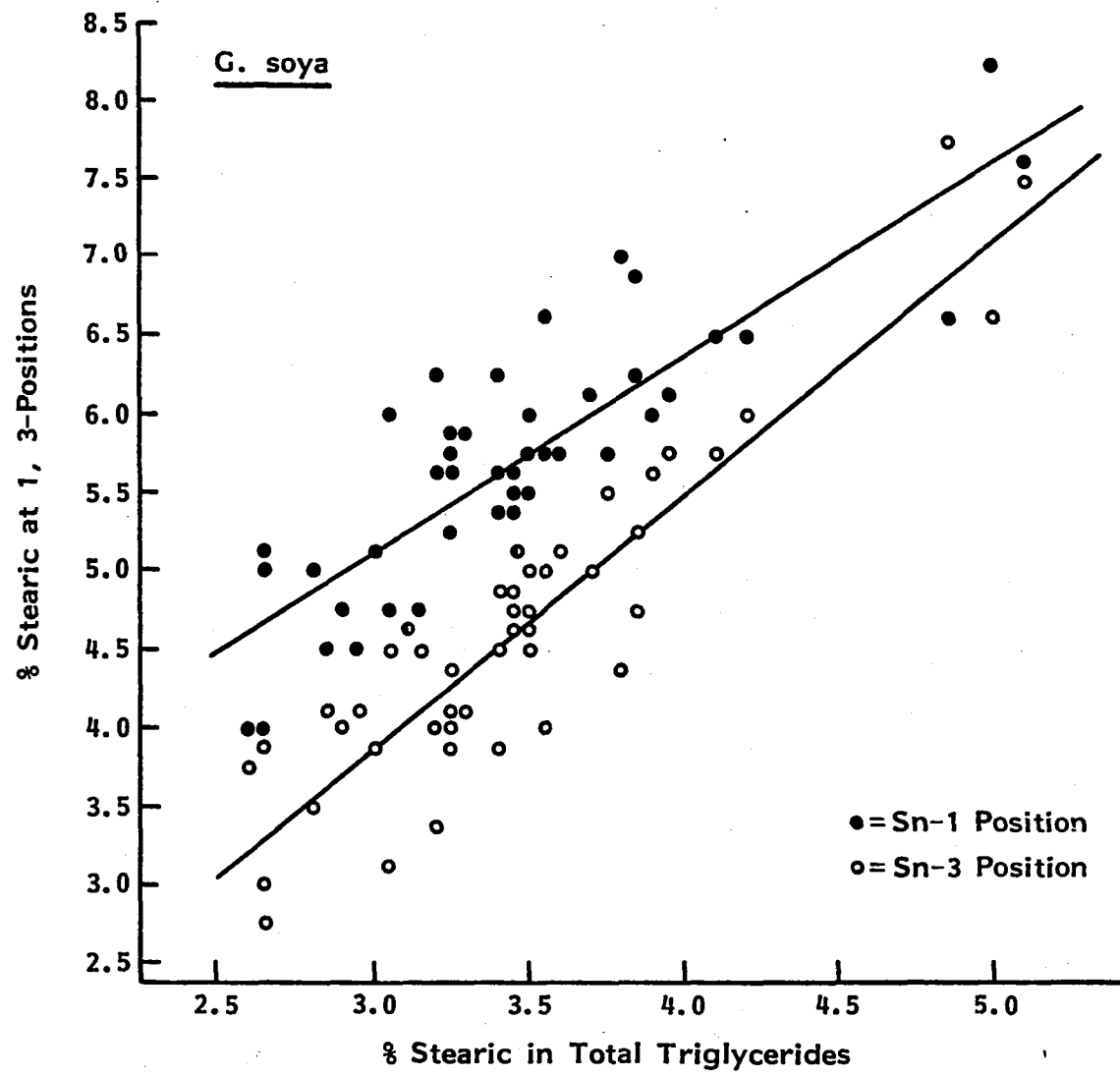


Figure 16. Percentage of oleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of oleic acid in the triglyceride

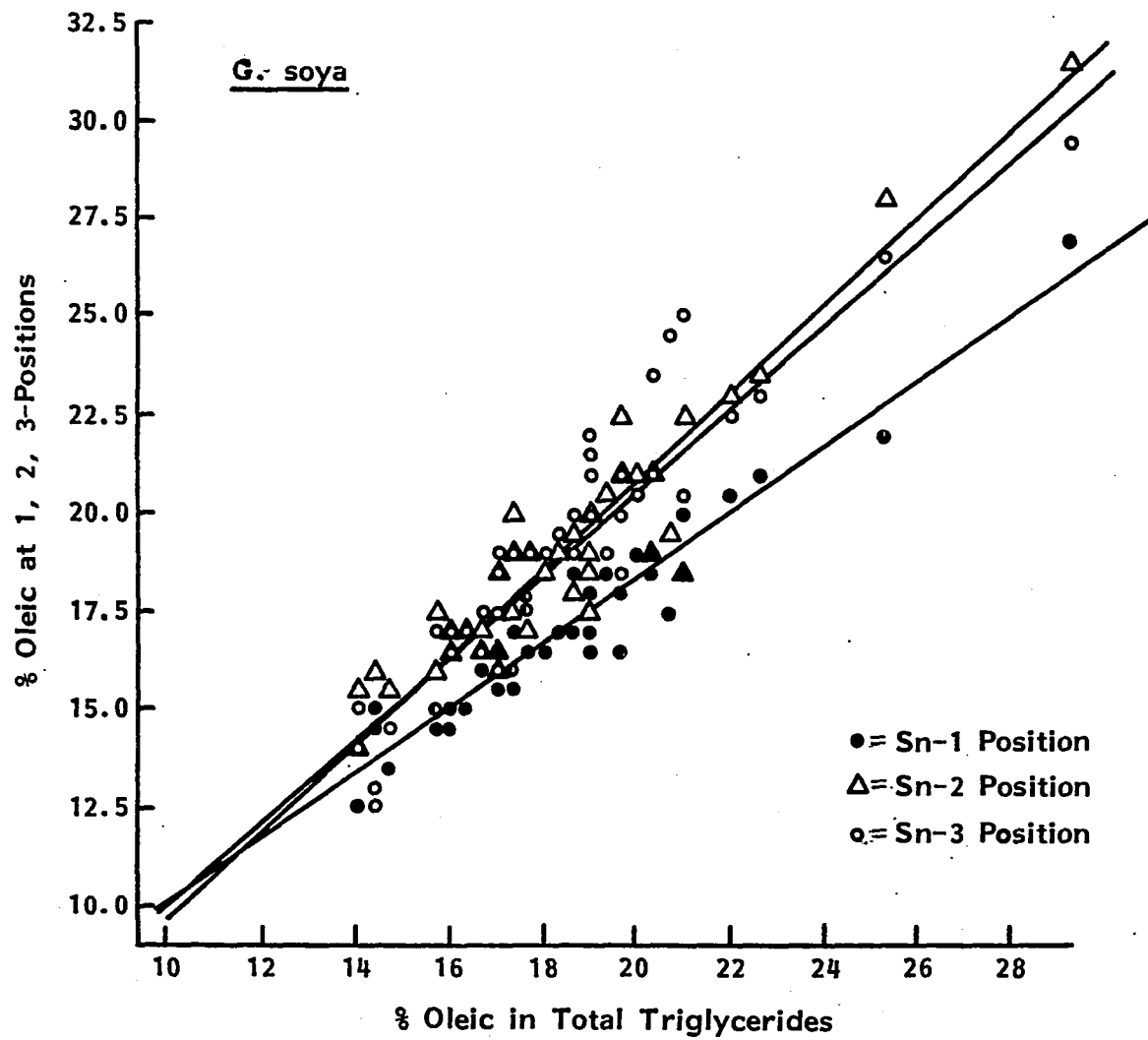


Figure 17. Percentage of linoleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linoleic acid in the triglyceride

Figure 18. Percentage of linolenic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linolenic acid in the triglyceride

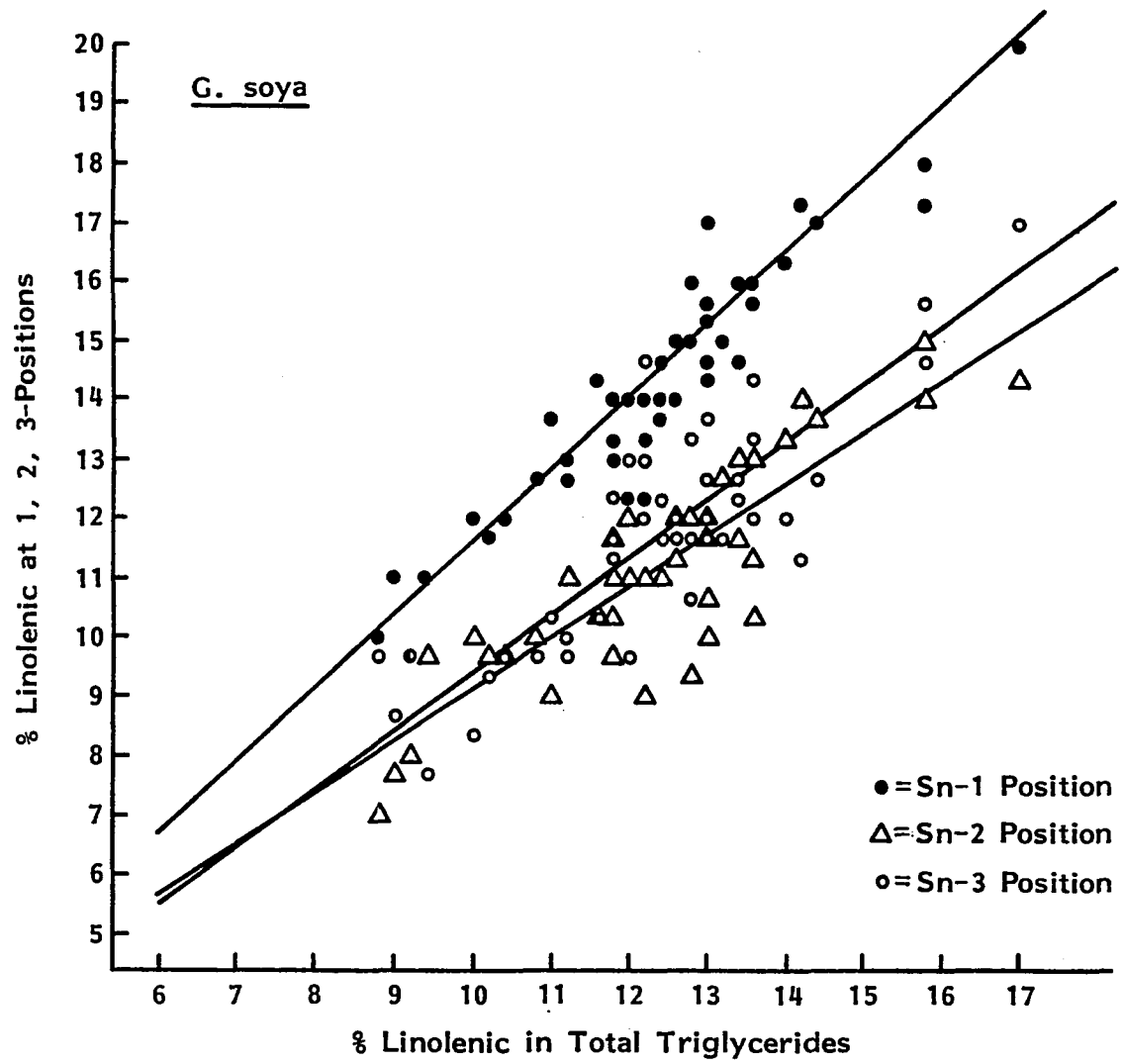


Table 6. Linear regression of the fatty acid compositions on three positions of triglycides vs. the fatty acid composition of the whole fat for the soybean varieties Glycine max

Fatty acid	Position	Slope	Intercept	Probability intercept $\neq 0$	R ²	Slope intercept 0	Number of deviants
16:0	1	1.67±0.02 ^a	0.83±1.21	0.50	0.82	1.75±0.01	3 ^b
	2	--	--	--	--	--	--
	3	1.27±0.12	-0.92±1.21	0.45	0.72	1.18±0.01	3
18:0	1	1.52±0.10	0.42±0.37	0.26	0.85	1.63±0.01	4
	2	--	--	--	--	--	--
	3	1.41±0.09	-0.21±0.36	0.56	0.83	1.36±0.01	4
18:1	1	0.86±0.02	0.46±0.63	0.47	0.97	0.88±0.01	4
	2	0.84±0.03	2.03±0.87	0.02	0.94	0.92±0.01	3
	3	1.30±0.04	-2.49±0.96	0.01	0.97	1.21±0.01	2
18:2	1	0.82±0.04	1.76±2.31	0.45	0.88	0.85±0.01	1
	2	0.79±0.05	26.92±2.42	0.0001	0.87	1.30±0.01	1
	3	1.39±0.05	-28.69±2.42	0.0001	0.95	0.85±0.01	1
18:3	1	1.01±0.04	0.69±0.25	0.01	0.94	1.11±0.01	4
	2	0.98±0.03	-0.30±0.21	0.15	0.96	0.94±0.01	3
	3	1.00±0.04	-0.39±0.30	0.21	0.92	0.95±0.01	3

^aThe probability that slope = 0 was 0.0001 in all instances.

^bBased on Proc Univariate (25). Those deviated two standard deviations from the regression lines were counted as deviants.

Table 7. The coefficient of variation (CV) of the deviations for the soybean varieties Glycine max

Position	Fatty acid				
	16:0	18:0	18:1	18:2	18:3
1	3.53	6.00	3.09	2.65	2.66
2	5.48	--	4.11	1.81	2.68
3	5.24	7.03	3.45	2.79	3.74

Table 8. Linear regression of the fatty acid compositions on three positions of triglycerides vs. the fatty acid composition of the whole fat for the soybean varieties Glycine soya

Fatty acid	Position	Slope	Intercept	Probability intercept $\neq 0$	R ²	Slope intercept 0	Number of deviants
16:0	1	1.52 \pm 0.14 ^a	3.94 \pm 1.68	0.02	0.71	1.86 \pm 0.01	1 ^b
	2	--	--	--	--	--	--
	3	1.42 \pm 0.15	-4.13 \pm 1.71	0.02	0.68	1.07 \pm 0.02	1
18:0	1	1.29 \pm 0.11	1.25 \pm 0.40	0.003	0.74	1.64 \pm 0.02	2
	2	--	--	--	--	--	--
	3	1.64 \pm 0.11	-1.04 \pm 0.39	0.01	0.83	1.35 \pm 0.02	3
18:1	1	0.83 \pm 0.04	1.81 \pm 0.72	0.02	0.91	0.92 \pm 0.01	2
	2	1.05 \pm 0.06	-0.45 \pm 1.11	0.69	0.87	1.03 \pm 0.01	1
	3	1.12 \pm 0.07	-1.36 \pm 1.28	0.29	0.85	1.05 \pm 0.01	2
18:2	1	0.98 \pm 0.07	-11.81 \pm 4.00	0.005	0.80	0.76 \pm 0.01	3
	2	0.70 \pm 0.07	31.09 \pm 3.80	0.0001	0.69	1.27 \pm 0.01	3
	3	1.32 \pm 0.10	-19.23 \pm 5.30	0.0007	0.80	0.96 \pm 0.01	3
18:3	1	1.18 \pm 0.06	-0.36 \pm 0.76	0.64	0.89	1.15 \pm 0.01	3
	2	0.86 \pm 0.08	0.51 \pm 0.95	0.59	0.74	0.90 \pm 0.01	3
	3	0.96 \pm 0.09	-0.15 \pm 1.12	0.89	0.71	0.95 \pm 0.01	1

^aThe probability that slope = 0 was 0.0001 in all instances.

^bBased on Proc Univariate (25). Those deviated two standard deviations from the regression lines were counted as deviants.

Table 9. The coefficient of variation (CV) of the deviations for the soybean varieties Glycine soya

Position	Fatty acid				
	16:0	18:0	18:1	18:2	18:3
1	5.17	7.56	4.35	4.42	4.84
2	7.80	--	6.03	2.52	7.72
3	9.18	9.11	6.84	4.66	8.73

This can best be accounted for by the restricted range of the data points as explained previously. Fatemi and Hammond (18) examined varieties with a much larger range of the major fatty acids (Table 10) and obtained intercepts much closer to zero. The slopes of the regressions forced through zero are probably more reliable indicators of the true regression and the preferential placement of the fatty acids on the glycerol positions.

Comparison of the glyceride structure of G. max and G. soya shows that they have similar triglyceride patterns but some differences in constituent fatty acid, especially linolenic acid. The R^2 as listed in Tables 6 and 8, and the CV from Tables 7 and 9, show that G. soya, in general,

Table 10. Range variations of fatty acid composition for the soybean varieties Glycine max and Glycine soya

Variety	Fatty acid and range (mole %)				
	16:0	18:0	18:1	18:2	18:3
<u>G. max</u>	9.05-11.99	2.54-4.98	18.46-36.52	43.77-60.21	5.06-8.53
<u>G. soya</u>	9.09-14.61	2.61-5.11	13.96-29.29	42.03-61.26	8.88-17.06
Fatemi & Hammond ^a	9.14-14.35	2.79-4.21	12.64-53.00	30.21-57.07	4.56-15.36

^aFatemi, S. H. and E. G. Hammond (18).

has smaller values of R^2 and larger CVs than G. max. This may indicate that G. soya shows more variation in glyceride structure, but also the smaller oil content in G. soya may have increased experimental error. The CVs of sn-3-position generally are larger than those in sn-1- and sn-2-positions. This is probably due to experimental error, because the sn-1- and sn-2-positions were determined directly from GLC analysis, while the sn-3-position was derived from calculations.

The number of plants which deviated from the regression by more than two standard deviations was small, and none deviated dramatically from the regression. Further testing of these outliers is necessary to verify whether they are true genetic deviants or only vary from the line because of analytical error or environmental factors.

Unfortunately, by the time the stereospecific analysis was worked out the crosses of PI68-788 and Amsoy examined by the lipase method were no longer viable and were not considered worthy of further analysis. PI68-788 deviated from the sn-1 and sn-3 regression lines by about the same amount as the deviants selected by the two standard deviations criterion.

Stereospecific Analysis of Oats:

Avena sativa and Avena sterilis

Oats are seldom used as a source of oil and A. sativa, therefore, has not been subjected to much selection for oil characteristics. A. sterilis introductions represent a vast reservoir of genetic material which can be crossed readily into A. sativa. It seemed possible that these oats might demonstrate greater or more frequent deviations in glyceride structure than G. max.

The percentages of specific fatty acids at the three positions of glycerol are plotted vs. the percentage of that fatty acid in the whole triglyceride and given in Figures 19-28. The results of stereospecific analysis are also listed in the appendix. The statistical analyses are summarized in Tables 11 and 13. The coefficients of variation (CV) are shown in Tables 12 and 14.

The salient distinctions in fatty acid composition between oat and soybean oils are the enrichment of palmitic acid (14 to 23%) and the relative low content of linolenic acid (<1 to 5%) in oat oil (21). In soybean oils, usually the sn-2-position is devoid of saturated fatty acids (palmitic and stearic acids), while in oat oils the palmitic acid usually accounts for 3.5 to 4.0% of the sn-2-position. The sn-2-position is consistently richer in unsaturated fatty acids (oleic, linoleic and linolenic acids)

Figure 19. Percentage of palmitic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of palmitic acid in the triglyceride

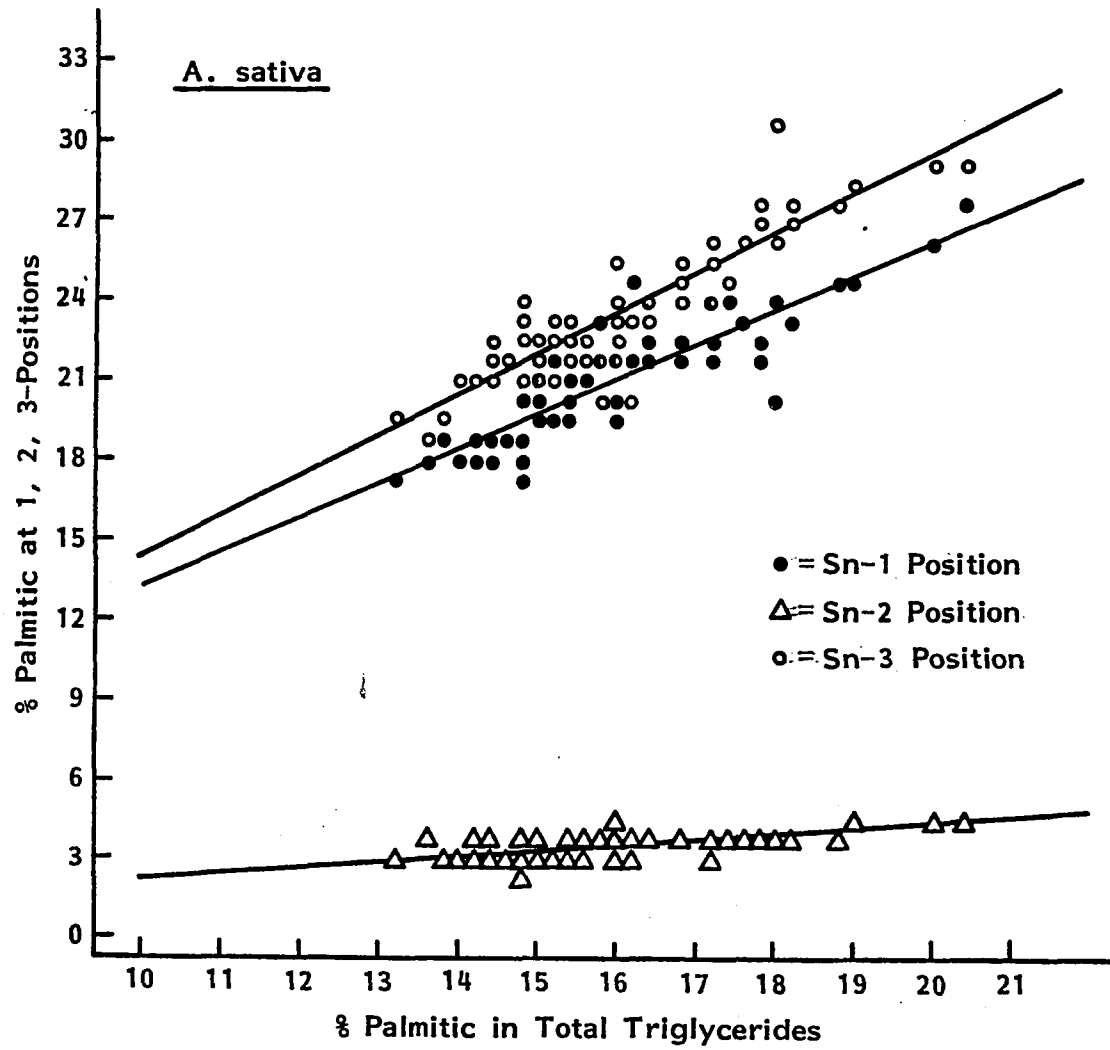


Figure 20. Percentage of stearic acid on the sn-1- and sn-3-positions of glycerol vs. the percentage of stearic acid in the triglyceride

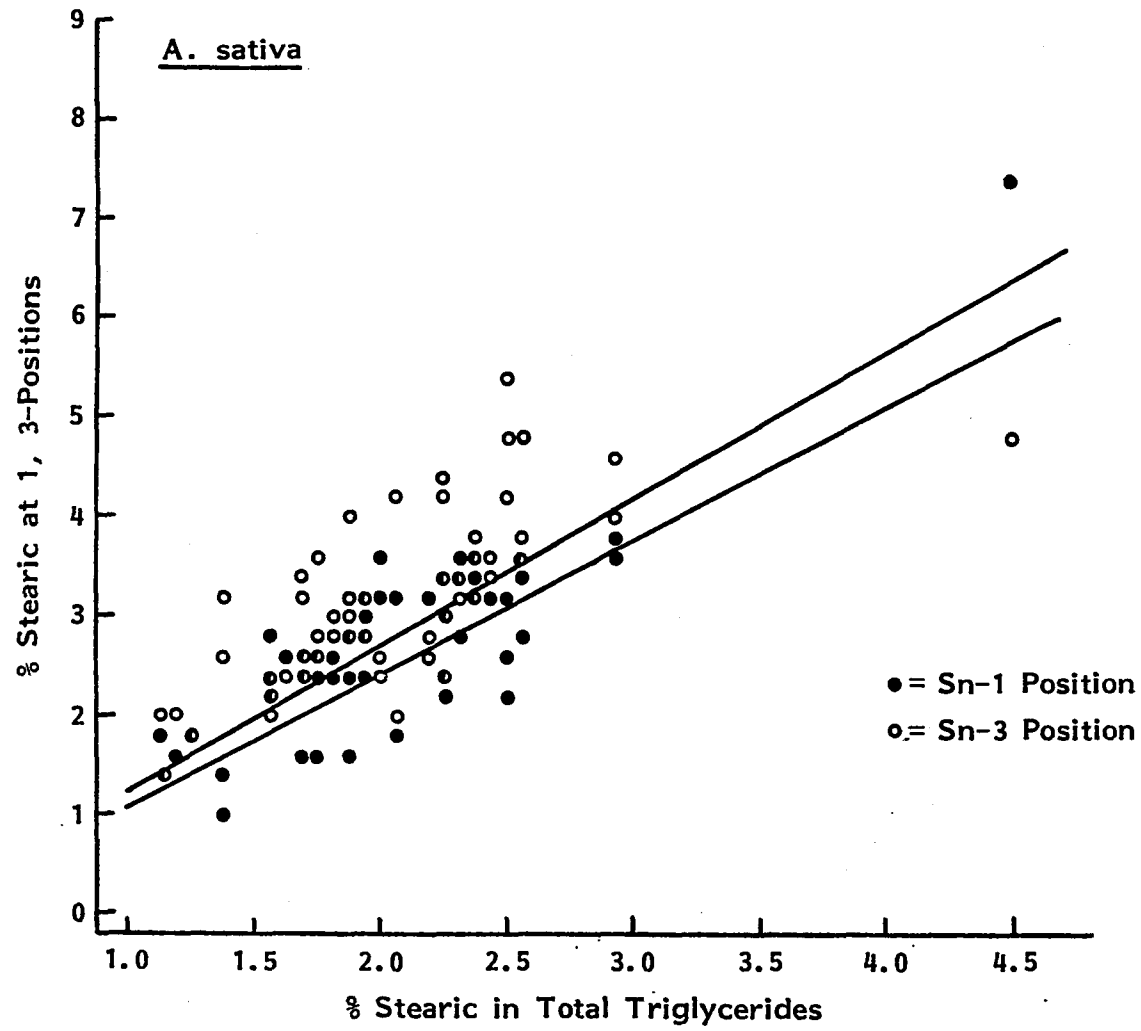


Figure 21. Percentage of oleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of oleic acid in the triglyceride

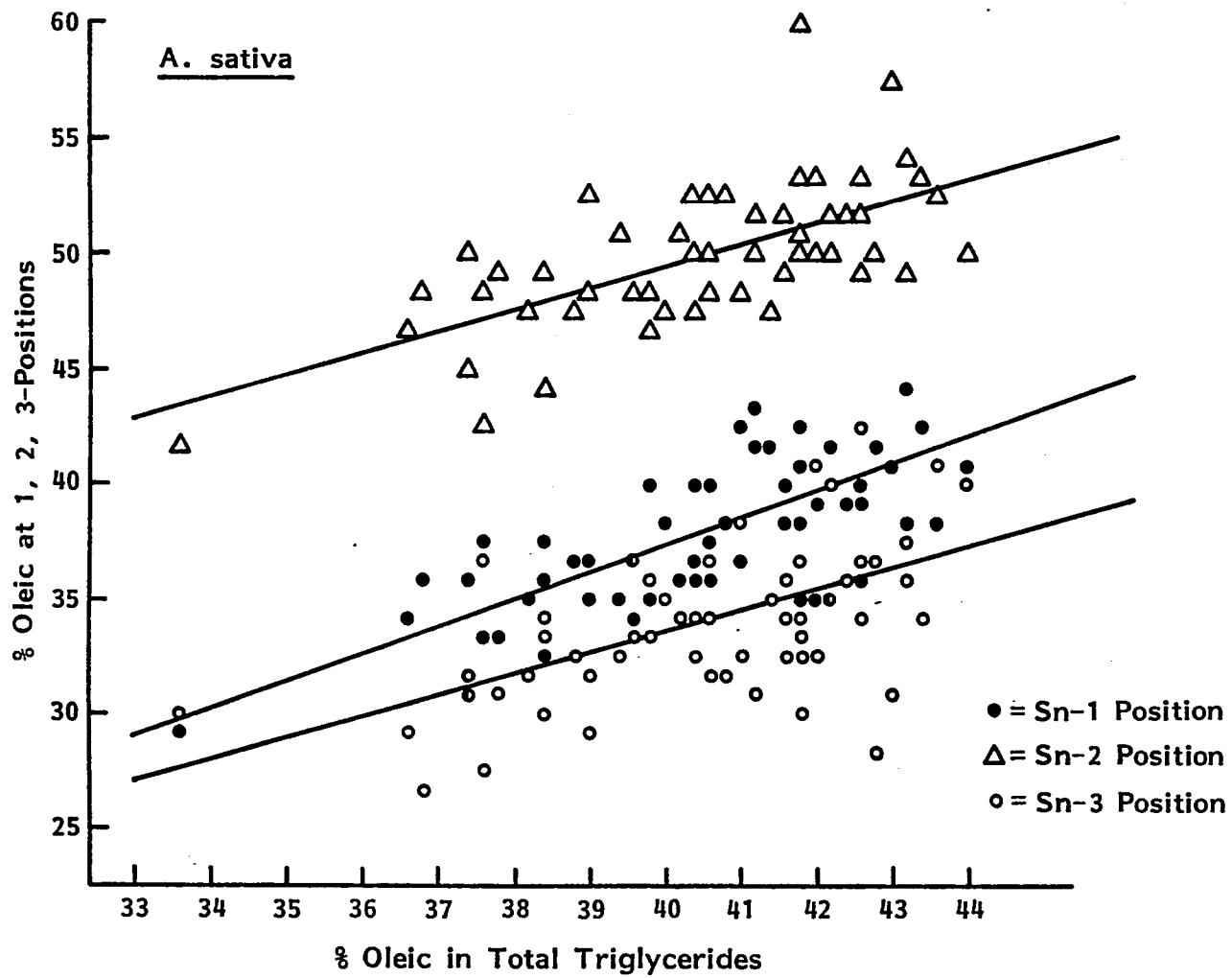


Figure 22. Percentage of linoleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linoleic acid in the triglyceride

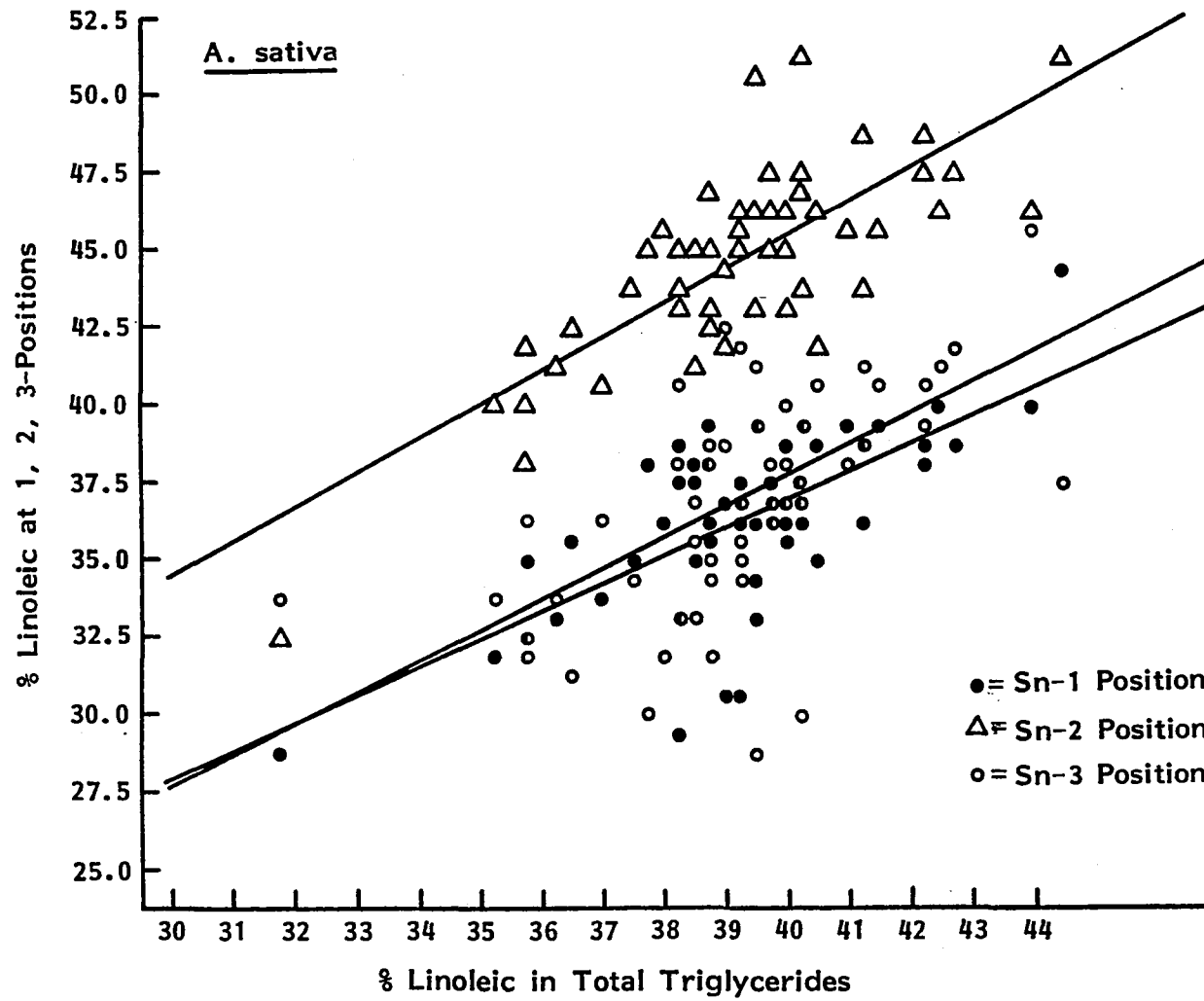


Figure 23. Percentage of linolenic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linolenic acid in the triglyceride

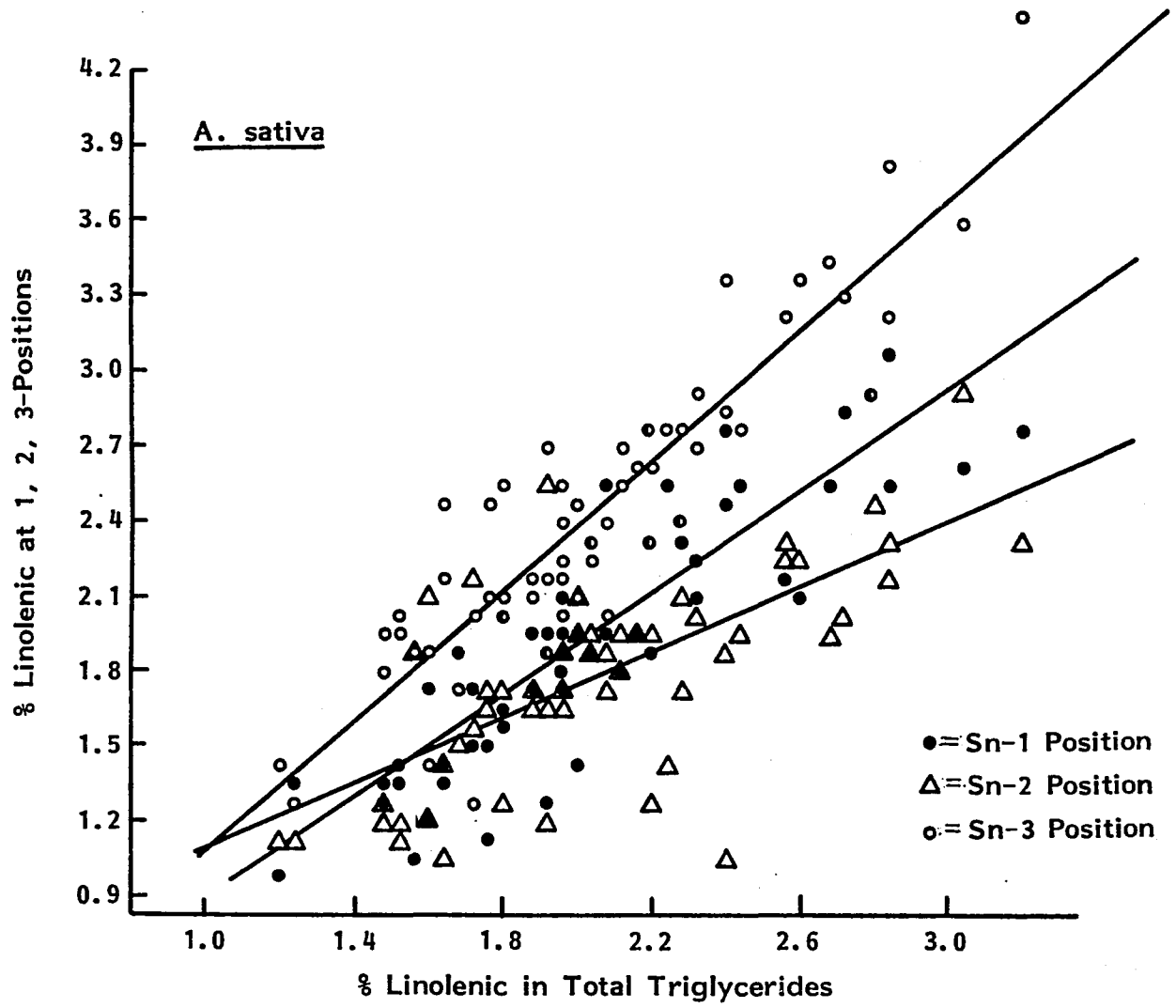


Figure 24. Percentage of palmitic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of palmitic acid in the triglyceride

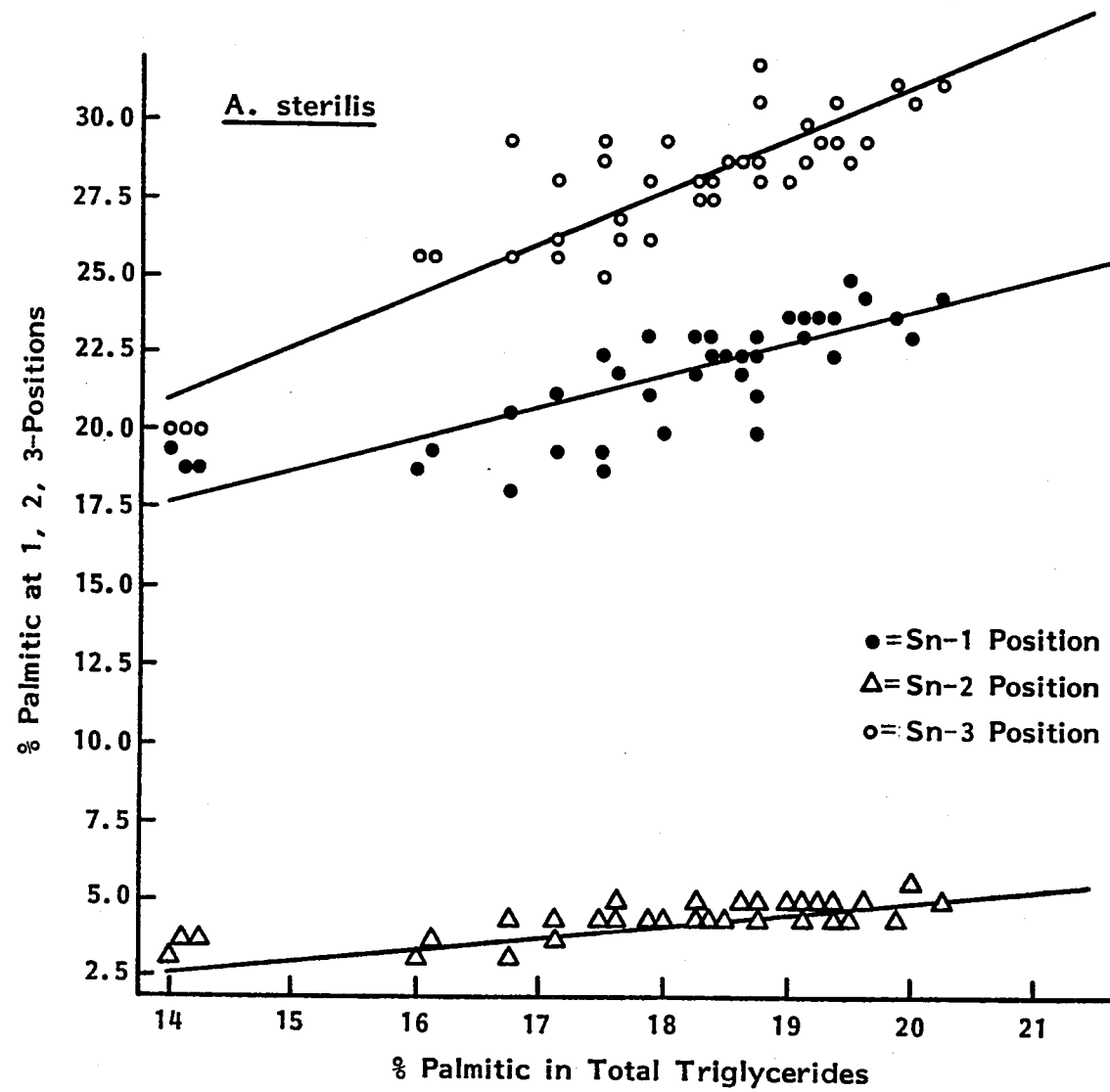


Figure 25. Percentage of stearic acid on the sn-1- and sn-3-positions of glycerol vs. the percentage of stearic acid in the triglyceride

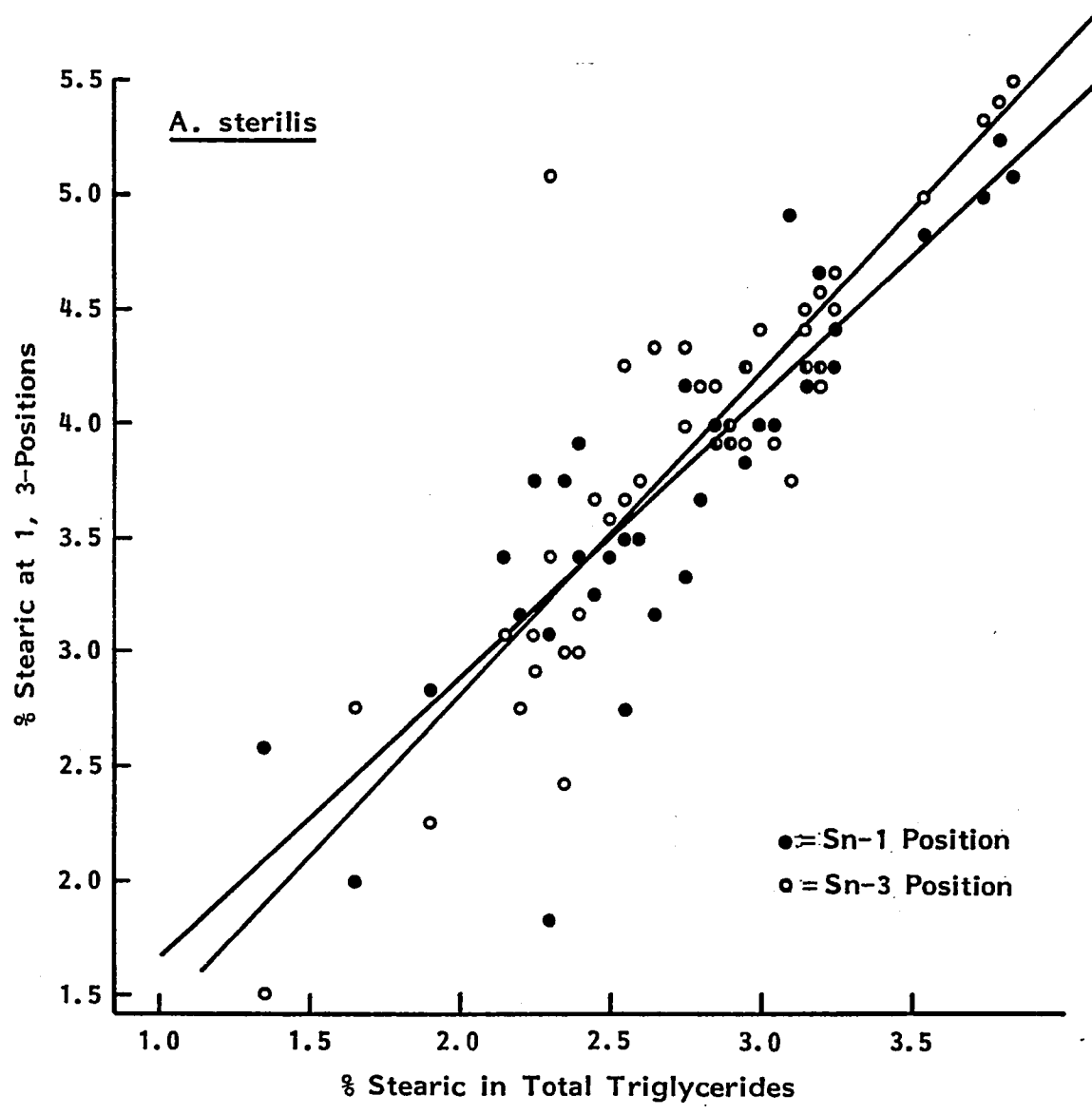


Figure 26. Percentage of oleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of oleic acid in the triglyceride

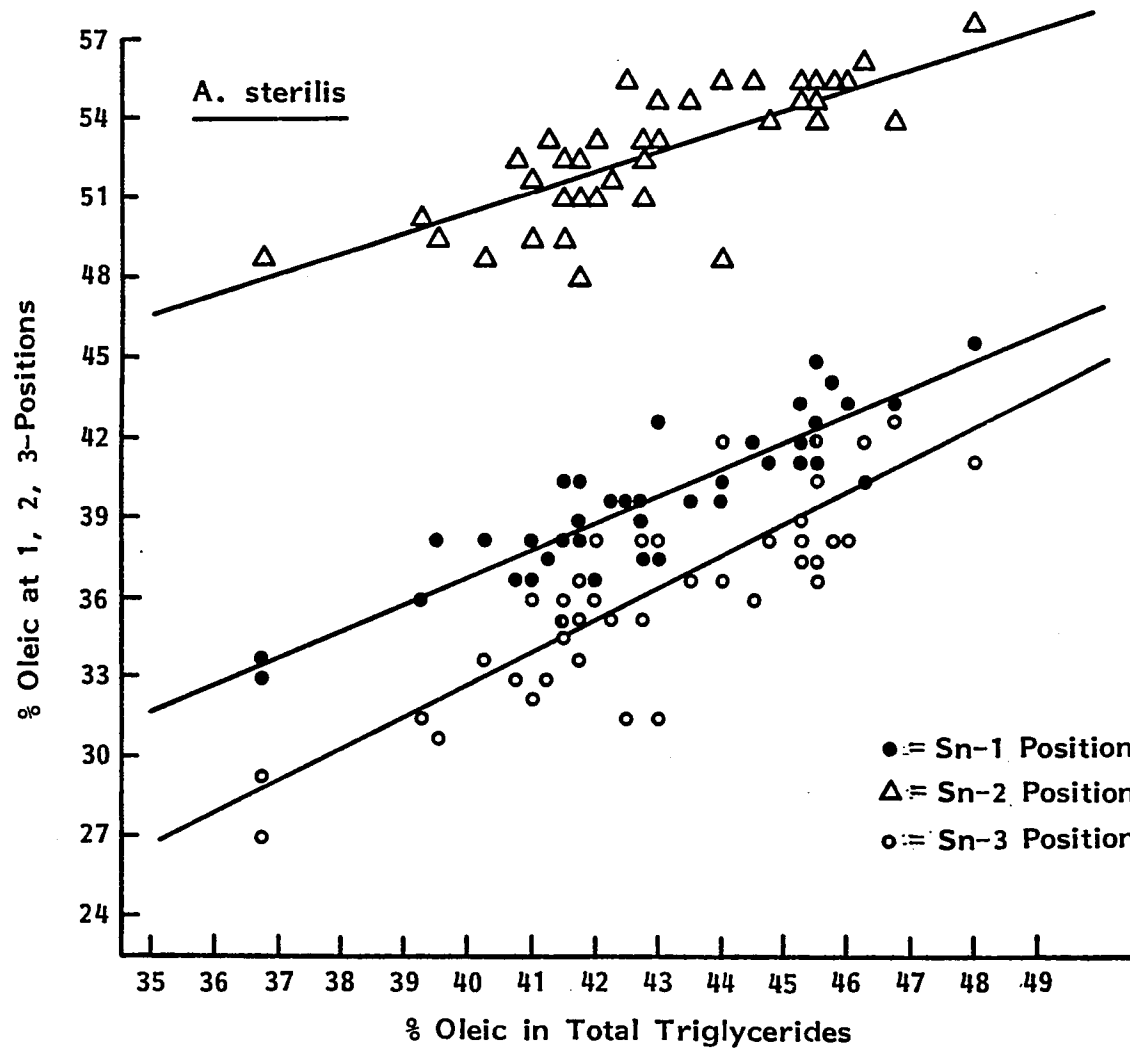


Figure 27. Percentage of linoleic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linoleic acid in the triglyceride

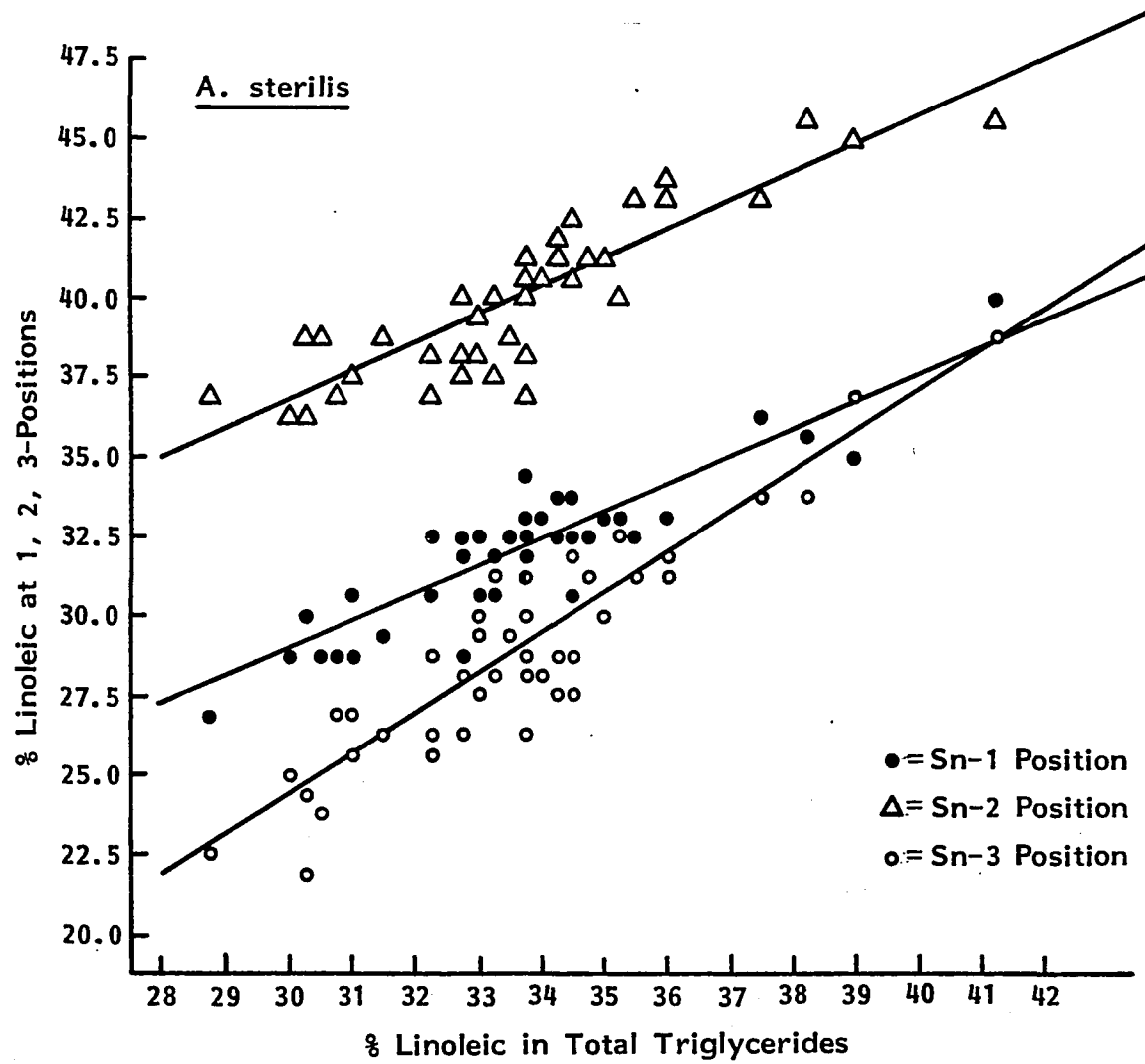


Figure 28. Percentage of linolenic acid on the sn-1-, sn-2-, and sn-3-positions of glycerol vs. the percentage of linolenic acid in the triglyceride

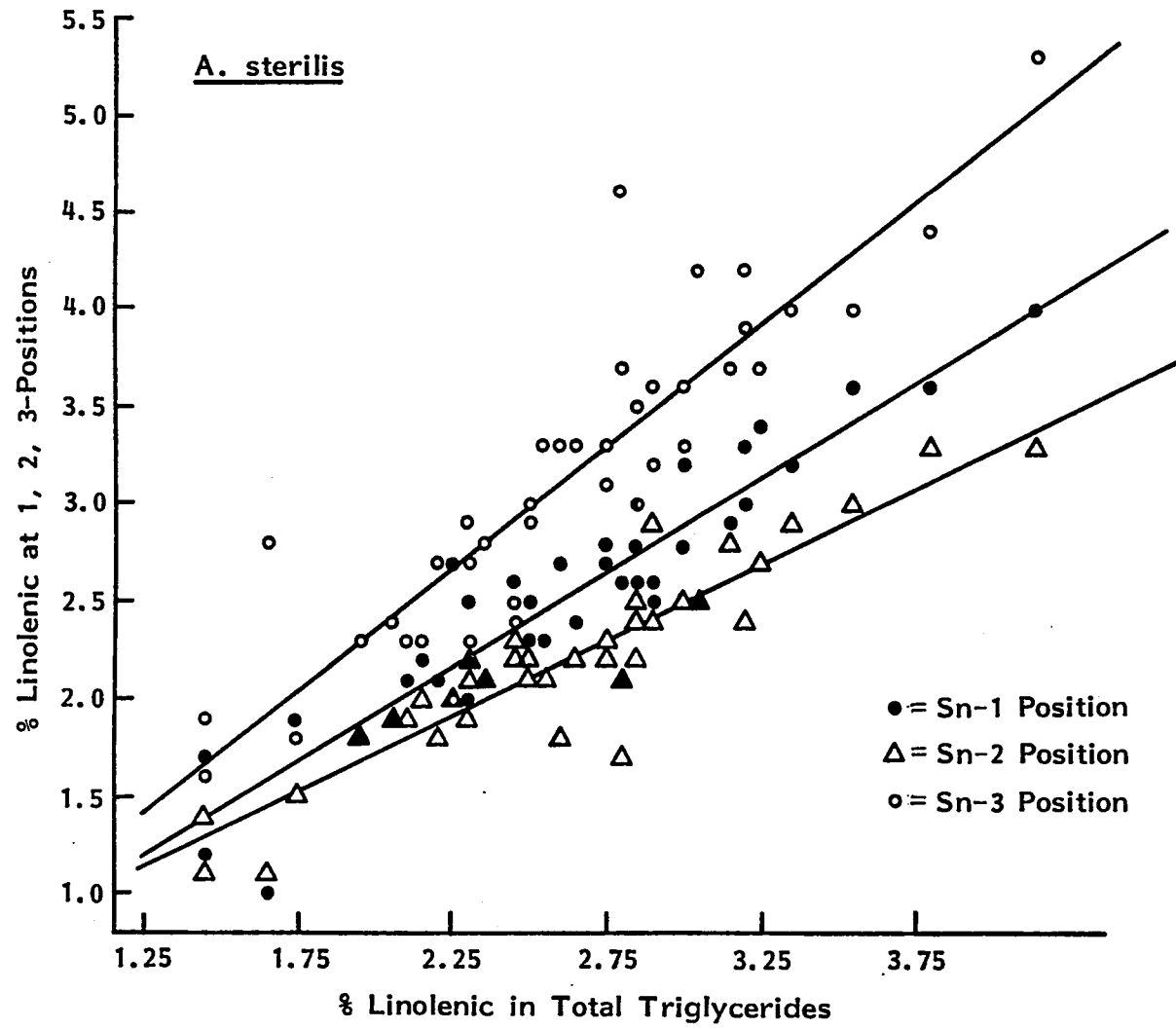


Table 11. Linear regression of the fatty acid compositions on the three positions of triglycerides vs. the fatty acid composition of the whole fat for the oat varieties Avena sativa

Fatty acid	Position	Slope	Intercept	Probability intercept $\neq 0$	R ²	Slope intercept 0	Number of deviants
16:0	1	1.28 \pm 0.09 ^a	0.47 \pm 1.40	0.74	0.79	1.31 \pm 0.01	4 ^b
	2	0.21 \pm 0.03	0.19 \pm 0.42	0.64	0.52	0.22 \pm 0.01	3
	3	1.51 \pm 0.10	-0.66 \pm 1.59	0.68	0.80	1.47 \pm 0.01	4
18:0	1	1.47 \pm 0.11	-0.26 \pm 0.24	0.30	0.74	1.35 \pm 0.03	2
	2	--	--	--	--	--	--
	3	1.10 \pm 0.14	0.92 \pm 0.30	0.004	0.50	1.52 \pm 0.04	2
18:1	1	1.17 \pm 0.16	-9.34 \pm 6.40	0.15	0.49	0.94 \pm 0.01	1
	2	0.93 \pm 0.15	12.37 \pm 5.98	0.04	0.41	1.23 \pm 0.01	3
	3	0.91 \pm 0.17	-2.97 \pm 7.10	0.68	0.32	0.83 \pm 0.01	4
18:2	1	0.91 \pm 0.13	0.45 \pm 5.12	0.93	0.46	0.92 \pm 0.01	3
	2	1.09 \pm 0.12	1.70 \pm 4.87	0.73	0.57	1.14 \pm 0.01	3
	3	1.00 \pm 0.18	-2.31 \pm 7.09	0.75	0.35	0.94 \pm 0.01	3
18:3	1	1.03 \pm 0.08	-0.16 \pm 0.16	0.32	0.75	0.95 \pm 0.02	4
	2	0.66 \pm 0.09	0.42 \pm 0.19	0.03	0.49	0.85 \pm 0.02	5
	3	1.31 \pm 0.08	-0.25 \pm 0.17	0.14	0.82	1.19 \pm 0.02	2

^aThe probability that slope = 0 was 0.0001 in all instances.

^bBased on Proc Univariate (25). Those deviated two standard deviations from the regression lines were counted as deviants.

Table 12. The coefficient of variation (CV) of the deviations for the oat varieties Avena sativa

Position	Fatty acid				
	16:0	18:0	18:1	18:2	18:3
1	4.93	16.73	6.59	5.79	13.27
2	8.86	--	4.67	4.49	16.29
3	5.01	18.30	8.18	7.89	10.98

Table 13. Linear regression of the fatty acid compositions on the three positions of triglycerides vs. the fatty acid composition of the whole fat for the oat varieties Avena sterilis

Fatty acid	Position	Slope	Intercept	Probability intercept $\neq 0$	R ²	Slope intercept 0	Number of deviants
16:0	1	1.02±0.12 ^a	3.56±2.08	0.09	0.66	1.21±0.01	3 ^b
	2	0.31±0.03	-1.20±0.58	0.04	0.70	0.25±0.01	3
	3	1.66±0.13	-2.14±2.35	0.37	0.80	1.54±0.01	2
18:0	1	1.22±0.11	0.43±0.31	0.17	0.75	1.37±0.02	2
	2	--	--	--	--	--	--
	3	1.41±0.12	-0.001±0.33	0.99	0.78	1.41±0.02	2
18:1	1	1.01±0.08	-3.78±3.54	0.29	0.79	0.93±0.01	3
	2	0.78±0.09	19.38±3.94	0.0001	0.64	1.23±0.01	3
	3	1.21±0.12	-15.60±4.96	0.003	0.73	0.85±0.01	3
18:2	1	0.85±0.07	3.35±2.29	0.15	0.79	0.95±0.01	1
	2	0.89±0.07	10.09±2.45	0.0002	0.78	1.19±0.01	1
	3	1.26±0.10	-13.44±3.28	0.0002	0.80	0.86±0.01	2
18:3	1	0.97±0.07	-0.01±0.18	0.95	0.84	0.96±0.01	3
	2	0.77±0.05	0.17±0.14	0.23	0.84	0.84±0.01	2
	3	1.26±0.09	0.16±0.25	0.52	0.82	1.20±0.02	2

^aThe probability that slope = 0 was 0.0001 in all instances.

^bBased on Proc Univariate (25). Those deviated two standard deviations from the regression lines were counted as deviants.

Table 14. The coefficient of variation (CV) of the deviations for the oat varieties Avena sterilis

Position	Fatty Acid				
	16:0	18:0	18:1	18:2	18:3
1	4.98	9.97	3.32	3.34	9.49
2	6.80	--	2.78	2.85	8.60
3	4.43	10.57	5.10	5.30	10.79

than saturated fatty acids. As for soybeans, the best fit regressions give large intercepts which may be caused by the limited range of the data. The slopes forced through zero give a better picture of the preferential placement of each fatty acid on the glycerol. Compared with soybeans, oats have a slight tendency to place more palmitic and stearic acids on sn-3 than sn-1. The reverse was true for soybeans. Oats have a greater tendency to place oleic acid and less tendency to place linoleic and linolenic acids on sn-2 than soybeans. Oats favor linolenic acid on sn-3, soybeans favor sn-1. A. sativa and A. sterilis differ little in glyceride structure pattern.

Table 15 gives the comparison of the ranges of fatty acid composition of A. sativa, A. sterilis and the results of Frey and Hammond (21). The 103 samples examined here

Table 15. Range variations of fatty acid composition for the oat varieties Avena sativa and Avena sterilis

Variety	Fatty acid and range (mole %)				
	16:0	18:0	18:1	18:2	18:3
<u>A. sativa</u>	13-20	1.0-3.0	33-44	32-44	1.2-3.2
<u>A. sterilis</u>	14-21	1.0-4.0	36-48	28-42	1.5-4.0
Frey & Hammond ^a	14-23	<1-4.0	29-53	24-48	<1-5.0

^aFrey, K. J. and E. G. Hammond (21).

have narrower ranges for the major fatty acids (oleic and linoleic acids), while for minor fatty acids (palmitic, stearic and linolenic acids) the ranges are similar. The reason is unknown.

The R^2 gives a general idea how well the regression lines fit in the linear model. The regression fits fairly well for both A. sativa and A. sterilis but not as well as for soybeans G. max and G. soya. The fit is poorest for oleic and linoleic of the A. sativa samples, especially on sn-3.

The CVs show a small variation for major fatty acids in both samples. The big CVs found in the minor fatty acids might be due to analytical error. The CVs for

sn-3 are greater than sn-1 or sn-2, probably because of the accumulation of analytical error in the sn-3 determination. Most of the deviants listed in Tables 11 and 13 depart from the regression by two to three standard deviations (SD). Approximately 12% of the deviants exceeds three SDs, but no extraordinary outlier is found. The number of deviants is similar to those found in soybeans.

Stereospecific Analysis of Palm Bactris gasipaes

Pulp Oil and Kernel Oil

The fruit of the palm Bactris gasipaes is consumed in many places in the American tropics, especially Costa Rica, where it is known as pejebaye. The results of the stereospecific analysis on pulp and kernel oil of pejebaye are given in Tables 16 and 17.

In pulp oil, the unsaturated fatty acids (palmitoleic, oleic, linoleic, and linolenic acids) are more prevalent as shown in Table 16. Oleic and linoleic are concentrated on sn-2-position. Similar to the patterns of soybean and oat oils, the saturated fatty acids (palmitic and stearic acids) are found at very low levels or not at all on the sn-2-position. The fatty acid compositions of the sn-1- and sn-3-positions are found to differ but to resemble each other more than they resemble that of the sn-2-position.

Table 16. Glyceride structure of palm Bactris gasipaes pulp oil

Compound or Position	Fatty acid (mole %)					
	16:0	16:1	18:0	18:1	18:2	18:3
TG	23.17	6.26	1.73	47.15	17.22	4.44
1	33.24	8.18	2.71	35.76	14.37	5.71
2	4.59	1.64	--	68.22	23.96	1.56
3	31.68	8.96	2.48	37.47	13.33	6.05

Table 17. Glyceride structure of palm Bactris gasipaes kernel oil

Compound or position	Fatty acid (mole %)							
	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
TG	0.70	0.72	34.43	30.64	9.18	2.75	17.09	4.48
1	0.71	0.70	27.25	34.76	14.41	4.19	13.97	4.01
2	--	--	46.35	18.95	1.75	0.57	24.35	8.02
3	1.39	1.46	29.69	38.21	11.38	3.49	12.95	1.41

The fatty acid composition of kernel oil as given in Table 17 is quite different from that of pulp oil. In addition to the common fatty acids (palmitic, stearic, oleic and linoleic acids), the kernel oil contains a considerable amount of short- and medium-chained saturated fatty acids (caprylic, capric, lauric and myristic acids). The sn-2-position is enriched in lauric, oleic and linoleic acids. Caprylic, capric, and myristic acids are concentrated on sn-1- and sn-3-positions, particularly the sn-3-position. Palmitic and stearic acids also are concentrated on sn-1- and sn-3-positions, but are more enriched on sn-1-position. These are generally in good agreement with those cited by Litchfield (38). After examining four species of Palmae family, he concluded the preferred positions for capric acid were sn-1 and sn-3, and for lauric acid was sn-2-position.

Overall Conclusions

In all the samples examined (soybeans, oats and palm), a general pattern was found. Saturated fatty acids (except lauric acid in palm kernel oils) were concentrated on sn-1- and sn-3-positions, while linoleic was enriched on sn-2-position.

Each species had considerable variation in fatty acid composition but a fairly consistent glyceride pattern.

The actual factors that select for this consistent pattern are unclear at present, but possibly these patterns may have some advantage to the plants that we do not understand. On the basis of the 1-random-2-random-3-random hypothesis, the Glycine and Avena oils would be enriched in sn-2-linoleodioleins and sn-2-linoleo disaturated glycerides. It is not clear how the enrichment of these glycerides would benefit the plant. Supposedly the pattern results from the specificities of the enzymes involved in glyceride synthesis (30, 54), but the pattern also may be affected by physical factors such as temperature (17, 44) and light.

Even if some of the deviation from the glyceride pattern that was found proves to be genetically controlled, apparently individual plants did not deviate too far from the general pattern. Seemingly, it will not be easy to find major glyceride structure deviants or produce them easily by mutation breeding. Thus, one cannot easily obtain oil samples of similar fatty acid content but different glyceride structure to test what effect glyceride structure has on stability, nutritional value and physical properties.

On the other hand, we do not understand how glyceride structure affects nutritional value or stability well enough to know what structure to breed for. Until these matters are clearer, it is not possible to launch a breeding program to change glyceride pattern.

SUMMARY

The pancreatic lipase deacylation of triglycerides was performed on a single TLC plate without any solubilizer or calcium chloride. Lipase hydrolysis was performed on 100 soybean varieties and a plot of the fatty acid composition of the sn-2-position vs. that of the whole oil was linear. There seemed little natural deviation from the regression lines. Mutagen treatment caused no great production of deviant strains.

Attempts to shorten the stereospecific analysis procedures by using the diglyceride kinase of Escherichia coli to carry out the phosphorylation on a TLC plate were unsuccessful, because the coating media inactivated the kinase activity. Instead, diglycerides were phosphorylated in a test tube and a procedure for the phospholipase A₂ hydrolysis of phospholipids and separation of the products on a single TLC plate was modified and applied to the samples.

Stereospecific analysis was carried out on 48 varieties of Glycine max and 47 varieties of Glycine soya. The results indicated the distribution of fatty acids among the three positions of glycerol molecule was clearly nonrandom. There was little palmitic and stearic acids on the sn-2-position, and the sn-1-position was richer in palmitic, stearic and linolenic acids than sn-3-position. The

sn-3-position was concentrated with oleic acid and the sn-2-position with linoleic acid. When the composition of each position of glycerol was plotted vs. the composition of the whole fat most observations fell into a straight line. The deviants were in the range of two to three standard deviations from the regression lines. The slight difference in slopes and intercepts of the regression lines between G. max and G. soya was probably genetically controlled.

The glyceride structure of oat varieties, which included 60 samples of Avena sativa and 43 samples of Avena sterilis, was determined by stereospecific analysis and showed a nonrandom distribution as well. Oleic and linoleic acids were enriched on sn-2-position, while palmitic, stearic and linolenic acids were concentrated on sn-3-position. Plots of the fatty acid composition of the glycerol positions vs. the composition of the whole fat were linear. Most of the outliers in the plots were between two to three standard deviations off the regression lines. About 12% of the deviants exceeded three standard deviations, but no extraordinary deviants were found.

The stereospecific results of palm pegebaye pulp oil and kernel oil were quite different. In pulp oil, oleic and linoleic acids were concentrated on sn-2-position, while in kernel oil the sn-2-position was enriched in lauric, oleic and linoleic acids and most saturated fatty

acids (caprylic, capric, myristic, palmitic and stearic acids) were concentrated on sn-1- and sn-3-positions.

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APPENDIX

Fatty Acid Analysis of Soybean and
Oat Varieties

- TG1 = percentage of palmitic acid in total triglyceride
TG2 = percentage of stearic acid in total triglyceride
TG3 = percentage of oleic acid in total triglyceride
TG4 = percentage of linoleic acid in total triglyceride
TG5 = percentage of linolenic acid in total triglyceride
MG1 = percentage of palmitic acid on the sn-2-position of
glycerol
MG2 = percentage of stearic acid on the sn-2-position of
glycerol
MG3 = percentage of oleic acid on the sn-2-position of
glycerol
MG4 = percentage of linoleic acid on the sn-2-position of
glycerol
MG5 = percentage of linolenic acid on the sn-2-position of
glycerol
S1 = percentage of palmitic acid on the sn-1-position of
glycerol
S2 = percentage of stearic acid on the sn-1-position of
glycerol
S3 = percentage of oleic acid on the sn-1-position of
glycerol
S4 = percentage of linoleic acid on the sn-1-position of
glycerol
S5 = percentage of linolenic acid on the sn-1-position of
glycerol
N1 = percentage of palmitic acid on the sn-3-position of
glycerol
N2 = percentage of stearic acid on the sn-3-position of
glycerol

- N3 = percentage of oleic acid on the sn-3-position of glycerol
- N4 = percentage of linoleic acid on the sn-3-position of glycerol
- N5 = percentage of linolenic acid on the sn-3-position of glycerol

GLYCERIDE STRUCTURE OF EMS TREATED 77-HARVEST

VARIETY	DBS	TG1	TG2	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5
STEEL 472	1	10.68	2.93	25.14	54.05	7.21	0.88	.	22.98	68.51	7.63
STEEL 473	2	10.36	2.89	26.83	52.06	7.85	0.96	.	24.72	66.09	8.23
STEEL 476	3	10.48	3.10	26.25	52.86	7.31	1.09	.	24.61	66.99	7.31
STEEL 477	4	10.85	3.26	24.53	54.04	7.32	1.22	.	25.98	66.32	6.49
STEEL 478	5	10.34	3.93	24.19	52.91	8.63	2.43	.	22.59	66.73	8.25
STEEL 479	6	10.18	3.36	29.11	50.13	7.22	1.02	.	28.13	65.13	5.72
STEEL 480	7	10.55	2.82	26.39	52.12	8.12	1.19	.	24.11	68.13	6.57
STEEL 481	8	10.60	3.11	25.03	53.37	7.90	1.33	0.08	25.50	65.19	7.90
STEEL 482	9	10.05	2.85	30.00	49.19	7.91	0.98	.	29.34	61.17	8.51
STEEL 483	10	10.56	3.59	26.09	52.41	7.35	1.14	.	25.74	67.93	5.19
STEEL 484	11	10.40	3.18	24.37	53.69	8.36	1.26	.	23.27	67.30	8.16
STEEL 485	12	10.10	3.13	28.02	51.44	7.31	1.24	.	28.01	63.41	7.34
STEEL 486	13	10.17	3.10	30.36	49.49	6.88	1.45	.	30.05	62.24	6.26
STEEL 487	14	10.45	3.18	26.06	51.98	8.34	1.52	.	27.27	62.99	8.23
STEEL 488	15	10.65	3.10	25.75	53.01	7.48	1.55	.	24.78	66.68	6.98
STEEL 489	16	10.00	3.23	28.20	51.62	5.94	1.01	.	27.42	65.15	6.43
STEEL 490	17	11.17	3.56	26.13	52.22	6.92	1.17	.	24.19	67.84	6.80
STEEL 493	18	10.44	3.38	27.65	51.24	7.29	1.05	.	27.11	65.91	5.93
STEEL 494	19	10.28	3.51	27.90	51.06	7.26	0.31	.	27.04	65.43	7.22
STEEL 495	20	10.77	3.27	27.50	51.15	7.30	0.91	.	24.22	66.74	8.12
STEEL 496	21	10.70	2.99	29.01	49.76	7.54	0.88	.	29.27	62.04	7.81
STEEL 497	22	10.20	2.58	29.42	50.75	7.07	0.69	.	28.74	63.88	6.68
STEEL 498	23	10.50	2.36	28.70	50.21	8.22	0.58	.	27.41	63.19	8.83
STEEL 499	24	10.26	2.58	28.60	51.00	7.56	0.54	.	27.76	63.86	7.84
STEEL 500	25	10.28	2.74	27.26	51.67	8.05	0.31	.	25.41	66.57	7.61
STEEL 501	26	10.45	2.96	25.92	54.48	5.19	0.48	.	25.68	68.10	5.74
STEEL 502	27	10.45	2.90	25.98	53.48	7.19	0.85	.	24.85	66.63	7.67
STEEL 503	28	10.65	2.77	27.58	52.05	6.94	0.85	.	26.86	66.02	6.27
STEEL 504	29	10.25	3.18	29.04	50.17	7.36	0.71	.	28.40	65.21	5.68
STEEL 505	30	10.22	2.81	26.23	53.06	7.68	0.95	.	27.03	66.02	6.00
STEEL 506	31	10.43	2.88	27.99	51.33	7.36	0.63	.	27.91	64.76	6.70
STEEL 508	32	9.93	2.88	28.88	51.50	6.81	0.46	.	27.85	65.30	6.39
STEEL 509	33	10.20	2.83	26.91	52.72	7.34	0.50	.	26.20	66.50	6.81
STEEL 510	34	10.17	2.67	26.38	53.32	7.46	0.94	.	25.10	68.24	5.71
STEEL 511	35	10.19	2.85	27.55	51.94	7.46	0.48	.	25.92	66.68	6.93
STEEL 514	36	11.08	2.58	25.78	52.38	8.17	0.98	.	26.24	65.88	6.90
STEEL 515	37	9.61	3.29	25.69	53.62	7.80	0.43	.	26.03	67.83	5.71
STEEL 516	38	8.61	3.40	28.93	50.40	8.66	0.98	.	27.92	63.12	7.97
STEEL 517	39	9.87	3.37	27.84	51.01	7.90	0.72	.	26.81	65.63	6.84
STEEL 518	40	8.29	3.62	28.66	51.49	7.94	0.60	.	28.15	65.60	5.65

GLYCERIDE STRUCTURE OF PLANT INTRODUCTION AMSOY AND CROSS

VARIETY	OBS	TG1	TG2	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5
PI68-788-1	1	11.04	3.63	36.80	42.25	6.28	1.05	.	37.12	55.88	5.95
PI68-788-2	2	10.83	4.14	36.94	41.94	5.15	1.02	.	35.86	56.56	6.56
PI68-788-3	3	11.17	4.08	34.90	43.60	6.26	1.06	.	32.92	59.47	6.55
PI68-788-4	4	11.09	3.83	35.60	43.30	6.17	1.10	.	34.55	58.08	6.27
PI68-788-5	5	12.05	3.49	29.56	48.02	5.88	1.57	.	26.01	65.08	7.34
PI68-788-6	6	11.98	3.59	27.51	49.73	7.19	1.21	.	24.63	67.35	6.82
PI68-788-7	7	12.06	3.53	28.71	48.71	7.00	1.18	.	26.47	65.86	6.50
PI68-788-8	8	11.69	3.37	31.84	46.32	6.77	0.55	.	33.25	59.78	6.42
PI68-788-9	9	11.98	3.49	27.91	49.36	7.26	0.91	.	26.75	65.68	6.66
PI68-788-10	10	11.89	3.69	28.11	48.98	7.34	1.03	.	26.79	65.28	6.89
PI68-788-11	11	11.61	3.46	29.36	48.30	7.27	1.32	.	27.31	63.92	7.46
PI68-788-12	12	10.70	4.07	34.44	44.05	6.74	0.80	.	33.34	59.43	6.42
AMSOY 01	13	10.27	3.34	29.05	49.56	7.79	1.14	.	27.48	63.23	8.16
AMSOY 02	14	10.67	4.42	22.06	54.71	8.14	1.16	.	21.32	70.46	7.06
AMSOY 03	15	10.20	3.41	22.72	57.34	5.33	1.30	.	21.99	70.68	6.03
AMSOY 04	16	9.60	3.59	22.57	56.38	7.86	0.58	.	21.16	72.04	6.22
AMSOY 05	17	10.81	3.19	25.36	52.53	8.11	0.89	.	23.18	68.76	7.17
AMSOY 06	18	10.46	3.30	28.66	50.02	7.56	0.19	.	27.04	66.24	6.53
AMSOY 07	19	10.30	3.41	29.18	49.54	7.58	0.13	.	28.66	64.30	6.90
AMSOY 08	20	11.02	3.16	24.16	53.40	8.26	0.95	.	21.89	70.51	6.65
AMSOY 09	21	10.44	3.81	27.63	50.33	7.99	0.39	.	25.53	66.98	7.10
AMSOY 10	22	10.84	3.45	24.40	53.36	7.94	0.70	.	22.19	69.96	7.16
AMSOY 11	23	10.73	3.55	27.90	49.77	8.05	1.10	.	25.78	64.84	8.27
AMSOY 12	24	10.41	4.07	26.25	50.98	8.29	0.33	.	26.20	66.78	6.70
CROSS 01	25	12.21	3.04	23.88	52.54	8.33	1.62	.	22.50	68.95	6.93
CROSS 02	26	12.22	3.18	24.48	52.39	7.73	1.51	.	24.13	67.84	6.53
CROSS 03	27	13.15	3.09	23.42	51.68	8.66	0.71	.	19.60	72.37	7.31
CROSS 04	28	11.19	4.04	35.52	43.34	5.91	0.46	.	34.22	60.15	5.17
CROSS 05	29	13.42	3.56	24.52	49.92	8.58	1.59	.	21.54	69.28	7.58
CROSS 06	30	10.96	3.29	28.51	50.00	7.25	0.14	.	28.99	64.65	6.22
CROSS 07	31	11.17	3.40	23.50	53.79	9.14	0.28	.	21.19	71.80	6.73
CROSS 08	32	10.89	3.31	32.73	47.92	5.15	0.14	.	31.31	62.30	6.26
CROSS 09	33	10.79	3.01	20.02	56.98	9.03	0.20	.	18.36	73.72	7.72
CROSS 10	34	11.14	3.29	24.13	53.04	8.39	0.85	.	21.97	69.72	7.46
CROSS 11	35	10.53	3.40	27.09	51.01	7.97	0.77	.	24.41	67.51	7.32
CROSS 12	36	10.95	3.77	29.33	48.85	7.10	0.04	.	26.87	66.67	6.42
CROSS 13	37	10.40	3.58	32.05	46.34	7.64	1.46	.	30.85	61.21	6.48
CROSS 14	38	10.46	3.58	22.46	55.05	8.45	0.65	.	21.46	70.81	7.08
CROSS 15	39	11.97	3.13	30.47	46.78	7.64	1.52	.	28.88	62.18	7.41

GLYCERIDE STRUCTURE OF PLANT INTRODUCTION ANSOY AND CROSS

VARIETY	OBS	TG1	TG2	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5
CROSS 16	40	11.01	3.82	37.02	41.07	7.08	0.87	0.23	34.34	57.86	6.70
CROSS 17	41	11.66	3.13	27.67	49.10	8.44	1.35	.	25.03	66.38	7.25
CROSS 18	42	11.20	3.83	30.40	47.78	6.79	1.55	.	28.95	63.97	5.53
CROSS 19	43	10.83	3.49	26.25	51.10	8.33	1.71	0.44	24.67	65.94	7.23
CROSS 20	44	11.44	3.48	30.67	45.97	8.44	1.85	0.45	26.24	63.40	8.06
CROSS 21	45	12.56	3.41	24.85	49.49	9.70	1.25	.	22.85	66.70	9.20
CROSS 22	46	10.95	3.45	27.41	50.64	7.55	1.22	.	25.86	67.02	5.90
CROSS 23	47	11.37	2.88	30.28	47.30	8.17	1.07	.	28.34	62.73	7.85
CROSS 24	48	10.36	3.41	24.02	54.11	8.09	0.65	.	23.13	69.28	6.96
CROSS 25	49	12.74	2.97	27.02	48.09	9.19	2.58	.	23.66	65.65	8.11
CROSS 26	50	10.27	2.38	29.41	50.21	7.72	1.01	.	28.21	64.43	6.35
CROSS 27	51	11.10	3.34	30.93	46.91	7.72	1.04	.	29.77	63.07	6.12
CROSS 28	52	10.98	3.04	27.09	51.01	7.87	0.61	.	28.42	65.59	5.38
CROSS 29	53	11.29	2.81	24.86	52.88	8.15	1.65	.	26.28	66.14	5.93
CROSS 30	54	11.05	2.92	26.54	51.18	8.32	0.57	.	26.65	66.54	6.24
CROSS 31	55	11.37	3.07	26.41	50.80	8.35	0.70	.	24.62	67.85	6.83
CROSS 32	56	11.14	3.06	27.06	50.73	8.00	0.93	0.17	26.23	66.17	6.50
CROSS 33	57	11.14	3.39	27.26	50.46	7.75	1.35	.	24.46	67.15	7.04
CROSS 34	58	11.88	2.60	25.46	51.21	8.84	1.13	.	23.20	67.03	8.64
CROSS 35	59	10.09	2.77	27.77	50.98	8.40	1.54	.	26.48	64.97	7.01
CROSS 36	60	11.53	3.25	26.94	49.71	8.58	0.63	.	24.36	66.26	8.75

GLYCERIDE STRUCTURE OF GLYCINE MAX

VARIETY	OBS	TG1	TG2	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5	S1	S2	S3	S4	S5	N1	N2	N3	N4	N5
PI54.604	1	10.52	2.54	19.50	59.97	7.45	0.74	.	17.74	74.57	6.93	18.41	4.04	17.16	52.25	8.11	12.41	3.58	23.60	53.09	7.31
PI54.607	2	9.05	4.98	26.96	53.40	5.58	0.68	0.14	25.12	68.84	5.20	16.12	8.74	23.81	45.17	6.14	10.35	6.06	31.95	46.19	5.40
PI54.608	3	9.31	3.73	33.76	47.64	5.53	0.64	0.11	30.78	63.41	5.04	15.98	6.10	31.03	40.51	6.36	11.31	4.98	39.47	39.00	5.19
PI54.619	4	9.14	4.93	26.04	53.80	6.07	0.71	.	23.85	69.74	5.68	16.28	8.01	22.41	45.43	6.86	10.43	6.78	31.86	46.23	5.67
PI54.809	5	10.49	2.92	25.85	53.75	6.96	0.72	.	24.67	68.15	6.44	17.14	4.76	23.31	46.42	7.74	13.61	4.01	29.57	46.68	6.70
PI60.279	6	9.57	3.40	27.72	53.18	6.11	0.67	.	25.83	67.90	5.58	16.75	5.34	23.84	47.18	6.87	11.29	4.86	33.49	44.46	5.88
PI63.271	7	10.52	3.33	26.50	52.63	6.99	0.74	.	22.37	70.37	6.50	18.31	5.57	23.78	44.34	7.98	12.51	4.42	33.35	43.18	6.49
PI65.338	8	9.95	4.24	23.79	55.08	6.92	0.66	0.22	23.44	69.43	6.23	17.42	6.84	20.46	47.55	7.71	11.77	5.66	27.47	48.26	6.82
PI65.341	9	9.63	3.42	33.80	47.03	6.10	0.71	0.12	30.97	62.21	5.97	16.95	5.46	29.07	41.71	6.79	11.23	4.68	41.36	37.17	5.54
PI65.346	10	9.65	3.78	33.57	47.21	5.77	0.67	.	32.71	61.28	5.32	16.63	6.23	29.17	41.43	6.52	11.65	5.11	38.83	38.92	5.47
PI68.457	11	10.56	3.85	27.07	51.86	6.64	0.78	.	25.89	67.04	6.27	17.58	6.18	23.82	44.91	7.49	13.32	5.37	31.50	43.63	6.16
PI68.461	12	10.71	3.65	22.21	56.26	7.15	0.74	0.10	19.84	72.46	6.84	18.94	5.84	20.61	46.51	8.08	12.45	5.01	26.18	49.81	6.53
PI68.465	13	9.85	3.44	20.89	57.86	7.94	0.77	.	20.13	71.25	7.83	16.83	5.57	17.99	50.82	8.77	11.98	4.75	24.55	51.51	7.22
PI68.466	14	9.91	3.37	19.33	58.89	8.46	0.68	.	19.17	72.15	7.98	19.34	6.94	16.14	48.13	9.43	9.71	3.17	22.68	56.39	7.97
PI68.475	15	10.56	3.76	22.25	54.87	8.53	0.71	.	20.14	71.05	8.08	18.83	5.04	19.05	47.92	9.14	12.14	6.24	27.56	45.64	8.37
PI68.562	16	9.51	3.51	27.15	53.64	6.16	0.75	.	23.66	69.79	5.78	16.75	5.83	23.94	46.49	6.97	11.03	4.70	33.85	44.64	5.73
PI68.564	17	10.62	3.99	24.59	54.26	6.51	0.72	0.08	22.52	70.51	6.15	18.44	6.37	21.41	46.60	7.16	12.70	5.52	29.84	45.67	6.22
PI68.572	18	10.52	3.50	18.46	59.59	7.91	0.73	.	14.89	76.46	7.90	18.52	6.71	17.58	48.93	8.24	12.31	3.79	22.91	53.38	7.59
PI68.576	19	9.83	3.92	25.43	54.18	6.66	0.69	0.12	23.92	69.24	6.01	17.92	6.04	22.96	45.81	7.25	11.03	5.30	29.41	47.49	6.72
PI68.585	20	9.32	3.87	25.71	54.38	6.70	0.74	.	23.97	68.95	6.32	16.13	6.31	21.76	48.60	7.18	11.09	5.30	31.40	45.59	6.60
PI69.501	21	9.15	3.47	25.13	55.50	6.73	0.63	0.12	23.65	69.30	6.28	16.84	5.40	21.62	48.18	7.94	9.98	4.89	30.12	49.02	5.97
PI69.503	22	9.85	3.27	31.95	48.65	6.86	0.66	.	29.44	63.56	6.32	16.83	5.83	26.38	43.18	7.76	12.06	3.98	40.03	39.21	6.50
PI69.507	23	8.95	4.53	25.89	53.67	6.74	0.67	0.18	23.84	68.92	6.37	16.46	7.14	22.62	46.24	7.52	9.72	6.27	31.21	45.85	6.33
PI69.512	24	9.89	3.42	27.48	52.79	6.39	0.72	.	23.86	69.45	5.95	17.02	5.41	24.63	45.90	7.02	11.93	4.85	33.95	43.02	6.20
PI69.532	25	9.91	2.57	19.44	60.21	7.86	0.75	.	18.87	72.58	7.78	17.54	3.98	17.14	52.34	8.98	11.44	3.73	22.31	55.71	6.82
PI70.016	26	9.69	4.11	26.13	52.63	7.52	0.64	.	22.93	69.46	6.95	15.48	6.65	23.74	45.62	8.49	12.68	5.68	31.72	42.81	7.12
PI70.017	27	11.42	3.96	26.10	52.45	6.05	0.88	0.11	23.85	69.46	5.68	18.49	5.96	22.64	45.95	6.94	14.89	5.81	31.81	41.94	5.53
PI70.021	28	10.58	4.77	25.18	53.27	6.17	0.73	0.16	24.61	68.65	5.83	18.35	7.16	21.76	46.07	6.64	12.66	6.99	29.17	45.09	6.04
PI70.027	29	11.27	4.47	26.37	51.56	6.30	0.74	0.18	24.51	68.70	5.85	19.70	6.98	23.76	42.48	7.06	13.37	6.25	30.84	43.50	5.99
PI70.036	30	10.46	3.84	31.53	46.55	7.59	0.72	.	27.66	64.75	6.85	18.32	6.52	28.14	38.83	8.17	12.34	5.01	38.79	36.07	7.75
PI72.328	31	10.42	3.81	26.25	52.09	7.42	0.81	.	24.40	67.86	6.91	18.92	6.14	23.75	42.71	8.46	11.53	5.29	30.60	45.70	6.89
PI72.341	32	9.78	4.34	23.30	56.35	6.24	0.68	.	22.43	70.93	5.94	16.88	7.17	19.74	49.21	6.98	11.78	5.85	27.73	48.91	5.80
PI72.342	33	9.83	3.65	35.15	44.87	6.49	0.64	0.09	31.14	62.28	5.83	17.20	5.96	31.22	38.22	7.38	11.65	4.90	43.09	34.11	6.26
PI73.585	34	10.07	3.82	29.03	50.40	6.68	0.73	.	26.85	66.15	6.25	18.12	6.12	26.49	42.11	7.14	11.36	5.34	33.75	42.94	6.65
PI79.712	35	11.13	4.06	29.11	47.88	7.83	0.82	.	27.16	65.02	6.98	18.37	6.67	25.02	41.44	8.48	14.20	5.51	35.15	37.18	8.03
PI79.737	36	11.64	4.29	22.12	54.45	7.49	0.84	.	21.25	70.92	6.97	20.39	6.76	19.81	44.54	8.48	13.69	6.11	25.30	47.89	7.02
PI79.745	37	11.49	4.47	22.79	53.96	7.29	0.78	0.24	20.97	71.20	6.97	19.98	7.19	19.61	45.07	8.13	13.71	5.98	27.79	45.61	6.77
PI79.746	38	11.99	3.72	25.01	53.18	6.09	0.83	.	22.91	70.38	5.86	21.68	6.24	22.50	42.85	6.98	13.46	4.92	29.62	46.58	5.43
PI81.035	39	11.67	3.91	26.15	50.85	7.40	0.86	0.13	23.95	68.26	6.78	20.12	6.16	22.98	42.09	8.63	14.03	5.44	31.52	42.20	6.79
PI81.037-4	40	11.35	3.61	30.89	48.08	6.06	0.79	.	26.92	66.84	5.43	20.64	5.74	26.57	40.11	6.93	12.62	5.09	39.18	37.29	5.82
PI81.040	41	9.93	4.27	36.52	43.77	5.43	0.67	.	30.44	63.86	5.01	17.85	6.98	31.41	37.72	6.02	11.42	5.83	47.71	29.73	5.26
PI81.763	42	10.32	4.83	33.18	46.59	5.06	0.73	0.24	30.19	63.94	4.86	18.46	7.91	28.54	39.25	5.82	11.77	6.34	40.81	36.58	4.48
PI81.765	43	11.17	3.14	24.44	53.50	7.72	0.75	.	22.83	69.12	7.28	19.33	5.43	23.41	43.65	8.16	13.43	3.99	27.08	47.73	7.72
PI84.668	44	11.85	3.57	19.64	57.31	7.59	0.84	0.11	17.99	73.69	7.35	21.35	5.82	16.89	48.04	7.88	13.39	4.78	24.04	50.20	7.54
PI84.688-1	45	10.73	2.98	33.15	47.27	5.84	0.71	.	31.32	62.53	5.42	17.67	5.16	29.59	40.95	6.61	13.81	3.78	38.54	38.33	5.49
PI84.673	46	10.55	2.84	33.04	47.49	6.06	0.69	.	28.26	65.39	5.64	18.16	4.77	28.41	41.71	6.93	12.80	3.75	42.45	35.37	5.61
PI84-674	47	11.51	3.58	24.50	53.71	6.68	0.80	0.14	23.49	69.14	6.41	21.14	5.86	19.97	45.68	7.33	12.59	4.74	30.04	46.31	6.30
PI84-681	48	11.23	3.89	22.36	55.76	6.69	0.82	.	21.47	71.37	6.32	19.37	6.43	20.12	46.55	7.51	13.65	5.24	25.49	49.36	6.24

GLYCERIDE STRUCTURE OF GLYCINE SOYA

VARIETY	OBS	T31	T32	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5	S1	S2	S3	S4	S5	N1	N2	N3	N4	N5
339.732	1	10.86	3.50	17.77	56.09	11.75	0.84	.	18.84	70.62	9.68	19.67	5.55	16.71	44.81	13.24	12.07	4.95	17.76	52.84	12.33
339.735	2	10.57	3.41	17.58	56.28	12.13	0.74	.	17.06	71.21	10.97	19.76	5.34	18.19	44.40	12.29	11.21	4.89	17.49	53.23	13.13
339.871	3	11.04	3.46	18.09	53.86	13.52	0.68	0.14	18.41	70.44	10.31	21.73	5.15	16.64	40.50	15.96	10.71	5.09	19.22	50.64	14.29
378.702	4	11.17	3.02	18.41	56.27	11.10	0.81	.	18.96	69.34	10.87	22.32	5.16	16.87	42.92	12.71	10.38	3.90	19.40	56.55	9.72
391.587	5	10.56	3.38	13.96	58.94	13.04	0.72	.	14.15	74.50	10.61	19.41	6.31	12.71	46.72	14.83	11.85	3.83	15.02	55.60	13.68
393.551	6	11.56	3.89	16.94	54.94	12.64	0.91	0.08	16.73	70.28	11.98	23.14	5.97	15.38	41.62	13.87	10.63	5.62	18.71	52.92	12.07
406.684	7	10.75	2.99	17.00	57.49	11.73	0.76	.	18.31	69.94	10.97	20.16	5.14	16.48	45.35	12.85	11.33	3.83	16.21	57.18	11.37
407.019	8	11.16	3.32	14.00	54.44	17.06	0.83	.	15.33	69.60	14.22	21.42	5.83	12.58	40.27	19.88	11.23	4.13	14.09	53.45	17.08
407.020	9	11.15	4.84	16.64	54.49	12.85	0.78	0.21	16.31	73.25	9.43	21.63	6.58	16.14	39.69	15.94	11.04	7.73	17.47	50.53	13.18
407.025	10	10.83	3.70	16.10	56.58	12.77	0.79	.	16.98	70.28	11.93	19.74	6.13	14.87	43.38	15.86	11.96	4.97	16.45	56.08	10.52
407.037	11	11.82	3.56	17.55	57.56	9.49	0.94	.	16.25	73.26	9.53	22.58	5.74	17.26	43.28	11.12	11.94	4.94	19.14	56.14	7.82
407.042	12	14.36	5.11	22.14	46.11	12.25	0.98	0.24	23.06	66.79	8.91	24.14	7.62	20.74	34.22	13.26	17.96	7.47	22.62	37.32	14.58
407.048	13	11.96	3.56	20.59	52.77	11.08	0.88	.	19.63	70.35	9.12	22.36	6.64	17.66	39.58	13.74	12.64	4.04	24.48	48.38	10.38
407.054	14	13.42	3.95	20.97	50.89	10.74	0.72	.	22.46	66.84	9.96	23.31	6.16	19.81	37.95	12.75	16.23	5.69	20.64	47.88	9.51
407.055	15	13.00	3.50	15.71	55.11	12.66	0.89	0.11	17.36	70.35	11.27	25.03	5.69	14.84	39.48	14.94	13.08	4.70	14.93	55.50	11.77
407.062	16	13.74	4.22	20.10	49.68	12.23	0.93	0.11	20.88	67.18	10.88	26.33	6.54	18.92	34.33	13.86	13.96	6.01	20.50	47.53	11.95
407.070	17	11.63	3.86	18.89	55.18	10.42	0.76	.	17.34	72.05	9.83	20.61	6.27	17.81	43.37	11.92	13.52	5.31	21.52	50.12	9.51
407.080	18	14.61	4.99	29.29	42.03	9.05	0.98	0.16	31.32	59.87	7.65	23.95	8.24	26.85	29.97	10.97	18.90	6.57	29.70	36.25	8.53
407.083	19	11.79	3.44	19.14	54.33	11.28	0.84	0.07	19.91	68.13	11.03	21.54	5.47	17.76	42.27	12.94	12.99	4.78	19.75	52.59	9.87
407.087	20	12.26	4.08	20.28	50.40	12.96	0.88	.	18.91	68.61	11.58	22.13	6.51	18.54	37.19	15.61	13.77	5.73	23.39	45.40	11.69
407.107	21	12.36	3.22	14.69	54.00	15.70	0.87	.	15.72	68.41	14.98	22.94	5.64	13.63	40.46	17.31	13.27	4.02	14.72	53.13	14.81
407.161	22	12.88	3.24	18.85	52.07	12.93	0.83	0.13	18.60	68.65	11.77	22.45	5.21	17.15	40.91	14.26	15.36	4.38	20.80	46.65	12.76
407.167	23	12.65	3.25	22.53	48.56	12.99	0.91	.	23.66	63.55	11.86	22.73	5.88	20.76	35.13	15.48	14.31	3.87	23.17	47.00	11.63
407.180	24	13.10	3.60	18.97	52.41	11.90	0.89	.	18.84	68.31	11.94	24.85	5.73	16.32	38.94	14.14	13.56	5.07	21.75	49.98	9.62
407.182	25	11.34	3.03	25.47	47.76	12.38	0.91	.	27.88	60.16	11.03	18.62	5.96	22.18	39.36	13.86	14.49	3.13	26.35	43.76	12.25
407.185	26	12.66	3.26	20.27	51.38	12.40	0.77	.	21.10	67.05	11.06	22.68	5.81	18.94	38.72	13.83	14.53	3.97	20.77	48.37	12.31
407.188	27	11.72	3.74	18.83	52.34	13.34	0.76	.	17.85	68.49	12.88	22.33	5.78	18.53	38.66	14.68	12.07	5.44	20.11	49.87	12.46
407.195	28	11.54	3.50	16.93	55.49	12.51	0.79	0.14	17.61	69.31	12.13	22.82	5.69	15.63	41.98	13.86	11.01	4.67	17.55	55.18	11.54
407.200	29	11.00	2.65	16.84	55.97	13.52	0.74	.	15.75	72.03	11.46	21.96	4.06	16.32	41.88	15.76	10.30	3.89	18.45	54.00	13.34
407.207	30	11.99	3.81	19.55	52.20	12.42	0.86	0.09	22.34	65.82	10.87	21.84	6.98	16.39	40.12	14.65	13.27	4.36	19.92	50.66	11.74
407.212	31	12.18	3.84	15.89	54.50	13.57	0.83	.	16.25	70.02	12.88	23.16	6.82	14.54	39.70	15.76	12.55	4.70	16.88	53.78	12.07
407.221	32	11.65	3.14	19.50	52.21	13.47	0.87	0.13	20.82	66.43	11.73	21.21	4.81	16.68	41.30	15.98	12.87	4.48	21.00	48.90	12.70
407.230	33	12.02	3.09	20.85	52.00	12.02	0.86	.	18.64	69.52	10.96	21.17	4.65	18.67	43.31	12.18	14.03	4.62	25.24	43.17	12.92
407.242	34	11.90	2.64	18.56	52.63	14.25	0.81	.	19.38	65.93	13.86	23.85	4.97	17.18	36.57	17.41	11.04	2.95	19.12	55.39	11.48
407.243	35	11.28	2.87	17.26	52.68	15.89	0.93	.	19.75	65.28	14.02	22.31	4.50	15.84	39.38	17.95	10.60	4.11	16.19	53.38	15.70
407.248	36	11.87	2.91	14.47	56.75	13.98	0.83	.	15.88	69.83	13.44	22.64	4.78	14.62	41.45	16.49	12.14	3.95	12.91	58.97	12.01
407.255	37	10.82	2.61	16.95	55.18	14.42	0.79	.	16.26	69.10	13.83	18.71	4.06	15.43	44.86	16.92	12.96	3.77	19.16	51.58	12.51
407.266	38	11.82	2.93	16.42	55.77	13.04	0.77	0.07	17.08	71.93	10.13	22.53	4.56	14.98	41.03	16.88	12.16	4.16	17.20	54.35	12.11
407.272	39	12.29	3.47	19.75	51.66	13.10	0.96	0.12	22.54	63.70	12.66	24.63	5.67	18.21	36.33	15.14	11.28	4.62	18.50	54.95	11.50
407.281	40	10.53	3.25	18.94	55.54	11.71	0.81	.	18.69	69.03	10.45	19.21	5.57	17.11	45.13	12.96	11.57	4.18	21.02	52.46	11.72
407.286	41	10.27	2.80	14.46	60.63	11.81	0.69	.	15.83	71.80	11.66	19.95	4.96	15.15	45.96	13.96	10.17	3.44	12.40	64.03	9.81
407.290	42	9.09	2.63	15.77	59.60	12.87	0.71	.	15.84	71.49	11.94	17.54	5.12	14.53	47.65	15.14	9.02	2.77	16.94	59.66	11.53
407.297	43	9.75	3.07	16.79	58.68	11.69	0.68	.	17.10	71.83	10.37	17.63	4.72	16.73	46.69	14.21	10.94	4.49	16.54	57.52	10.49
407.303	44	10.66	3.50	19.38	56.36	10.07	0.74	0.06	20.51	68.71	9.96	19.67	5.95	18.64	43.84	11.88	11.57	4.49	18.99	56.53	8.37
407.304	45	9.17	3.21	17.19	61.26	9.15	0.63	.	17.34	73.87	8.14	16.51	6.28	15.46	51.97	9.76	10.37	3.35	18.77	57.94	9.55
423.988	46	11.01	3.39	17.24	58.06	10.27	0.93	.	18.95	70.44	9.66	22.65	5.62	16.86	43.09	11.76	9.45	4.55	15.91	60.65	9.39
423.991	47	10.66	3.44	19.16	57.84	8.88	0.81	.	19.83	72.22	7.12	19.78	5.41	16.48	48.45	9.86	11.39	4.91	21.17	52.85	9.66

GLYCERIDE STRUCTURE OF AVENA SATIVA

VARIETY	OBS	TG1	TG2	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5	S1	S2	S3	S4	S5	N1	N2	N3	N4	N5
GRUNDY	1	17.81	1.37	43.37	36.16	1.26	3.87	.	53.43	41.52	1.16	21.86	1.43	42.25	33.10	1.34	27.70	2.68	34.43	33.86	1.28
Y286-23	2	18.12	1.66	42.92	35.80	1.47	3.43	.	57.28	37.98	1.28	23.41	1.69	40.77	32.75	1.36	27.52	3.29	30.71	36.37	1.77
Y286-323	3	17.67	1.68	42.52	35.28	2.80	3.74	.	53.31	40.21	2.51	23.14	1.56	40.24	32.10	2.94	26.19	3.48	33.81	33.53	2.95
Y349-23	4	17.17	2.05	40.60	37.59	2.56	2.98	0.92	50.31	43.46	2.31	22.06	3.16	37.64	34.96	2.16	26.47	2.07	33.85	34.35	3.21
CI8044	5	18.99	1.90	38.41	38.57	2.10	4.24	0.33	48.96	44.71	1.74	24.47	2.38	32.69	37.91	2.53	28.26	2.99	33.58	33.09	2.03
CI9170	6	18.22	2.17	39.58	37.95	2.05	3.96	0.86	47.96	45.33	1.87	23.51	3.12	34.48	36.53	2.34	27.19	2.53	36.30	31.99	1.94
Y492-3-12	7	17.15	2.56	39.65	38.81	1.81	3.58	.	48.51	46.64	1.25	22.49	2.89	36.83	35.71	2.06	25.38	4.79	33.61	34.08	2.12
Y502-2-13	8	14.93	2.31	41.91	38.85	1.92	2.78	.	53.48	42.50	1.22	19.68	3.49	39.44	35.53	1.84	22.48	3.44	32.81	38.52	2.70
B652-13	9	16.24	1.16	43.65	36.48	2.45	3.48	.	52.11	42.42	1.97	21.67	1.56	38.26	35.91	2.58	23.57	1.92	40.58	31.11	2.80
B652-253	10	16.13	1.77	41.77	38.26	2.04	3.36	.	49.88	44.87	1.86	24.59	1.62	42.59	29.20	1.98	20.44	3.69	32.84	40.71	2.28
B657-39	11	15.81	2.22	41.27	39.10	1.58	3.66	0.43	49.86	44.15	1.87	23.38	2.11	43.09	30.38	1.02	20.39	4.12	30.86	42.77	1.85
B657-170	12	15.09	1.70	42.29	38.17	2.73	3.58	.	51.55	42.84	2.02	20.68	2.67	35.02	38.73	2.88	21.01	2.43	40.30	32.94	3.29
B657-555	13	14.82	1.39	42.72	39.28	1.77	3.04	.	49.66	45.59	1.69	17.28	0.95	50.03	30.61	1.11	24.14	3.22	28.47	41.64	2.51
B654-67	14	18.10	1.90	33.62	44.44	1.91	4.11	.	42.01	51.32	2.54	23.64	1.66	28.77	44.62	1.28	26.55	4.04	30.08	37.38	1.91
B654-279	15	17.87	2.28	37.66	40.13	2.02	4.03	.	42.36	51.47	2.12	22.52	3.41	33.54	39.07	1.43	27.12	3.43	37.08	29.85	2.51
B656-55	16	14.49	2.04	42.51	38.73	2.21	3.72	.	51.81	43.20	1.24	18.12	1.89	38.80	38.42	2.75	21.63	4.23	36.92	34.57	2.64
B656-85	17	17.96	2.49	38.39	39.40	1.73	3.42	.	43.97	50.39	2.19	19.92	2.12	37.14	39.08	1.72	30.54	5.35	34.06	28.73	1.28
B656-98	18	14.81	1.79	41.85	39.87	1.65	2.49	.	51.23	45.22	1.04	18.12	2.41	40.78	37.21	1.46	23.82	2.96	33.54	37.18	2.45
B656-110	19	14.84	1.94	42.75	38.50	1.95	2.99	0.13	50.19	44.92	1.74	19.11	2.46	41.28	35.03	2.10	22.42	3.23	36.78	35.55	2.01
B655-189	20	14.27	1.10	43.95	39.14	1.52	3.46	.	50.39	44.96	1.16	18.36	1.36	41.19	37.61	1.46	20.99	1.94	40.27	34.85	1.94
B655-203	21	14.80	2.55	42.13	38.31	2.18	3.54	0.36	50.18	43.96	1.93	20.18	3.45	41.51	32.86	1.97	20.68	3.84	34.70	38.11	2.64
D609-14	22	15.31	1.95	41.70	38.63	2.39	3.34	.	50.07	44.69	1.87	19.65	2.71	38.65	36.51	2.46	22.94	3.14	36.38	34.69	2.84
D622-12	23	18.90	2.23	39.08	36.90	2.86	3.86	0.71	52.58	40.51	2.31	24.96	2.98	35.24	33.75	3.04	27.88	3.00	29.42	36.44	3.23
D623-13	24	15.16	1.87	41.63	39.50	1.81	3.17	.	51.82	43.23	1.75	19.34	2.84	40.22	35.94	1.65	22.97	2.77	32.85	39.33	2.03
D623-15	25	15.79	1.87	41.99	38.69	1.63	3.68	0.04	49.70	45.16	1.40	21.77	2.38	35.29	39.20	1.33	21.92	3.19	40.98	31.71	2.16
D623-17	26	15.15	2.16	41.21	39.57	1.87	3.21	0.48	51.38	43.18	1.72	19.61	3.14	41.30	34.18	1.74	22.66	2.86	30.95	41.35	2.15
D623-18	27	16.09	1.83	40.65	39.13	2.28	3.59	.	48.23	46.42	1.74	21.45	2.46	37.43	36.33	2.31	23.23	3.03	36.29	34.64	2.79
B709-15	28	14.96	1.10	41.88	40.45	1.59	2.73	.	53.47	41.66	2.11	20.16	1.83	37.97	38.81	1.21	21.99	1.47	34.20	40.88	1.45
B709-20	29	14.53	2.38	40.67	40.11	2.24	3.27	0.50	49.92	44.88	1.45	18.75	3.13	40.06	35.51	2.53	21.72	3.51	32.03	39.99	2.74
B709-38	30	14.31	1.25	43.16	39.55	1.71	3.21	.	48.75	46.42	1.60	18.98	1.86	44.52	33.14	1.48	20.74	1.89	36.21	39.09	2.05
B709-43	31	15.37	1.67	39.97	39.92	3.05	3.36	.	47.23	46.50	2.89	20.82	2.33	38.08	36.12	2.63	21.93	2.68	34.60	37.14	3.63
B709-58	32	13.59	1.96	42.66	40.26	1.51	3.57	.	51.48	43.74	1.19	18.16	3.14	39.70	37.63	1.35	19.04	2.74	36.80	39.41	1.99
B709-85	33	16.07	1.73	42.52	37.77	1.89	3.98	.	49.22	45.13	1.65	21.76	2.46	35.46	38.37	1.93	22.47	2.76	42.88	29.81	2.09
B709-98	34	15.31	1.56	40.72	39.99	2.41	3.48	.	52.45	42.97	1.08	19.94	2.23	38.11	36.93	2.77	22.51	2.45	31.60	40.07	3.38
B709-103	35	15.60	1.78	40.29	39.98	2.33	3.70	.	51.12	43.17	1.99	20.93	2.41	35.87	38.44	2.28	22.17	2.88	33.88	38.33	2.72
B709-123	36	13.77	1.57	41.50	41.33	1.79	3.15	.	51.68	43.89	1.26	18.87	2.46	38.45	38.65	1.54	19.35	2.25	34.37	41.45	2.57
B709-225	37	14.05	2.96	41.63	39.33	2.01	2.87	0.98	48.78	45.41	1.94	18.14	3.89	39.99	35.97	1.98	21.14	4.01	36.12	36.61	2.11
B709-227	38	16.35	2.03	40.36	39.12	2.11	3.78	0.26	52.25	41.86	1.83	21.92	3.16	36.14	36.95	1.81	23.35	2.67	32.69	38.55	2.69
B709-248	39	15.12	2.44	40.97	39.49	1.97	3.23	0.48	48.47	46.17	1.62	19.41	3.16	42.35	33.19	1.87	22.72	3.68	32.09	39.11	2.42

GLYCERIDE STRUCTURE OF AVENA SATIVA

VARIETY	OBS	TG1	TG2	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5	S1	S2	S3	S4	S5	N1	N2	N3	N4	N5
B709-252	40	16.01	2.37	37.68	42.74	1.19	3.14	0.07	48.31	47.33	1.13	19.35	3.17	37.56	38.89	1.01	25.54	3.87	27.17	42.00	1.43
B709-261	41	16.32	2.39	39.79	39.35	2.12	3.56	0.37	48.50	45.60	1.95	22.50	3.65	35.18	36.81	1.83	22.90	3.15	35.69	35.64	2.58
B709-271	42	15.92	2.35	37.37	42.14	2.21	3.96	0.76	44.82	48.52	1.92	20.53	3.13	36.07	38.34	1.91	23.27	3.16	31.22	39.56	2.80
B709-286	43	15.94	2.95	40.59	38.54	1.95	4.16	0.95	52.46	41.13	1.63	21.65	3.68	35.43	37.53	1.69	22.01	4.58	33.88	36.96	2.53
B709-291	44	16.33	2.43	38.82	40.32	2.07	3.42	0.61	47.28	46.82	1.84	21.60	3.21	36.32	36.87	1.98	23.97	3.47	32.86	37.27	2.39
B709-296	45	14.49	2.34	36.77	44.08	2.29	2.87	0.32	48.55	46.11	2.13	18.35	3.52	35.48	40.26	2.37	22.25	3.18	26.28	45.87	2.37
B709-366	46	15.50	2.56	40.41	39.81	1.70	3.29	0.35	49.86	44.95	1.53	21.18	3.65	37.08	36.23	1.84	22.03	3.68	34.29	38.25	1.73
D226-30-3	47	17.17	1.65	38.97	40.59	1.60	3.89	.	48.73	46.14	1.21	23.96	2.57	36.80	34.91	1.73	23.66	2.38	31.38	40.72	1.86
D226-30-8	48	17.69	1.73	37.71	40.93	1.92	3.74	.	49.23	45.34	1.67	22.94	2.43	33.40	39.26	1.95	26.39	2.76	30.50	38.19	2.14
D277-32-2	49	16.83	2.30	43.28	35.76	1.76	3.41	0.77	54.21	39.91	1.68	21.83	2.86	38.53	35.24	1.52	25.40	3.27	37.10	32.13	2.08
D277-32-6	50	16.85	2.38	42.39	35.69	2.67	3.66	0.62	51.94	41.77	1.98	22.15	3.41	39.26	32.58	2.58	24.74	3.11	35.97	32.72	3.45
D227-35-5	51	17.43	2.25	39.37	38.87	2.05	3.86	0.93	50.65	42.57	1.97	23.84	3.48	35.06	35.73	1.87	24.59	2.34	32.40	38.31	2.31
D236-29-4	52	20.47	4.51	41.77	31.73	1.49	4.87	1.43	59.88	32.56	1.23	27.45	7.33	35.07	28.85	1.28	29.09	4.77	30.36	33.78	1.96
LANG	53	14.73	1.95	39.86	41.22	2.21	3.05	0.07	46.39	48.51	1.96	18.17	2.96	39.59	36.52	2.33	22.97	2.82	33.20	38.63	2.34
NOBLE	54	14.97	1.59	38.27	42.55	2.59	3.76	.	47.58	46.38	2.25	19.33	2.84	35.41	40.27	2.13	21.82	1.93	31.82	41.00	3.39
N.M.	55	15.22	2.03	41.05	39.71	1.96	3.36	0.16	48.42	46.16	1.88	21.61	3.51	36.44	36.63	1.79	20.69	2.42	38.29	36.34	2.21
OGLE	56	16.81	1.79	36.57	42.26	2.55	3.49	.	46.65	47.58	2.26	22.71	2.66	33.76	38.66	2.19	24.23	2.71	29.30	40.54	3.20
STOUT	57	19.95	2.53	37.38	38.15	1.95	4.63	0.11	49.87	43.69	1.67	26.34	3.19	30.93	37.56	1.96	28.91	4.29	31.34	33.20	2.22
B605-1085	58	15.97	1.74	38.30	41.62	2.34	3.24	.	49.38	45.35	2.01	20.61	2.63	35.49	39.14	2.11	24.06	2.59	30.03	40.37	2.90
LANCER	59	14.21	2.48	40.30	40.13	2.86	3.02	.	47.24	47.56	2.15	18.93	2.61	39.89	35.97	2.58	20.68	4.83	33.77	36.86	3.85
B605-1805	60	13.27	2.24	41.41	39.85	3.21	2.87	.	47.46	47.28	2.36	17.18	2.38	41.47	36.14	2.81	19.76	4.34	35.30	36.13	4.46

GLYCERIDE STRUCTURE OF AVENA STERILIS

VARIETY	OBS	TG1	TG2	TG3	TG4	TG5	MG1	MG2	MG3	MG4	MG5	S1	S2	S3	S4	SS	N1	N2	N3	N4	N5
PI282731	1	17.50	1.92	45.92	32.87	1.77	4.57	0.67	55.64	37.56	1.53	22.77	2.80	43.62	29.85	1.94	25.16	2.29	38.50	32.20	1.84
PI320837	2	18.83	2.35	45.78	31.12	1.93	4.69	0.83	55.28	37.32	1.75	19.97	3.74	44.12	30.39	1.76	31.74	2.38	37.94	25.65	2.28
PI324750	3	14.21	1.35	43.93	38.16	2.32	3.54	.	49.06	45.45	1.92	18.91	2.55	40.86	35.49	2.17	20.18	1.50	41.87	33.54	2.87
PI324804	4	14.13	2.80	41.75	39.00	2.29	3.67	0.57	48.23	45.28	2.22	18.97	3.69	40.55	34.81	1.96	19.75	4.14	36.47	36.91	2.69
PI324819	5	16.11	2.65	45.44	33.72	2.06	3.47	0.45	55.73	38.39	1.93	19.37	3.16	42.81	32.75	1.89	25.49	4.34	37.78	30.02	2.36
PI411540	6	14.01	1.67	46.86	35.22	2.13	3.14	0.31	54.25	40.31	1.96	19.35	1.96	43.68	32.83	2.16	19.81	2.74	42.65	32.52	2.27
PI411580	7	20.21	3.10	39.36	34.49	2.80	4.88	0.60	50.35	42.41	1.73	24.65	4.91	35.91	32.45	2.06	31.10	3.79	31.82	28.61	4.61
PI411971	8	16.79	2.30	45.39	33.84	1.66	2.95	.	54.62	41.29	1.12	18.14	1.84	44.68	34.28	1.03	29.28	5.06	36.87	25.95	2.83
PI412364	9	17.83	2.57	48.11	30.01	1.46	4.35	0.76	57.42	36.37	1.07	21.14	2.73	45.75	28.64	1.72	28.00	4.22	41.16	25.02	1.59
PI412365	10	19.96	3.03	41.60	33.92	1.47	5.68	1.12	51.34	40.45	1.38	23.35	4.02	38.21	33.23	1.17	30.85	3.95	35.25	28.08	1.86
PI412428	11	17.44	2.39	45.20	32.36	2.58	4.08	0.74	55.19	38.17	1.79	19.02	3.41	42.08	32.81	2.66	29.22	3.02	38.33	26.10	3.29
PI412443	12	17.51	2.18	45.21	32.87	2.20	4.37	0.70	54.77	38.29	1.84	19.27	3.13	43.17	32.34	2.07	28.89	2.71	37.69	27.98	2.69
PI324761	13	19.34	3.82	40.65	33.67	2.50	4.78	0.79	52.39	39.84	2.17	23.82	5.23	36.54	32.11	2.28	29.42	5.44	33.02	29.06	3.05
PI324732	14	15.95	3.24	41.62	36.10	3.06	3.14	0.78	49.66	43.86	2.54	18.87	4.43	40.78	33.42	2.48	25.84	4.51	34.42	31.02	4.16
PI324733	15	18.95	2.47	41.70	33.07	3.78	4.87	0.51	52.86	38.43	3.31	23.64	3.26	38.59	30.85	3.64	28.34	3.64	33.65	29.93	4.39
PI412237	16	18.53	3.17	39.57	35.90	2.81	4.31	0.83	49.85	42.86	2.13	22.32	4.18	38.11	32.82	2.56	28.96	4.50	30.75	32.02	3.74
PI411996	17	18.60	3.74	41.17	33.59	2.88	4.74	0.85	53.29	38.71	2.39	22.14	5.03	37.46	32.70	2.65	28.92	5.34	32.76	29.36	3.60
PI324818	18	17.66	3.25	40.21	35.53	3.33	4.84	0.77	48.64	42.86	2.87	21.91	4.29	37.92	32.71	3.16	26.23	4.69	34.07	31.02	3.96
PI318002	19	19.67	3.22	45.24	28.66	3.19	4.98	0.81	55.01	36.74	2.44	24.65	4.28	41.34	26.72	2.98	29.38	4.57	39.37	22.52	4.15
PI411942	20	18.23	2.27	41.65	34.95	2.88	4.81	.	50.78	41.46	2.93	21.86	3.76	38.71	33.14	2.51	28.02	3.05	35.46	30.25	3.20
PI317995	21	17.64	3.56	45.62	30.39	2.76	4.23	0.83	54.08	38.56	2.28	21.61	4.86	42.07	28.76	2.68	27.08	4.99	40.71	23.85	3.32
PI318001	22	19.66	3.20	43.49	30.80	2.84	5.13	0.75	54.44	37.13	2.53	24.35	4.63	39.57	28.46	2.97	29.50	4.22	36.46	26.81	3.02
PI412480	23	17.10	2.76	36.80	41.24	2.09	4.08	0.14	48.57	45.34	1.85	21.16	4.13	32.72	39.83	2.14	26.06	4.01	29.11	38.55	2.28
PI412645	24	18.64	2.38	44.68	31.44	2.84	4.84	.	53.99	38.91	2.23	22.63	3.94	41.45	29.16	2.79	28.45	3.20	38.60	26.25	3.50
PI412724	25	17.63	3.14	42.37	33.81	2.98	4.53	0.96	51.66	40.36	2.47	21.64	4.21	40.04	31.27	2.83	26.87	4.25	35.41	29.80	3.64
PI412227	26	19.14	2.85	45.51	30.15	2.34	4.58	0.66	53.68	38.96	2.08	22.98	3.98	41.01	29.87	2.15	29.86	3.91	41.84	21.62	2.79
PI317710	27	18.75	2.73	40.88	34.48	3.13	4.54	0.51	49.81	42.36	2.76	23.32	3.36	36.60	33.81	2.89	28.42	4.32	36.23	27.27	3.74
PI317716	28	18.31	2.96	41.95	34.29	2.44	4.46	0.72	50.98	41.64	2.18	22.80	3.87	36.93	33.76	2.62	27.76	4.29	37.94	27.47	2.52
PI411642	29	17.96	3.14	42.38	33.74	2.76	4.11	0.73	55.74	37.16	2.24	20.25	4.28	40.05	32.72	2.69	29.52	4.41	31.35	31.34	3.35
PI324800	30	19.09	2.17	46.20	30.27	2.24	4.98	.	56.52	36.51	1.97	23.84	3.43	40.19	29.80	2.71	28.45	3.08	41.89	24.50	2.04
PI282780	31	17.89	2.52	42.78	34.28	2.49	4.34	0.61	51.32	41.53	2.18	22.86	3.39	38.69	32.77	2.27	26.47	3.56	38.33	28.54	3.02
PI411615	32	16.78	3.22	41.65	34.80	3.54	4.37	0.84	50.85	40.96	2.96	20.63	4.63	38.66	32.43	3.63	25.34	4.19	35.44	31.01	4.03
PI317712	33	19.48	2.55	41.46	33.77	2.73	4.67	0.41	52.75	39.88	2.26	24.76	3.54	35.59	33.25	2.84	29.01	3.70	36.04	28.18	3.09
PI317739	34	18.73	2.62	42.92	33.14	2.56	4.51	0.63	55.07	37.68	2.08	20.96	3.51	42.51	30.65	2.35	30.72	3.72	31.18	31.09	3.25
PI317733	35	19.84	2.32	44.48	31.03	2.30	4.63	0.45	55.14	37.62	2.14	23.81	3.10	41.93	28.68	2.46	31.08	3.41	36.37	26.79	2.30
PI317747	36	19.31	2.88	41.89	33.05	2.84	4.76	0.76	52.96	39.14	2.36	23.74	3.94	37.04	32.65	2.61	29.43	3.94	35.67	27.36	3.55
PI324800	37	17.07	2.27	44.09	32.36	4.17	4.11	0.13	55.30	37.16	3.28	21.51	3.75	39.89	30.86	3.97	25.65	2.93	37.08	29.06	5.26
PI324759	38	18.21	3.86	42.78	32.65	2.46	4.37	0.98	52.55	39.74	2.34	22.85	5.11	37.59	31.81	2.62	27.41	5.49	38.20	26.40	2.42
PI324749	39	19.38	3.01	36.70	37.62	3.25	4.65	0.66	49.03	42.93	2.71	22.56	3.98	34.08	35.97	3.39	30.93	4.39	26.99	33.96	3.65
PI317729	40	18.77	2.89	42.77	33.04	2.51	4.81	0.71	53.19	39.16	2.11	22.52	3.96	40.09	30.85	2.55	28.98	4.00	35.03	29.11	2.87
PI282777	41	17.14	2.87	45.13	32.19	2.65	3.98	0.59	54.97	38.23	2.21	19.57	3.88	41.35	32.74	2.44	27.87	4.14	39.07	25.60	3.30
PI324816	42	18.42	2.34	42.95	33.28	2.99	4.56	0.27	52.94	39.72	2.48	23.41	3.71	37.83	31.87	3.16	27.29	3.04	38.08	28.25	3.33
PI411501	43	18.41	2.96	40.90	34.50	3.21	4.42	0.73	51.74	40.73	2.36	22.96	4.24	38.58	30.86	3.34	27.85	3.91	32.38	31.91	3.93