December 7, 1976

To the Graduate Council:

I am submitting herewith a thesis written by Richard Edward Moyers entitled "Lipids of Defatted Soy-Flours During Storage." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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S. L. Melton, Major Professor

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and recommend its acceptance:

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LIPIDS OF DEFATTED SOY-FLOURS DURING STORAGE

A Thesis

Presented for the

Master of Science

Degree

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Richard Edward Moyers

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ABSTRACT

The objective of this study was to investigate the effect of storage on lipids present in defatted soy-flours (fully toasted, white and enzyme active).

Five replicates of each soy-flour were stored for 0, 60, 120 and 180 days at 23°C and then analyzed for lipoxygenase activity, nitrogen solubility index, thiobarbituric acid number, and percent extractable lipid. Both enzyme active and white flours had lipoxygenase activity; the enzyme active flour had the highest activity. Lipoxygenase activity of the enzyme active soy flour decreased at an increasing rate during storage. TBA number decreased during storage and thus was of little value in measuring the amount of oxidation that had occurred. The average percent of extractable lipid material decreased from approximately 3.0 to 2.8 percent during storage.

Lipid extracts were separated into three fractions by silicic acid column chromatography by eluting lipids with 300 ml portions of chloroform-methanol 20:1, chloroform-methanol 1:1, and methanol. The type of lipids in each fraction was analyzed by TLC and the fatty acid distribution was determined by GLC. Approximately 30, 57 and 14 percent of the lipids were eluted into Fractions I, II, and III, respectively. Fraction I lipids consisted mainly of glycerides, glycolipids, and steroids while Fraction II lipids consisted mainly of phospholipids, glycolipids, and steroids. As expected, lipid in Fraction III was almost pure lecithin. Of significance in the fatty acid analysis, the

relative percent of linoleic acid in the fully toasted and white flour decreased during storage while the relative percent of linoleic acid in the enzyme active flour did not change. A considerably lower initial level of linoleic acid was found in the enzyme active flour than in the fully toasted and white flours.

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CHAPTER I

INTRODUCTION

During the past decade, increased attention has been devoted to the use of numerous plant protein supplements as a potential source of additional protein in human food. The soybean, in particular, has received much attention because of the quantity produced, the developed technology associated with its processing, its low cost, and its high nutritive value. Although the soybean possesses numerous desirable attributes, soybean protein products have a major problem with development of objectionable off-flavors. Characteristic off-flavors of soybean protein products generally have been described as bitter or beany.

The total cause of the off-flavor has not been determined, but past research has indicated that lipid degradation by both enzymatic and autoxidative means contributes heavily. Enzymatic oxidation of lipids by lipoxygenase has been shown to be important in the generation of off-flavor compounds during the processing of soybeans under high moisture conditions. Less is known of the effect of lipoxygenase on lipids when soybeans are processed under low moisture conditions as in the commercial extraction of oil.

Since many of the soybean protein products are produced as a by-product of the oil extraction process, lipoxygenase action during this process is given great importance. Equally important to certain soy protein products is whether lipoxygenase plays a significant role in lipid oxidation during storage.

This study investigates the storage effects on lipids extracted by a chloroform-methanol extraction procedure from a lipoxygenase active defatted flour, a defatted soy-flour processed with minimum heat, and a toasted defatted soy-flour with no lipoxygenase activity.

CHAPTER II

LITERATURE REVIEW

I. LIPOXYGENASE ACTION AND ITS RELATION TO SOY FLAVOR PROBLEMS

Past work has shown that lipoxygenase catalyzes the oxidation of fatty acids possessing cis methylene interrupted double bonds to respective hydroperoxides (37). Dolev et al. (10) proved that the oxygen introduced into the hydroperoxide molecules comes from the gaseous phase and not from the aqueous phase.

The enzyme's molecular weight has been estimated at 102,000 by Theorell et al. (41) and 108,000 by Stevens et al. (34). Lipoxygenase's isoelectric point has been estimated at pH 5.4 by Theorell et al. (41) and pH 5.65 by Catsimpoolas (7).

The optimum pH for lipoxygenase activity has been shown to be dependent on whether the enzyme was in a crude extract or in the crystalline form. For crystalline lipoxygenase, investigators reported an optimum pH of 9.0 when sodium linoleate was the substrate (15, 41). Other pH optima, reported for crude extracts, were later proved to result from multiple forms of lipoxygenase in soybeans (8, 9, 14, 15, 45). These other forms, isoenzymes or isozymes, have been reported to exhibit either greater activity for free linoleic acid or to show greater activity for linoleic acid in the triglyceride form in addition to the free fatty acid (8, 19, 45).

Off-flavor in soybean protein products has been connected with lipid oxidation (11, 20, 30, 31, 43). The oxidation reactions that are catalyzed by lipoxygenase are believed to be responsible for a considerable amount of the off-flavor problem (21, 24, 43, 44). Investigations have shown that soy-flour produced from soybeans in which the lipoxygenase was inactivated before or soon after dehulling had a significantly better flavor score than normally treated soy-flour (2, 24, 25, 27).

The type of lipids which can be utilized by lipoxygenase as a substrate might have some effect on the flavor produced. Lecithin has been questioned as a source of bitter off-flavor in soybean protein products (31). It has not been reported whether oxidation of lecithin can be catalyzed by lipoxygenase.

II. ASSAYS OF LIPOXYGENASE ACTIVITY

Lipoxygenase activity has been assayed by spectrophotometric methods for the measurement of lipid oxidation products (19, 35, 40) and by measurement of oxygen uptake of the substrate by various techniques (23, 38, 42). The spectrophotometric methods are used to measure either the increase in conjugated diene formation at 234 nm (40) or the increase in peroxide formation at 480 nm (19, 35). The spectrophotometric method to measure conjugated diene formation has been found to have the advantages of simplicity and speed but optically clear solutions are required for measurement in the ultraviolet region. Several workers have developed substrate preparation procedures achieving optically clear solutions (6, 12, 36). The peroxide measurement seems to be less

accurate and reproducible than the conjugated diene measurement. This is due to the lower stability of the peroxides.

III. TEST FOR LIPID OXIDATION IN SOYBEAN OIL AND PRODUCTS

The thiobarbituric acid test (TBA) has been used to determine the extent of oxidation in various soy products, and attempts have been made to correlate the flavor of soy products with TBA values. In the test, 2-thiobarbituric acid reacts with dienals to produce colored products that have maximum absorption at 532 nm (red pigment) only, or with saturated aldehydes to produce products that have maximum absorption at 452 nm (yellow pigment) (18). The peak at 532 nm is often considered to be malonaldehyde which is prepared for standard curves by the hydrolysis of 1, 1, 3, 3 tetraethoxypropane (18, 39). Reaction of thiobarbituric acid with other end products of lipid oxidation such as 2, 4-heptadienal, 2, 4-hexadienal, 2-hexenal, and crotonaldehyde results in products which also may absorb at 532 nm (18).

Jacobson et al. (18) reported that absorbance of TBA reaction products at 532 nm might be of value in assessing the flavor of soybean oil aged at 60°C. Using both 452 nm and 532 nm, Fioriti et al. (13) reported a poor correlation of TBA value with flavor scores of hydrogenated soybean oil. Sessa et al. (30) stated that flavor of soy flakes could not be correlated with degree of oxidation as measured by TBA at 532 nm. No other reports of investigations were found in which the oxidation of soy-flour lipids was objectively measured by TBA.

IV. STORAGE OF SOY PRODUCTS

Only one study was found in which the effect of storage on soy product lipids was reported. Maga and Johnson (20) investigated the effect of processing and storage on lipids in soy flakes and in a soy isolate. They reported that storage of these products at room temperature for periods up to 12 months resulted in decreased unsaturated fatty acid levels. Maga and Johnson (20) used a fat extraction procedure, a modification of which was found by Melton et al. (22) to extract less lipid material than a chloroform-methanol extraction procedure of Ostrander and Dugan (26). Maga and Johnson did not measure the amount of oxidation by objective or subjective methods except for a fatty acid analysis. Their investigation was not designed to test the effect of lipoxygenase action on lipid oxidation during storage.

An attempt also was made by Maga and Johnson (20) to quantitate and qualitate soy lipids through thin layer chromatographic (TLC) procedures. However, no preliminary column chromatographic separation was performed on their lipid extract before TLC analysis. The number of lipids present in soy products makes their methods questionable. Quantitation by Maga and Johnson was made by a char densitometric technique in which lipids are considered to char equally. More recent work has shown this concept to be invalid (1).

V. LIPID EXTRACTION PROCEDURES

Many procedures have been reported for the extraction of total lipids from soybeans and soy products. Privett et al. (28) and Zukov and Vereschagin (46) recently have reported procedures for quantitative

extraction of total lipids from full fat soy-flour and whole soy beans. These procedures, which extracted lipids with chloroform-methanol solvents, required purification of the lipids after the extraction and were rather lengthy to be used for extraction of a large number of samples.

Honig et al. (16) extracted lipids from soybean flakes with a hexane-absolute ethanol azeotrope by stirring the flakes with the azeotrope for six hours. Maga and Johnson (20) modified the procedure by blending the soy products with the azeotrope for five minutes in a Waring Blendor.

Melton et al. (22) extracted defatted soy meal, soy concentrate and soy isolate by four different extraction procedures. Their results showed that the modified Ostrander and Dugan (26) procedure extracted as much lipid material from soy concentrate and soy isolate as the two more stringent Soxhlet extraction procedures. Of the four procedures, the modified procedure of Maga and Johnson (20) was found to extract the least amount of lipid material from soy products. The modified Ostrander and Dugan procedure did not remove as much lipid from defatted soybean meal as the Soxhlet procedures. The difference between the procedures was attributed to non-lipid material extracted by the Soxhlet procedures.

CHAPTER III

MATERIALS AND METHODS

I. SOURCE OF SOY-FLOURS

Three defatted soy-flours representing three treatments (Soyafluff-200T, a fully toasted soy-four; Soyafluff 200W, a white soy-flour made with minimum moist heat treatment; and Hizyme 280, an enzyme active soy-flour) were obtained from Central Soya. These three flours represented varying degrees of lipoxygenase activity. The three flours were produced at different locations in the United States and five samples representing different production dates were obtained for each flour.

II. PREPARATION OF SOY-FLOURS FOR STORAGE

It was assumed that the flours from the manufacturing plants were completely randomized and were representative of the general population. The age of the sample with the earliest production date upon arrival acted as the base for the beginning of the storage period for each sample. At 92 days after production, each sample was split into four equal portions and each portion was randomly assigned a storage time of 0, 60, 120, or 180 days. All sample portions were stored in clear plastic bags placed inside brown paper bags at 23°C.

III. TREATMENT OF SAMPLES AFTER STORAGE

At each storage time (0, 60, 120 and 180 days), a sample portion was taken from storage and kept at -10°C under nitrogen in a freezer until tested. Portions from each flour sample were taken and subjected to the following analyses and/or procedures: lipoxygenase assay; nitrogen solubility index; thiobarbituric acid test; and lipid extraction.

Lipoxygenase Assay

Lipoxygenase activity was tested according to the Ben-Aziz et al. (6) on a crude water extract of the flour. A Perkin Elmer Model 202 DB spectrophotometer set at a wavelength of 234 nm was used to monitor the enzyme reaction.

A. Extraction Procedure

Five g of defatted flour were mixed with 100 ml of a precooled buffer, 0.0533M in citrate and 0.0935M in phosphate, pH 4.5 and blended in a Waring blendor for 45 seconds. The extract was filtered through cheese cloth and the filtrate was centrifuged at 30,000 x gravity at 4°C for 15 minutes. Extracts of the Hizyme flour were diluted with McIlvaines buffer (0.00275 M in citrate and 0.1945 M in phosphate, pH 9.0) to contain 1 mg flour/ml. These dilutions were adjusted to pH 9.0[1] prior to assay. Extracts of the toasted and white flours (50 mg flour/ml) without any dilution were adjusted

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[1]The pH was adjusted to 9.0 by addition of concentrated NaOH; less than one ml was required for adjustment of 100 ml of extract or solution.

to pH 9.0 prior to assay. The diluted extracts of Hyzyme flour and the extracts of toasted and white flours were the enzyme solutions in the assay procedure.

B. Assay Procedure

The following solutions were prepared:

1. Stock Solution A: 1 percent (w/v) linoleic acid in absolute ethanol.
2. Stock Solution B: to 7.1 ml of stock solution A, 0.25 ml Tween 20 was added. The ethanol was evaporated under 635 torr vacuum by a rotary evaporator and the residue was dissolved in 100 ml of 0.05M Na_2HPO_4. The solution was adjusted to pH 9.0. For the lipoxygenase assay, stock solution B was diluted 10 fold with McIlvaines buffer and adjusted to pH 9.0.

The sample and reference cuvettes were filled with 2.4 ml of diluted stock solution B and 0.1 ml deionized water and were inserted into the constant temperature cell (T = 25°C) of the spectrophotometer. The spectrophotometer was zeroed at 234 nm. The sample cuvette was emptied and filled with 2.4 ml of diluted stock solution B and 0.1 ml of enzyme solution, both of which were 25°C. The solution was mixed and aerated by turning the cuvette upside down. The solutions were added to the cuvette, mixed and inserted into the constant temperature cell of the spectrophotometer within 10 seconds. The absorbance at 234 nm was recorded immediately. At the end of five minuted the absorbance at 234 nm was recorded again. Lipoxygenase activity was determined as the change in absorbance per minute (ΔA/min). The activity for Hyzyme flour was

multiplied by 50 in order that the enzyme activity would be on an equal flour weight basis (50 mg flour/ml extract) with that of the white and toasted flours.

Nitrogen Solubility Index

Nitrogen solubility index value was determined by A.O.C.S. method, BA 11-65 (5). Five g of defatted soy-flour were weighed into a 400 ml beaker. Two hundred ml of deionized water at 30°C were added in the described manner. The mixture was stirred at 120 rpm with a mechanical stirrer for 120 minutes at 30°C with the beaker immersed in a water bath. The mixture was transferred quantitatively to a 250 ml volumetric flask. One or two drops of silicone defoamer (Dow Corning Antifoam A) were added to the flask, and then it was diluted to the mark with deionized water and mixed thoroughly. After standing a few minutes, about 40 ml were decanted into a 50 ml centrifuge tube. The tubes were centrifuged at about 380 x gravity for 10 minutes and the supernatant was decanted through a funnel containing a plug of glass wool. The clear filtrate was collected in a 100 ml beaker. Twenty-five ml of clear liquid were pipetted into a Kjeldahl flask and the protein determined by A.O.A.C. Method 2.051 (3). Total nitrogen of the sample was also measured using A.O.A.C. Method 14.0 8 (3). Calculations were made according to the following formulas:

$$\text{Percent Water Soluble Nitrogen} = \frac{(S-B) \times N \times 0.014 \times 100}{\text{Wt. of Sample}}$$

where B = ml of acid for blank

S = ml of acid for sample

N = normality of acid.

$$N.S.I. = \frac{\text{Percent Water Soluble Nitrogen}}{\text{Percent Total Nitrogen}} \times 100.$$

Duplicate Kjeldahl determinations were made on each sample extract and only one extract was made per sample.

Thiobarbituric Acid Test

The distillation method of Tarladgis et al. (39), which was modified by Rhee and Watts (29), was used to determine lipid oxidation of the defatted soy-flours by measuring absorbance of the thiobarbituric acid (TBA) reaction with oxidation products at 532 nm. Absorbance values for the reaction products of the distillate from the flour extracts with TBA were converted to mg malonaldehyde per 1000 g of flour and recorded as "TBA number."

The following solutions were prepared for the TBA number determinations:

1. TBA Reagent: 0.02M 2-thiobarbituric acid in 90 percent glacial acetic acid.
2. TEP Standard: 1×10^{-3}M 1, 1, 3, 3-tetra ethoxypropane in deionized water.
3. HCl Solution: One part of concentrated HCl to two parts of deionized water.

Four g of defatted soy-flour were mixed with 60 ml of deionized water to which enough hydrochloric acid solution was added to make the final 100 ml volume a pH of 1.1 to 1.2. The mixture was blended for two minutes with a Virtis homogenizer at high speed. The mixture was quantitatively transferred to a Kjeldahl flask with 40 ml of deionized water. Boiling chips or beads and 0.5 ml of Dow Corning Antifoam AF

were added to prevent bumping and foaming. Flasks were heated on a Kjeldahl distillation rack and 50 ml of distillate were collected in 50 ml graduate cylinders in 10 to 12 minutes after distillation started. Flasks were swirled at intervals of approximately 5 minutes to prevent the soy-flour from sticking and burning on the bottom of the Kjeldahl flask.

Duplicate 5 ml samples were pipetted into test tubes and mixed with 5 ml of the TBA reagent. A TEP standard and a blank were run with each group of samples. The test tubes were capped with marbles which acted as condensers and placed in boiling water for 35 minutes. The tubes were cooled and the absorbance values (A) of the samples versus the blank were determined at 532 nm on a Perkin Elmer Model 202 DB spectrophotometer.

One standard curve was prepared by making appropriate dilutions of the TEP standard to yield 1×10^{-8} to 7×10^{-8} moles of malonaldehyde in 5 ml of distillate and reacting it with the TBA reagent. A second standard curve was prepared by the same procedure but without the distillation step. The second standard curve served as the base to determine the percent recovery of malonaldehyde from distillation. The standard curve regression equation with distillation had a regression coefficient of 0.99 and was:

$$\text{Malonaldehyde } (10^{-8} \text{ moles}) = 0.008427 + 10.84386\ (A).$$

Percent recovery of malonaldehyde from distillation was determined to be 72 percent.

Lipid Extraction

Lipids were extracted and quantitated by a modified procedure of Ostrander and Dugan (26). Fifty g of defatted soy-flour were blended with 130 ml of methanol (reagent grade) for five minutes in a Waring blendor at medium speed. Sixty-five ml of chloroform (reagent grade) were added to the blendor jar and the mixture reblended for five minutes. Sixty-five ml of chloroform were added to the jar and this mixture was reblended for 20 seconds. Sixty-five ml of deionized water containing 1.5 g of zinc acetate were added and this mixture was reblended for 10 seconds. The contents of the blendor jar were filtered through Whatman No. 1 filter paper in a Buchner funnel with suction. An atmosphere of nitrogen was maintained over the lipid extract at all times. Filter paper, residue and one-half facial tissue (used to wipe the funnel) were reblended with 100 ml of chloroform for two and one-half minutes. The blendor jar contents were filtered as above, and 75 ml of chloroform were used to rinse. The filtrate was quantitatively transferred to a 500 ml graduate cylinder and the filter flask was rinsed with 25 ml of methanol.

The graduate cylinder with filtrate was placed in a freezer (-10°C) under nitrogen until a sharp interface appeared between the methanol-water and chloroform layers. The volume of the chloroform layer was recorded and the cylinder contents were emptied into a 500 ml separatory funnel which was kept at -10°C until the chloroform and methanol-water layers were clearly separated. The chloroform layer was drained off. Five ml portions of the chloroform solution were pipetted into pre-weighed 50 ml beakers for determination of solid weight.

Beakers were air dried in a hood and then heated for two hours at 65°C under 635 torr vacuum to remove the solvent. Weights of solids were determined and percent lipid was calculated:

$$\text{Percent lipid} = \frac{\text{ml } CHCL_3 \text{ layer} \times 5 \text{ ml} \times \frac{\text{g solid}}{100}}{50 \text{ g sample}} .$$

IV. LIPID ANALYSIS

One lipid extract of each sample at each storage time was concentrated to less than 10 ml total volume on a rotary evaporator at 35° to 40°C under 635 torr vacuum and transferred quantitatively to a 25 ml volumetric flask. The concentrate was made to volume with chloroform-methanol 20:3, v/v, and the amount of lipid extract per ml of concentrate was determined in triplicate by drying a 1 ml aliquot of the solution in a beaker of known weight. Concentrated lipid extracts were subjected to silicic acid column chromatography as described later and fractionated into three fractions. Each fraction was concentrated to less than 10 ml and transferred to a 25 ml volumetric flask. After filling each flask to volume with its corresponding eluting solvent, two 3 ml aliquots were dried in preweighed beakers to determine the weight of lipid containing material in each fraction. Total lipid and recovery from the column were determined from these weights. From each fraction a 2 ml portion was set aside for thin layer chromatographic investigation and the remainder was saved for gas liquid chromatographic analysis of the fatty acids in the lipids of each fraction. All of these samples were stored at -10°C under nitrogen until needed for analysis.

Silicic Acid Column Chromatography

Silicic acid column chromatography was carried out according to the method of Hornstein et al. (17). Fifty g of silicic acid (Mallinckrodt AR 100-Mesh), heated overnight at 130°C, were slurried with chloroform-methanol 3:1, v/v, for 30 minutes. Forty ml of the mixture were decanted to remove the finer particles of silicic acid and the remainder was poured into a 2.5 x 90 cm column fitted with a sintered glass disc. Forty ml of chloroform methanol 3:1, v/v, were used to rinse the silicic acid container and the rinse was poured into the column. Three 10 ml aliquots of the chloroform methanol 3:1, v/v, solution were used to rinse down the column. Slight pressure of N_2 was applied to pack the column of silicic acid and 15 cm of liquid were allowed to stand above the interface of the silicic acid. Enough anhydrous sodium sulfate was added to the column to form a 2.5-cm-thick layer on top of the silicic acid. After the column was washed with 300 ml of chloroform the concentrated lipid extract containing from 0.8 to 1.2 g was added to the column and washed in with 15 ml of chloroform-methanol 20:1, v/v. The column was developed with successive 300 ml portions of chloroform-methanol 20:1, v/v, chloroform-methanol 1:1, v/v and methanol. A flow rate of approximately 3 to 5 ml/minute was maintained.

Thin Layer Chromatography Analysis

The lipids in the three fractions were further investigated by thin layer chromatography on 250 microns thick Absorbosil 5 thin layer plates (Prekotes, Applied Science Lab., State College, PA). Fraction I was developed by solvent systems of chloroform-methanol-water 65:25:4,

v/v/v, and petroleum ether-ethyl ether-acetic acid 70:20:4, v/v/v. Fractions II and III were developed only by the solvent system of chloroform-methanol-water 65:25:4, v/v/v. Preliminary test showed that no lipids in Fraction II or Fraction III were developed by the petroleum ether-ethyl ether-acetic acid solvent system. Identification of specific lipids in each fraction was made by comparison of R_f values with R_f values of appropriate standards and by specific detection tests (32): antimony trichloride test for steroids; molybdenum blue test for phospholipids; ninhydrin test for free amino groups; diphenylamine test for glycolipids, gangliosides, and sulfatides; and Dragendorff test for choline containing phospholipids.

Fatty Acid Analysis by GLC

Methyl esters of the fatty acids were made from lipids in the three fractions eluted from the silicic acid columns and relative fatty acid distribution of C_{12} to C_{20} fatty acid methyl esters were determined in duplicate by gas liquid chromotography (GLC). A volume from each fraction containing 100 mg of lipid material was dried on a rotary evaporator at 35° to 40°C for 635 torr vacuum and methyl esters were made by the A.O.C.S. Method Ce 2-66 (4). Each methyl ester solution was concentrated and 4 to 6 µl were injected with a 10 µl syringe into a Bendix GLC Model 2600 equipped with a flame ionization detector and Dohrman recorder with a built-in disc intergrator. A 1.83 m by 4 mm inside diameter stainless steel column packed with 15 percent diethylene glycol succinate on 60/80 mesh acid washed chromosorb W (Applied Science Lab., State College, PA) was used to separate the esters. Conditions

of the GLC were: nitrogen flow rate, 40 ml/min; hydrogen flow rate, 40 to 50 ml/min; air flow rate, 400 to 500 ml/min; injection temperature, 245°C; detector temperature, 240°C; column temperature, 195°C. Standards of methyl esters of fatty acids that contained known percentages of the fatty acids, C_{12} to C_{20}, including $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$, were obtained (Applied Science Lab, State College, PA) and used to check accuracy and precision of the GLC procedure.

Fatty acid C_{20} was present only in Fraction I but it was in such small amount that no relative percent was reported.

V. MOISTURE DETERMINATION

Moisture was determined on the soy-flour samples by A.O.A.C. Method 14.076 in order to report results on a dry matter basis (3). All results were reported on a dry matter basis except for NSI, which was reported on an "as is" basis.

VI. STATISTICAL METHODS

The experimental design of this experiment was a split plot design with replication. The whole plot was the treatments (fully toasted, minimum moist heat, and enzyme active) and the split plots were the storage times (0, 60, 120, and 180 days). Five replications were completed. (See Table 1.)

TABLE 1

ANALYSIS OF VARIANCE TABLE

Source	df
Treatment	2
Sample/Treatment	12
Time	3
Treatment x Time	6
(Sample x Time)/Treatment	36
TOTAL	59

Where effects of treatment x time interactions were significant for a dependent variable the sum of squares for time plus treatment x time were partitioned by orthogonal polynomials (linear, quadratic, and cubic) for each treatment. By testing the sequential effect of the additional terms in the polynomial equation, it was possible to determine the order of the model to use for each variable. The polynomial equation was:

$$y = a + bT + cT^2 + dT^3 + \ldots$$

where y = value for the response variable;

$T = (\frac{z + 1}{60})$;

z = days of storage at 23°C.

The parameters (a, b, c, and d) of the equation for the model were then estimated.

When the effect of time was significant and the treatment x time effect was not significant for a dependent variable the effects of time across treatments were partitioned by orthogonal polynomials and the order of the model and parameters of the equation were determined as previously described. When the effect of treatment was significant and the treatment x time interaction was not significant for a dependent variable, the treatment means across time were separated by the Student-Newman-Keuls test (33).

Dependent variables for which equations of the model were estimated will be shown in graphical form. The response of the variable is plotted as a function of days storage, and the actual means at 0, 60, 120 and 180 days storage are plotted on the graph. Treatment means for dependent variables separated by the Student-Newman-Keuls test are presented in tabular form.

Equations for the model of each variable are given in Appendix A and significant mean squares and error mean square for each source of variation for a variable are shown in Appendices B and C, respectively.

CHAPTER IV

RESULTS AND DISCUSSION

I. LIPOXYGENASE ACTIVITY

The three treatments of soy-flour were originally selected in the hope that: Treatment 1 (fully toasted flour) would not have any lipoxygenase activity; Treatment 2 (white flour) would have some lipoxygenase activity; and Treatment 3 (enzyme-active flour) would have a high lipoxygenase activity. Analysis of variance showed a significant effect on lipoxygenase activity for treatment, storage time, and treatment x storage time (Appendix B). The equations for lipoxygenase activity of each treatment as a function of storage time are graphed in Figure 1. Treatment 1, subject to a high heat treatment during processing, showed no lipoxygenase activity. Treatment 2, subjected to minimum heat during processing, had a low lipoxygenase activity that did not change significantly over time. Treatment 3 had the highest lipoxygenase activity and the activity decreased significantly at an increasing rate during storage.

II. NITROGEN SOLUBILITY INDEX

Nitrogen Solubility Index (NSI) is also a measure of severity of heat treatment of soy-flour. No significant difference in NSI was found for storage time, but a significant difference due to treatment was

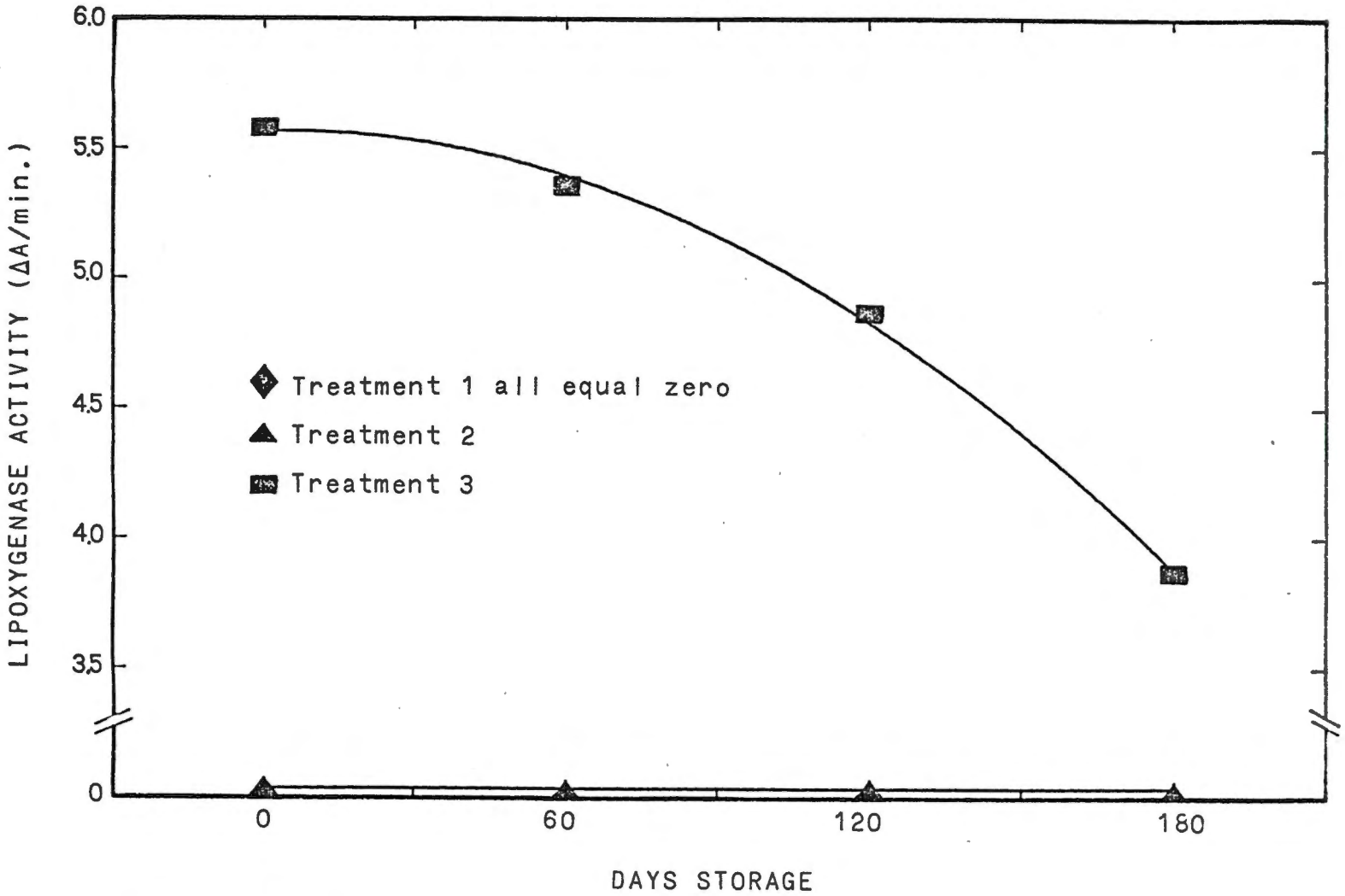


FIGURE 1. The mean lipoxygenase activity for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

found (Appendix B). Mean NSI values of Treatment 1, 2 and 3 across time were found to be significantly different from each other (Table 2). As the heat treatment of the flour increased in severity, the NSI value decreased in a corresponding pattern. The treatment x time interaction for NSI had an alpha risk level of about 6 percent. To avoid the possibility of making a type II statistical error, NSI values were considered to have a significant treatment x time interaction and NSI for each treatment as a function of time are graphed in Figure 2. The NSI values of Treatment 3 increased with time while the NSI values of Treatment 2 decreased. NSI values of Treatment 1 remained constant through the 180 days storage.

III. THIOBARBITURIC ACID NUMBER

Thiobarbituric acid (TBA) number (mg malonaldehyde/1000 g flour), a measure of oxidation, was significantly different for treatment, time, and treatment x time (Appendix B). Graphs of the equations for TBA number of each treatment as a function of storage time are shown in Figure 3. The TBA number of Treatments 1 and 3 decreased through 120 days storage and then started to increase. The TBA number of Treatment 2 decreased linearly with time. Separation of treatment means for TBA number across time by the Student-Newman-Keuls test showed that the mean TBA number of Treatment 1 (2.35) was significantly lower than Treatment 2 (3.55) or Treatment 3 (3.45) (Table 2).

Sessa et al. (30) reported that toasted defatted flakes had lower TBA numbers than non-heated defatted flakes. They also reported that the TBA number of raw soybean flakes did not change more than three

TABLE 2

MEANS OF SELECTED DEPENDENT VARIABLES FOR EACH TREATMENT AVERAGED ACROSS STORAGE TIMES AND REPLICATES

Variable	Treatment[d] Means		
	1	2	3
Nitrogen Solubility Index	21.44[a]	66.08[b]	86.05[c]
TBA Number	2.35[a]	3.55[b]	3.45[b]
% Lipid Dry Matter Basis	2.70[a]	2.77[a]	3.12[b]
% Lipid in Fraction I	34.79[b]	25.61[a]	28.33[a]
% Lipid in Fraction II	53.07[a]	60.99[b]	56.05[a]
% Lipid in Fraction III	12.14[a]	13.40[a]	15.62[b]
Relative % C16:0 in Fraction III	20.05[a]	22.12[b]	21.54[b]

[a,b,c]Means in a row followed by the same superscript letter are not significantly different at the 5 percent level; 5 replicates x 4 storage times in each mean.

[d]Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

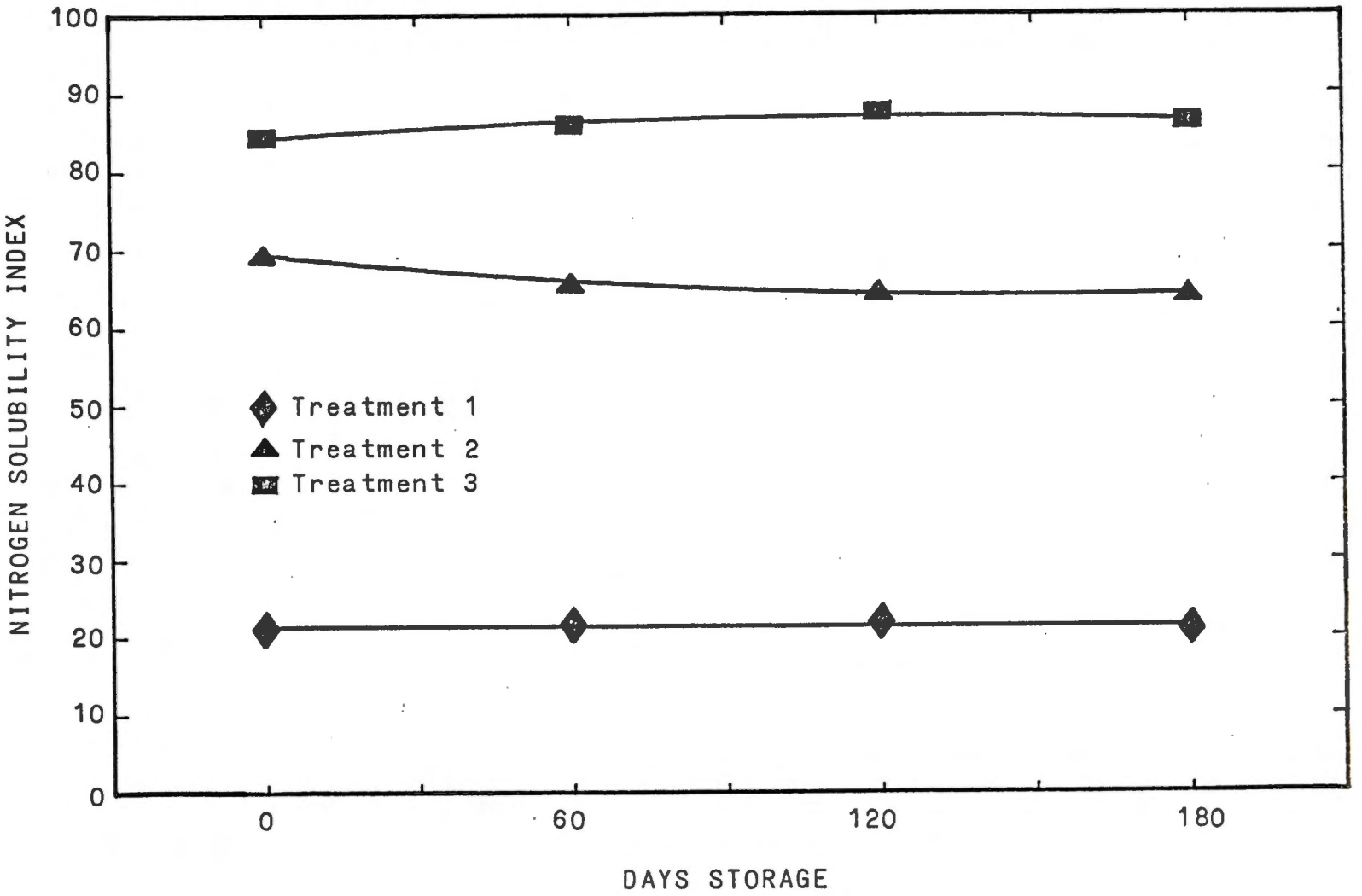


FIGURE 2. The mean nitrogen solubility index for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

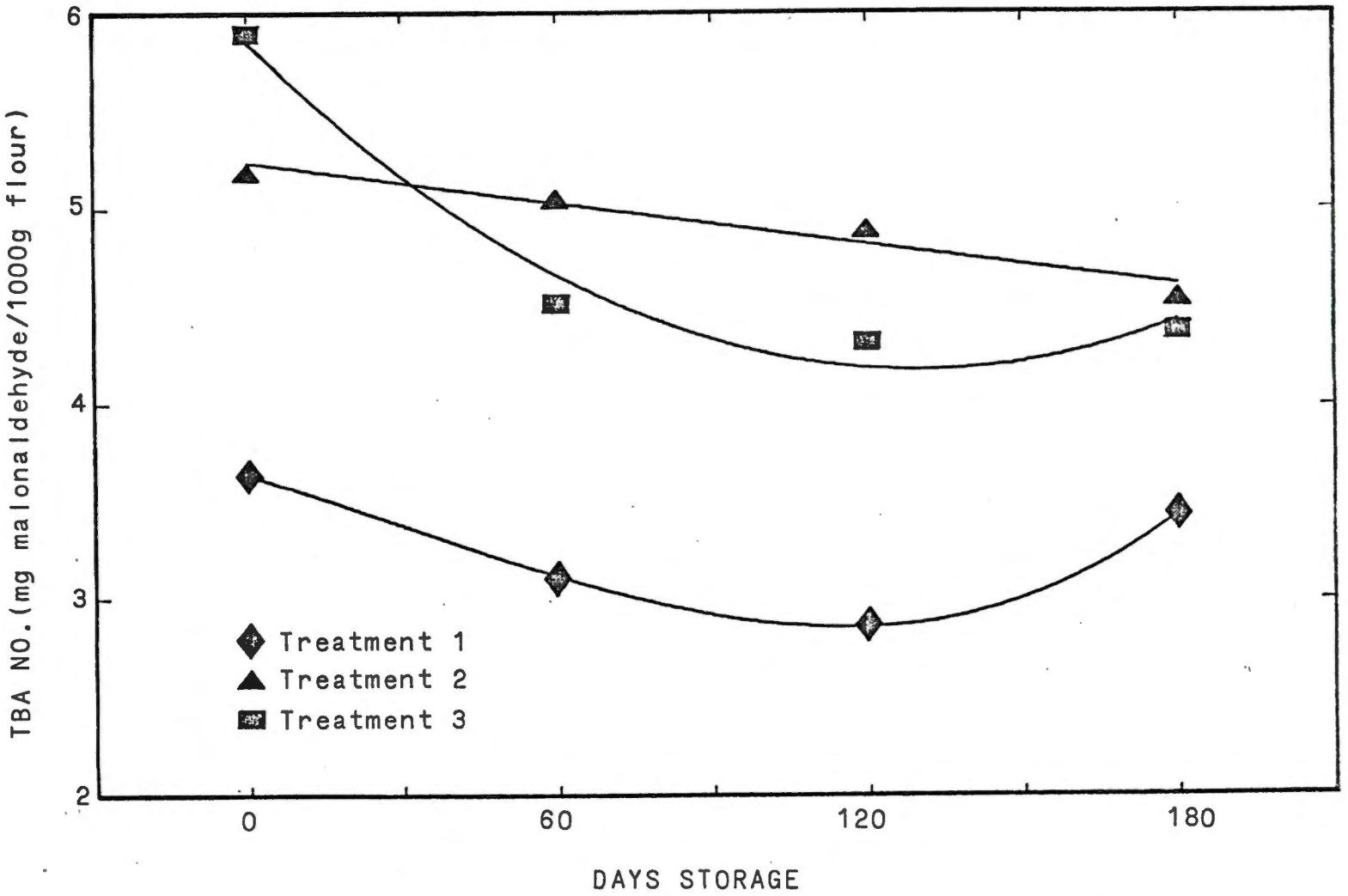


FIGURE 3. The mean TBA number for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

units during storage for seven months at room temperature. However, they did not report whether this change was an increase or decrease. The TBA number of the enzyme active flour (Treatment 3) decreased approximately 1.5 units up to 120 days storage. Treatments 1 and 2 decreased less than a unit during storage. Results are inconclusive why the TBA decreased, but it might be due to loss of volatile TBA reactive substances over time.

IV. LIPID EXTRACTION--PERCENT LIPID DRY MATTER BASIS

Significant differences in extracted lipids were found for treatment and time but the treatment x time effect was not significant (Appendix B). A significantly higher percent lipid was extracted from Treatment 3 than from Treatment 1 or 2 (Table 2, page 24). An average of the lipids extracted across treatments at each storage time showed a slight linear decrease (Figure 4). The decrease possibly was due to oxidation of lipids and thus to a reduction in extractable material.

V. SILICIC ACID FRACTIONS

No significant treatment x time interaction was found in the percent lipid in any of the silicic acid fractions (I, II, or III). The percent lipid in Fraction I and II changed significantly with time (Figures 5 and 6, and Appendix B). In Fraction I, the percent lipid decreased to a minimum and then increased to a maximum value at 180 days storage (Figure 5). The percent lipid in Fraction II was maximum at 120 days and minimum at 180 days storage (Figure 6). It must be considered that the amount of lipid in each fraction might be affected by

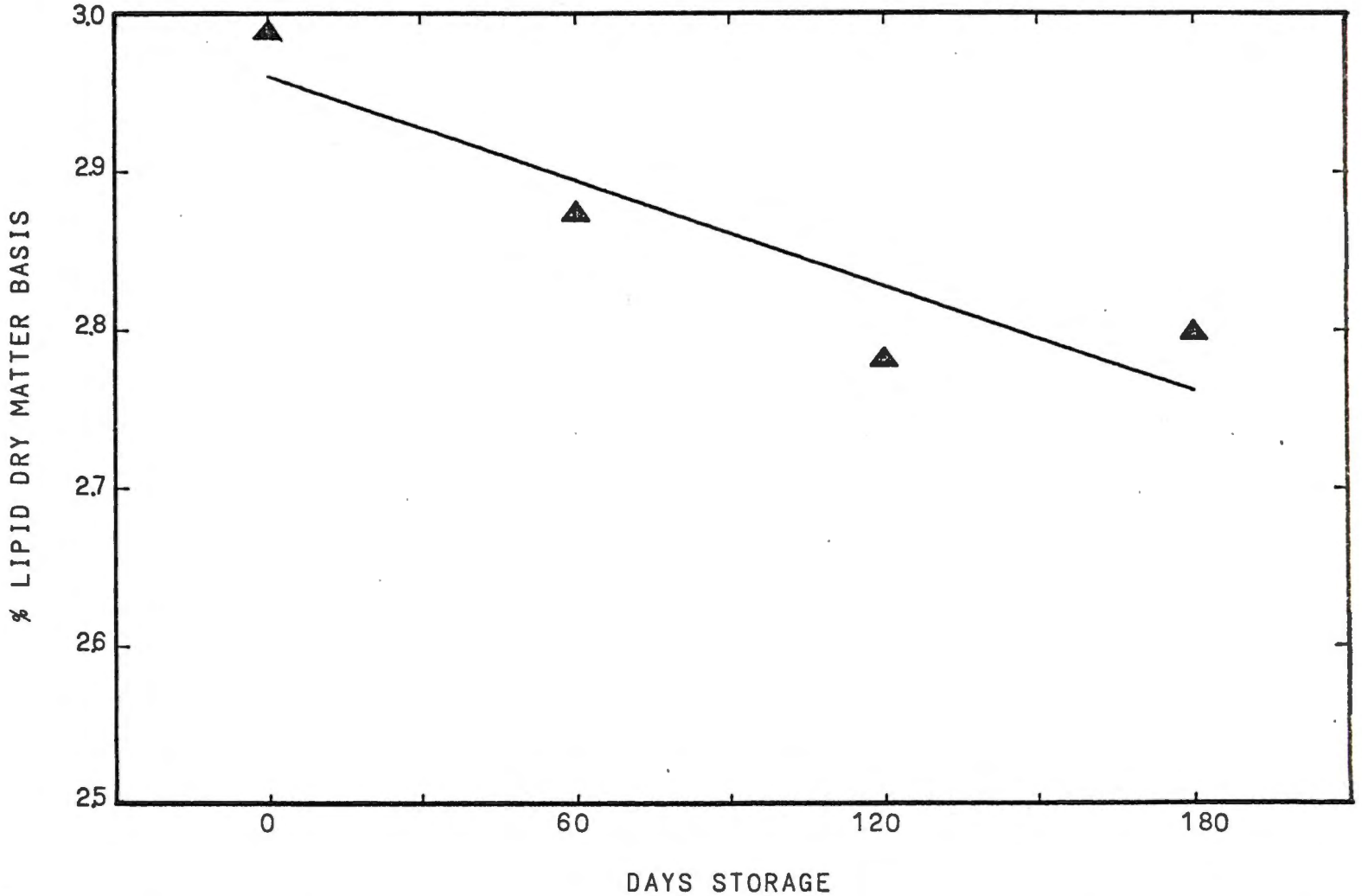


FIGURE 4. The mean percent extractable lipid of three treatments of defatted soy-flour plotted as a function of storage time. Each point represents the mean of three treatments x five replicates.

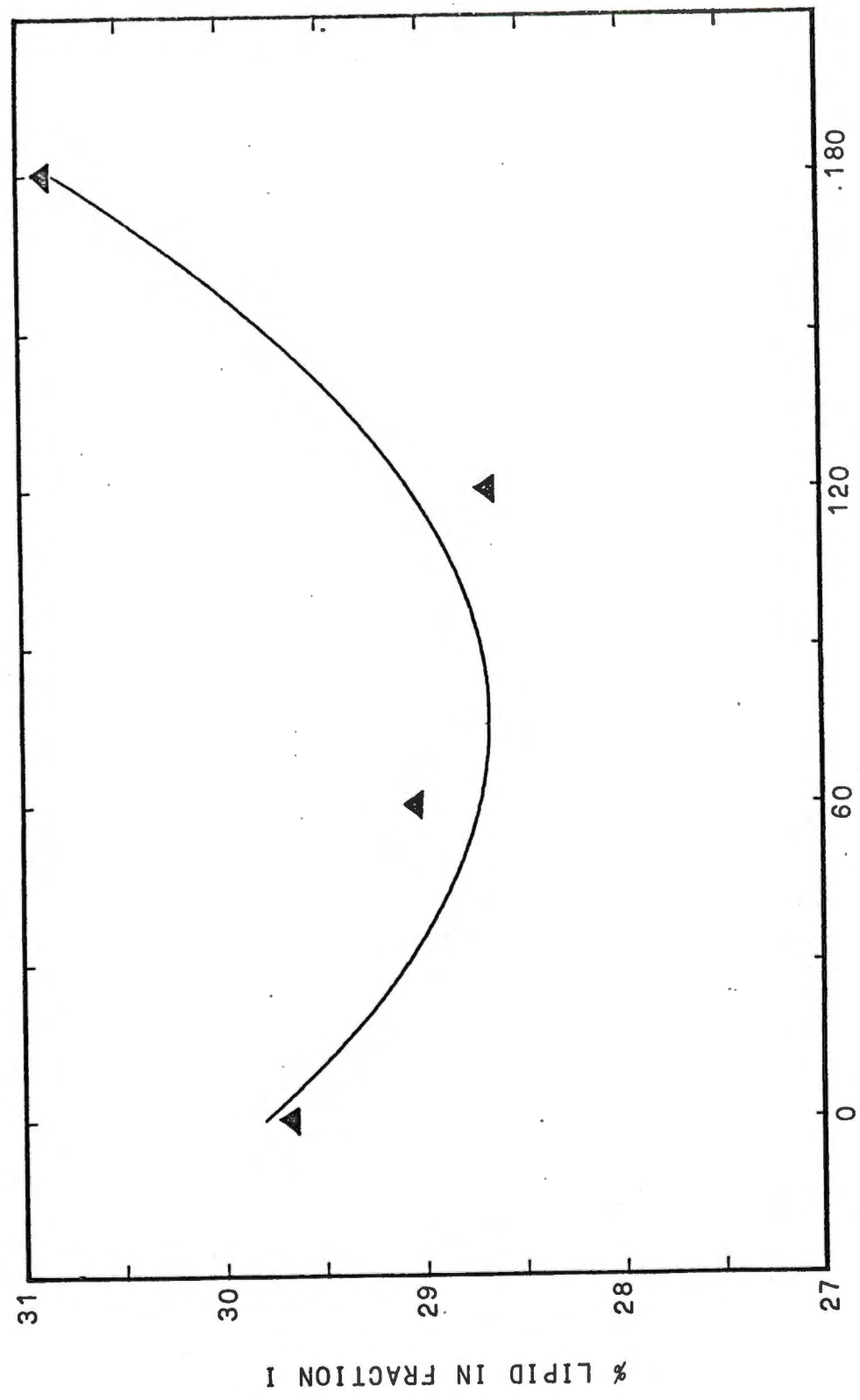


FIGURE 5. The mean percent lipid in Fraction I of three treatments of defatted soy-flour plotted as a function of storage time. Each point represents the mean of three treatments x five replicates.

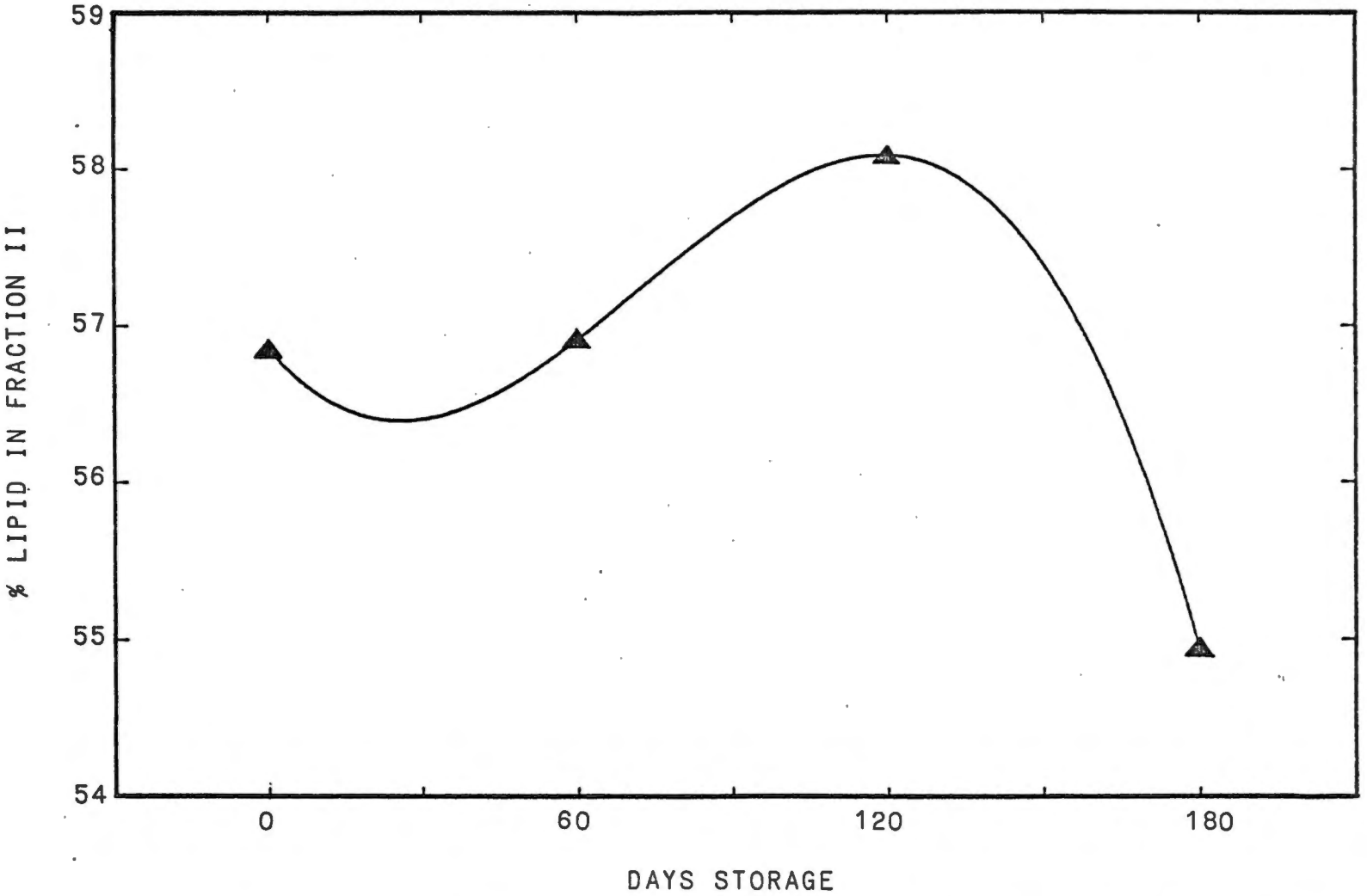


FIGURE 6. The mean percent lipid in Fraction II of three treatments of defatted soy-flour plotted as a function of storage time. Each point represents the mean of three treatments x five replicates.

the amount of lipid extracted and oxidation of lipids. Percent lipid in Fraction I changed approximately plus or minus 1.5 and percent lipid in Fraction II changed approximately plus or minus 2. No significant change with time was found in the percent lipid in Fraction III.

The mean percent of lipid across time and treatment in each fraction was: 29.58 percent for Fraction I; 56.70 percent for Fraction II; and 13.72 percent in Fraction III. In all three fractions, there was a significant difference by treatment in the amount of lipid eluted (Appendix B). The percent of lipid eluted into Fraction I from Treatment 1 was significantly more than from Treatment 2 or 3 (Table 2, page 24). Fraction II had a higher percent of lipid for Treatment 2 than for Treatment 1 or 3 (Table 2). Fraction III had a higher percent of lipid for Treatment 3 than for Treatment 1 or 2 (Table 2).

VI. TLC ANALYSIS OF LIPIDS IN THE SILICIC ACID FRACTIONS

Analysis of the lipids in Fraction I of Treatment 1, 2, and 3 by thin layer chromatography with the solvent system of petroleum ether-ethyl ether-acetic acid showed the presence of mono-, di-, and triglycerides (Figure 7). For demonstration purposes, only 0 and 180 day storage samples were spotted and photographed.

Figure 8 shows the thin layer chromatogram of Fraction I and II of Treatments 1, 2, and 3 which was developed by the chloroform-methanol-water solvent system. Lipids in Fraction I and II were numerous which made identification of only a few lipids possible.

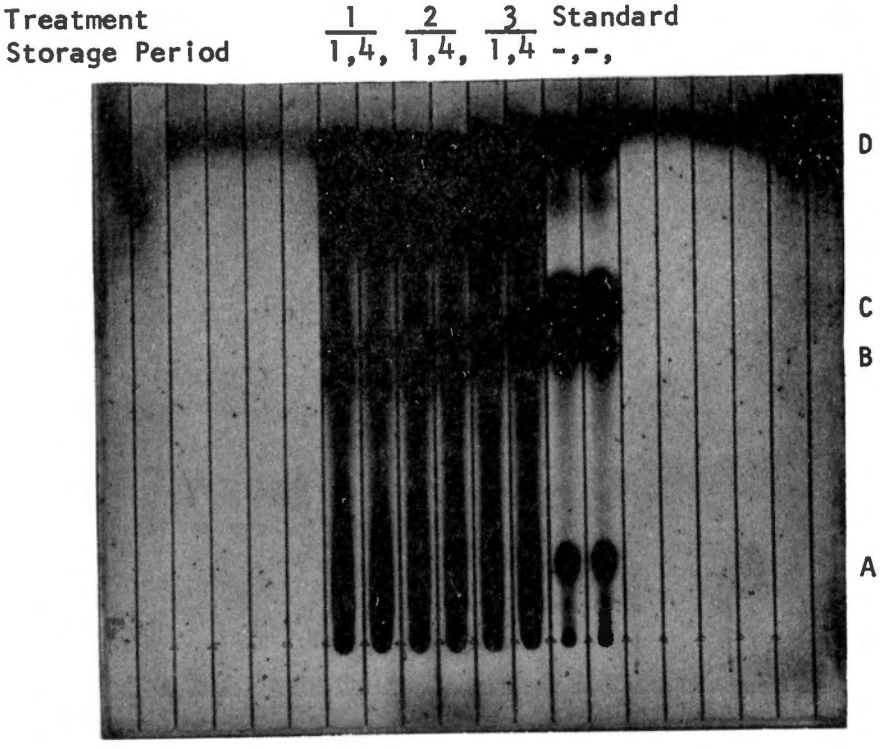


FIGURE 7. Thin layer chromatogram of Fraction I lipids developed by petroleum ether:ethyl ether:acetic acid, 70:20:4. Treatments 1, 2 and 3 represent fully toasted, white and enzyme active defatted soy-flours, respectively. Storage 1 and 4 are 0 and 180 days, respectively.

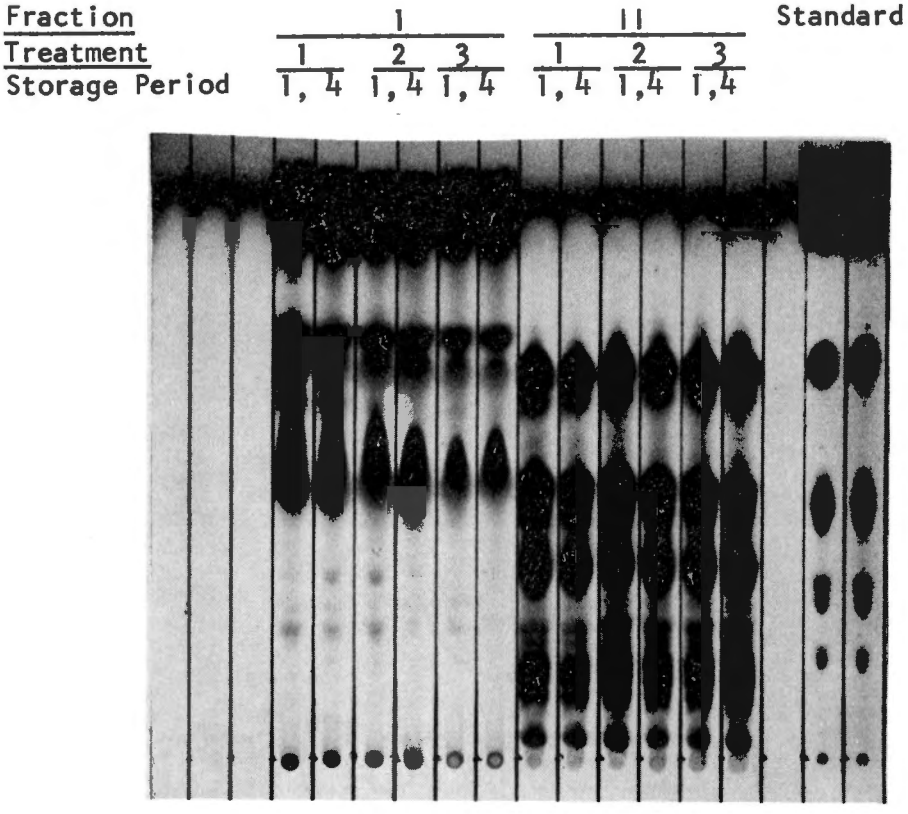


FIGURE 8. Thin-layer chromatogram of Fraction I and Fraction II lipids developed by chloroform:methanol:water, 65:25:4. Treatments 1, 2 and 3 represent fully toasted, white and enzyme active defatted soy-flours, respectively. Storage 1 and 4 are 0 and 180 days, respectively.

Sample lanes from Fraction I and II are shown in Figures 9 and 10, respectively, with results from the detection test. Most of the lipids in Fraction I (Figure 9) reacted to the diphenylamine test for glycolipids (A, B, C, D, F, H, and J). Lipids H and J also gave a positive reaction to the antimony trichloride test for steroids as well as for the diphenylamine test. A lipid in the area of F gave a positive reaction to the molybdenum blue test for phospholipids. A large number of lipids in Fraction II (Figure 10) either reacted positively to the diphenylamine test (A, B, C, D, E, F, G, K, and M) or the antimony trichloride test (B, C, D, E, F, J, and K). By visual observation, the three most concentrated lipids in Fraction II (H, I, and L) reacted positively to the molybdenum blue test (Figure 10). Lipid L reacted positively to the ninhydrin test for phospholipids containing free amino groups and Lipid I reacted positively to the Dragendorff's test for choline containing phospholipids. Identifications were made for phosphatidyl choline (Lipid I), phosphatidyl ethanolamine (Lipid L), and phosphatidyl inositol (Lipid H).

Fraction III lipids, developed by the chloroform-methanol-water solvent system, are shown in Figure 11. One major lipid in high concentration is shown in this fraction. It was identified to be phosphatidyl choline because of its positive reaction to the molybdenum blue and Dragendorffs tests and its R_f value. The solvent system for the silicic acid column chromatography originally was selected for phosphatidyl choline to be the main lipid in the last fraction. Other lipids also were present in Fraction III, but not in large enough concentrations to be classified by the specific detection test.

LIPIDS IN FRACTION I

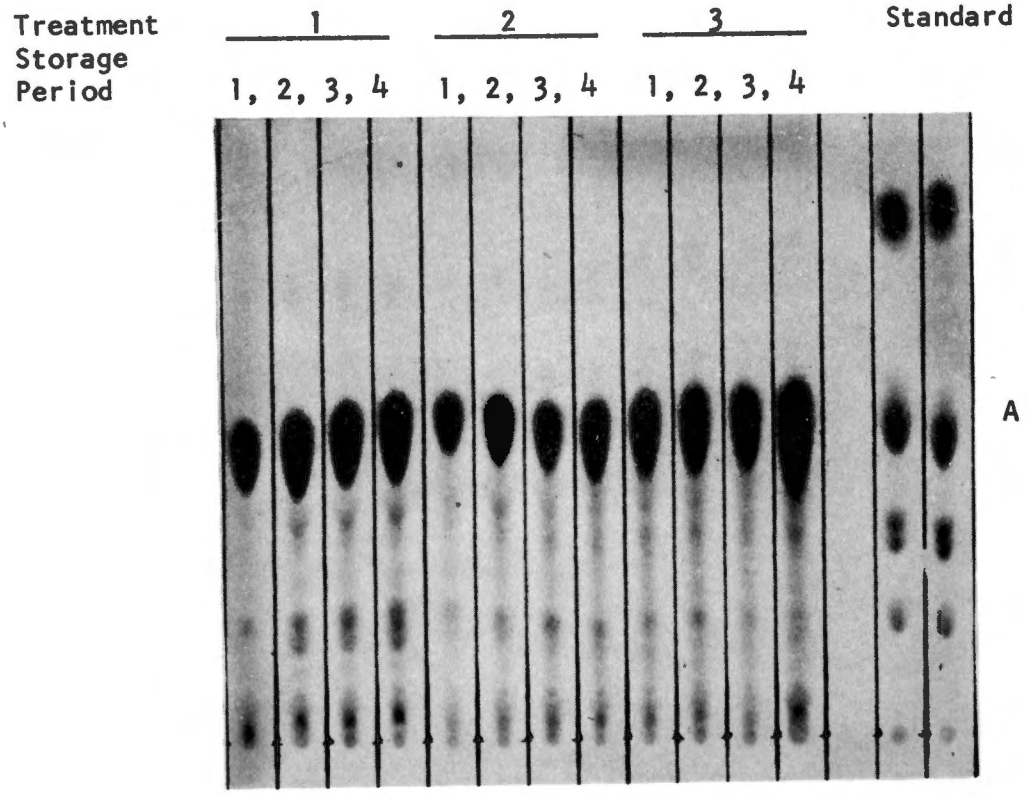
	Detection Test				
	Molybdenum Blue	Diphenyl-amine	Ninhydrin	$SbCl_3$	Dragen-dorff
K					
J		+			
I				+	
H		+		+	
G					
F	+	+			
E					
D		+			
C		+			
B		+			
A		+			

FIGURE 9. A representative sample lane of a thin layer chromatogram of Fraction I lipids developed by chloroform:methanol:water, 65:25:4.

LIPIDS IN FRACTION II

	Dectection Test				
	Molybdenum Blue	Diphenyl-amine	Ninhydrin	$SbCl_3$	Dragen-dorff
M		+			
L	+		+		
K		+		+	
J				+	
I	+				+
H	+				
G		+			
F		+		+	
E		+		+	
D		+		+	
C		+		+	
B		+		+	
A		+			

FIGURE 10. A representative sample lane of a thin layer chromatogram of Fraction II lipids developed by chloroform:methanol:water, 65:25:4.



A. Phosphatidyl choline

FIGURE 11. Thin layer chromatogram of Fraction III lipids developed by chloroform:methanol:water, 65:25:4. Treatments 1, 2 and 3 represent fully toasted, white and enzyme active defatted soy-flours, respectively. Storage 1, 2, 3 and 4 are 0, 60, 120 and 180 days, respectively.

VII. FATTY ACID ANALYSIS

In the fatty acid analysis of the lipids in the three silicic acid fractions for each treatment, it must be acknowledged that a decrease in the relative percent of one or more of the fatty acids in a fraction during storage will always be off-set by an increase in one or more different fatty acids in the same fraction. A decrease in the relative percent of a fatty acid actually shows a decrease in the amount of that fatty acid where an actual increase is not probable.

VIII. FATTY ACID DISTRIBUTION IN FRACTION I

The relative percents of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) of Fraction I of Treatment 1, 2, and 3 had a significant treatment x time interaction within the range of the data (Figures 12 through 16, and Table 4, Appendix B). All of the above fatty acids had significant mean squares for treatment and time except for palmitic acid (Appendix B).

The relative percent of linoleic acid in Treatments 1 and 2 decreased at a decreasing rate while the relative percent of linoleic acid in Treatments 3 remained constant (Figure 15). It should be noted that the relative percent of linoleic acid in Treatment 3 at 0 day was about 6 percent lower than in Treatment 1 and 2 at 0 day. At 180 days, the level of relative percent linoleic acid in Treatment 3 remained lower than in Treatment 1 or 2. The relative percent of linolenic acid in Treatment 3 decreased linearly over the range of the data as the relative percent

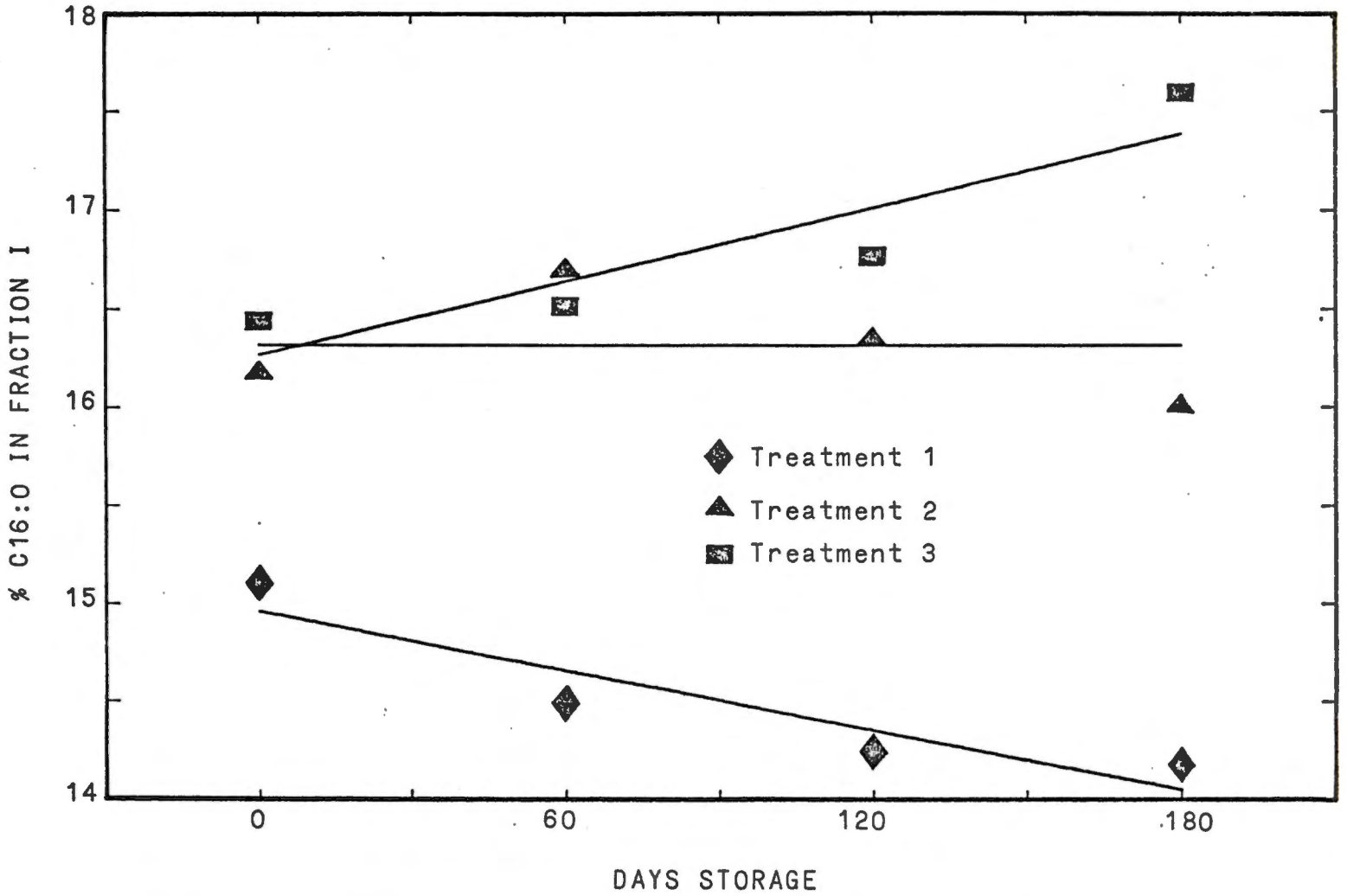


FIGURE 12. The mean relative percent of palmitic acid in Fraction I for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

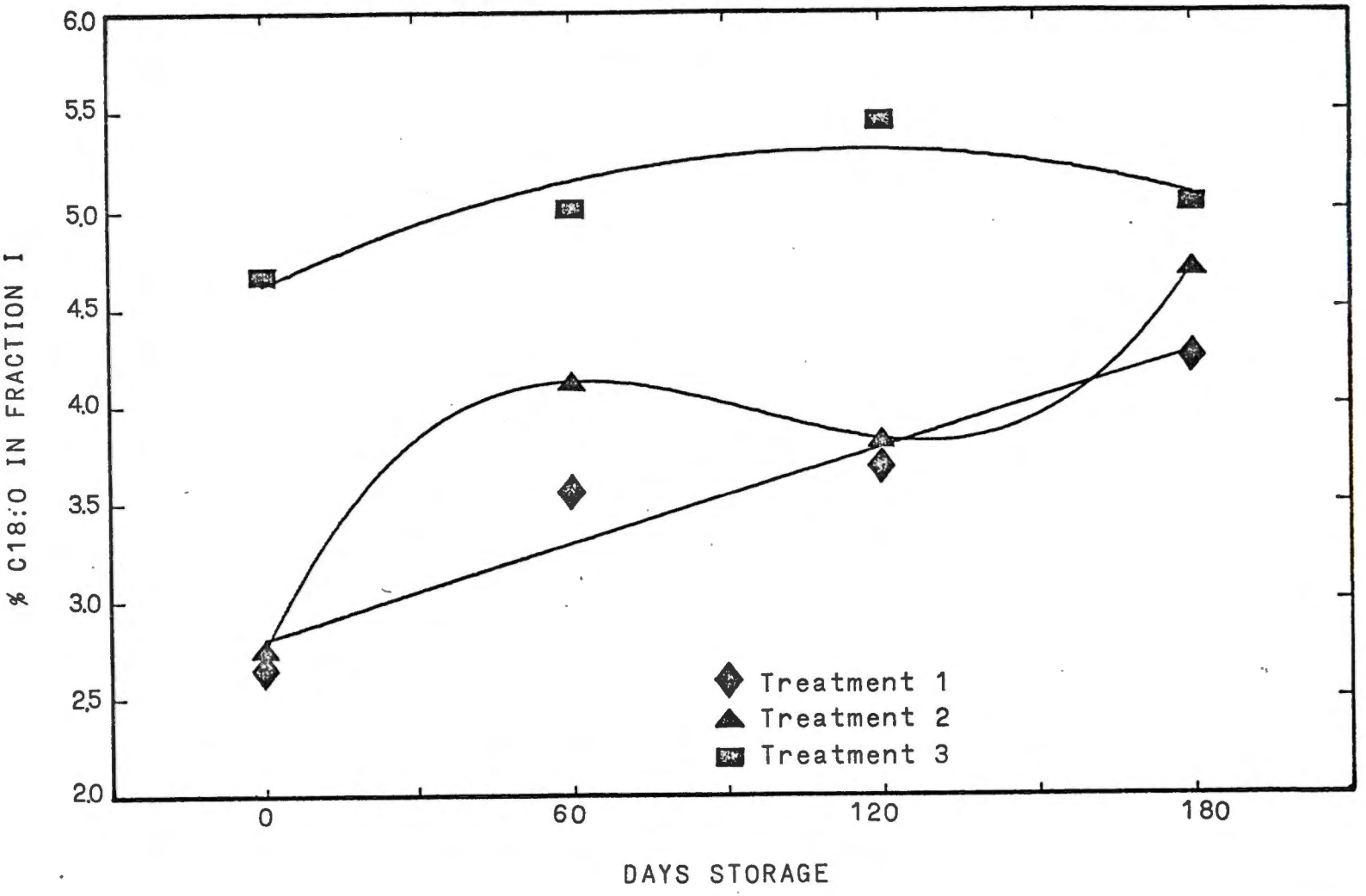


FIGURE 13. The mean relative percent of stearic acid in Fraction I for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

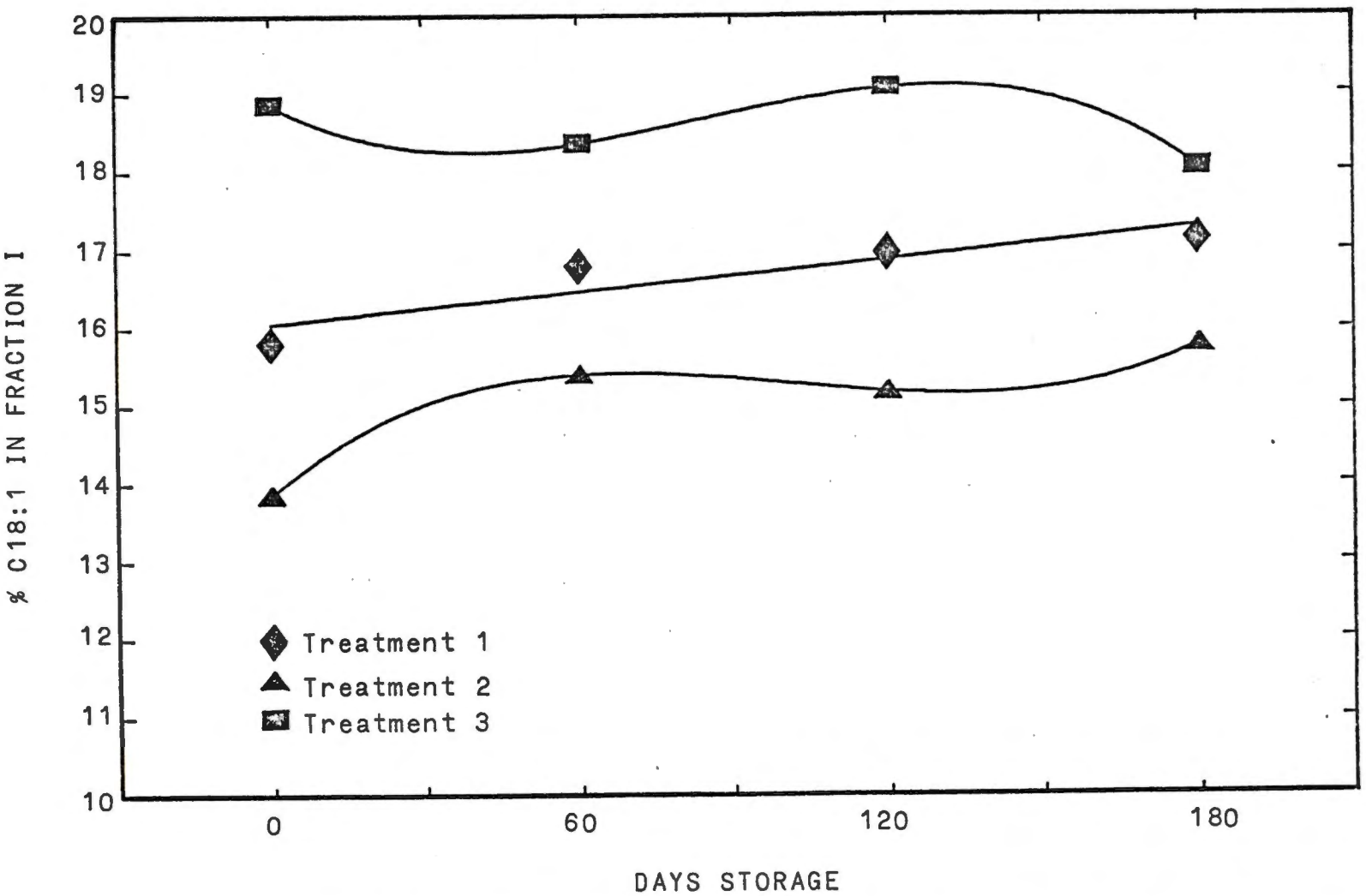


FIGURE 14. The mean relative percent of oleic acid in Fraction I for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

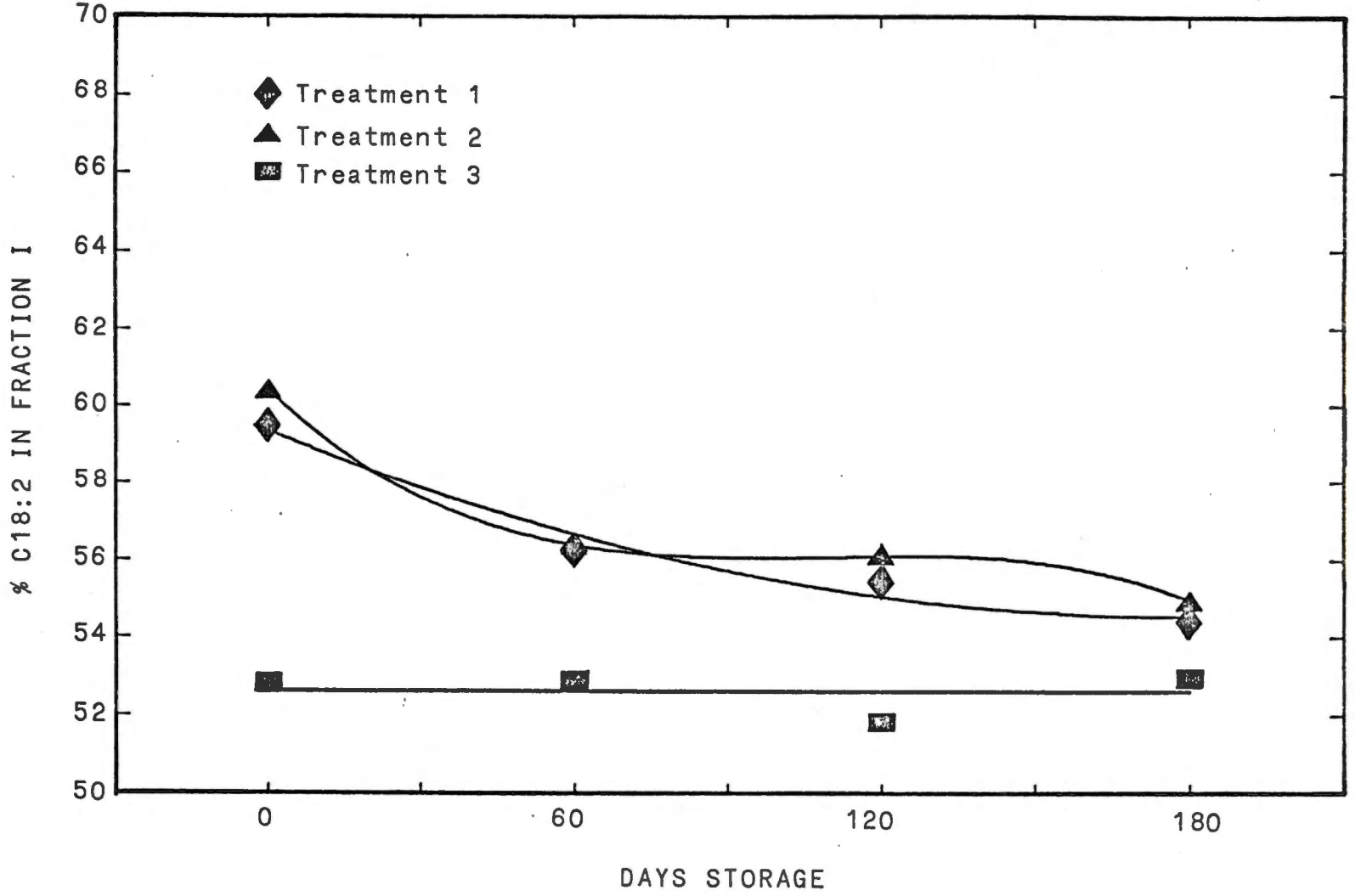


FIGURE 15. The mean relative percent of linoleic acid in Fraction I for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

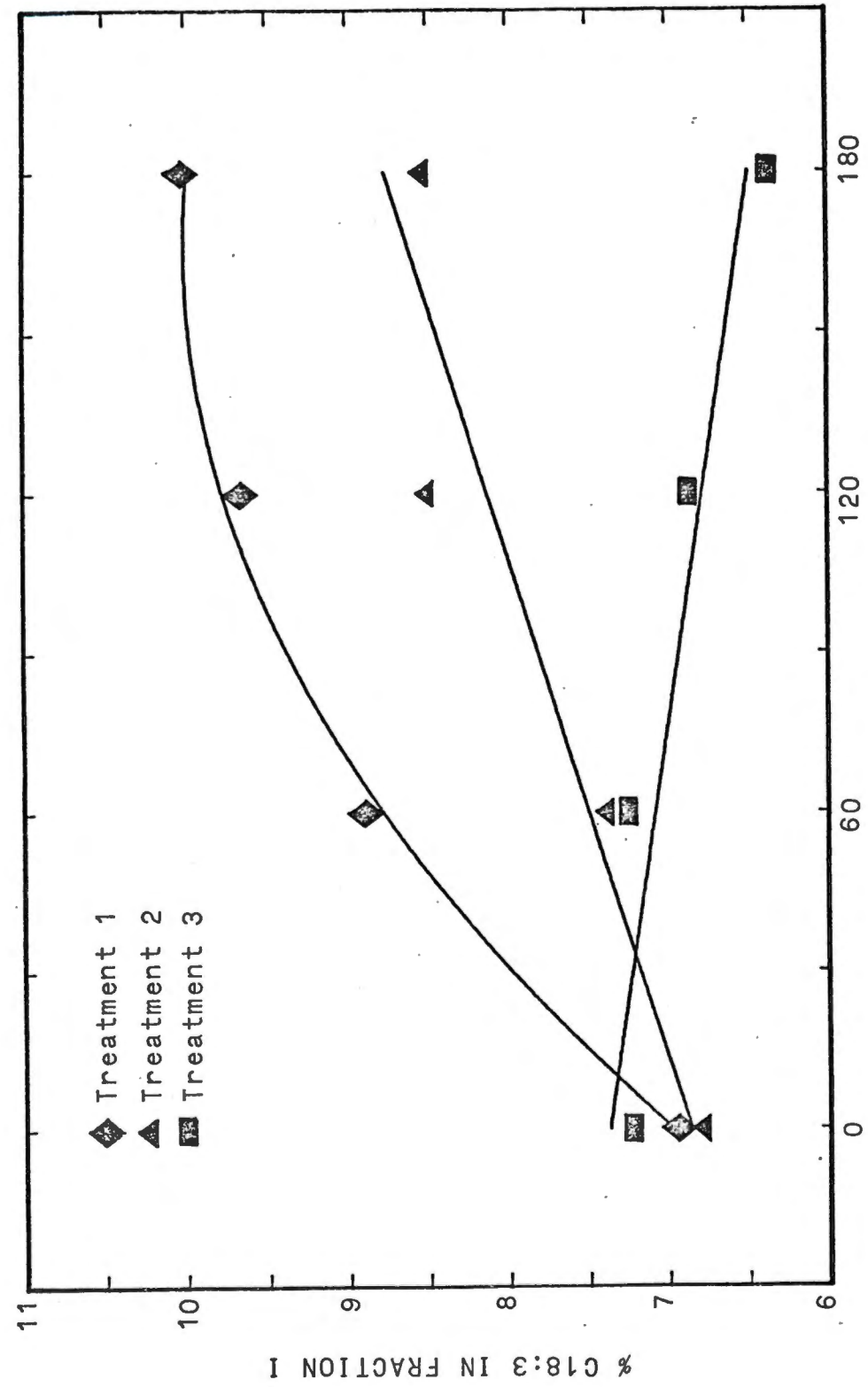


FIGURE 16. The mean relative percent of linolenic acid in Fraction I for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

of linolenic acid in Treatment 1 and 2 increased (Figure 16). Relative percents of stearic acid and oleic acid either increased or changed very little (Figures 13 and 14). The relative percent of palmitic acid decreased linearly in Treatment 1, increased linearly in Treatment 3, and remained constant in Treatment 2 (Figure 12).

IX. FATTY ACID DISTRIBUTION IN FRACTION II

The relative percent of all five fatty acids in Fraction II had significant mean squares for storage time and the relative percent of oleic acid and linoleic acid had significant mean squares for treatment (Appendix B). At the 5 percent alpha risk level, the relative percent of palmitic acid, stearic acid, oleic acid, and linolenic acid had a significant treatment x time interaction (Figures 17 through 20, and Table 4, Appendix B). Because of the consistent behavior of the relative percent of linoleic acid in Fraction II compared with that in Fraction I and III, a higher alpha risk level with treatment x time was considered significant but with less confidence ($p < 0.11$).

Taking into consideration the low degree of confidence of the treatment x time interaction of the relative percent of linoleic acid in Fraction II, the relative percent of linoleic acid in Treatment 1 and 2 decreased while the relative percent of linoleic acid in Treatment 3 remained constant (Figure 21). As in Fraction I, the initial level of linoleic acid in Fraction II for Treatment 3 was considerably lower than for Treatments 1 and 2. To offset the decreases in linoleic acid in Treatments 1 and 2, the relative percent of stearic acid, oleic acid, and linolenic acid increased in a fairly constant fashion (Figures 18,

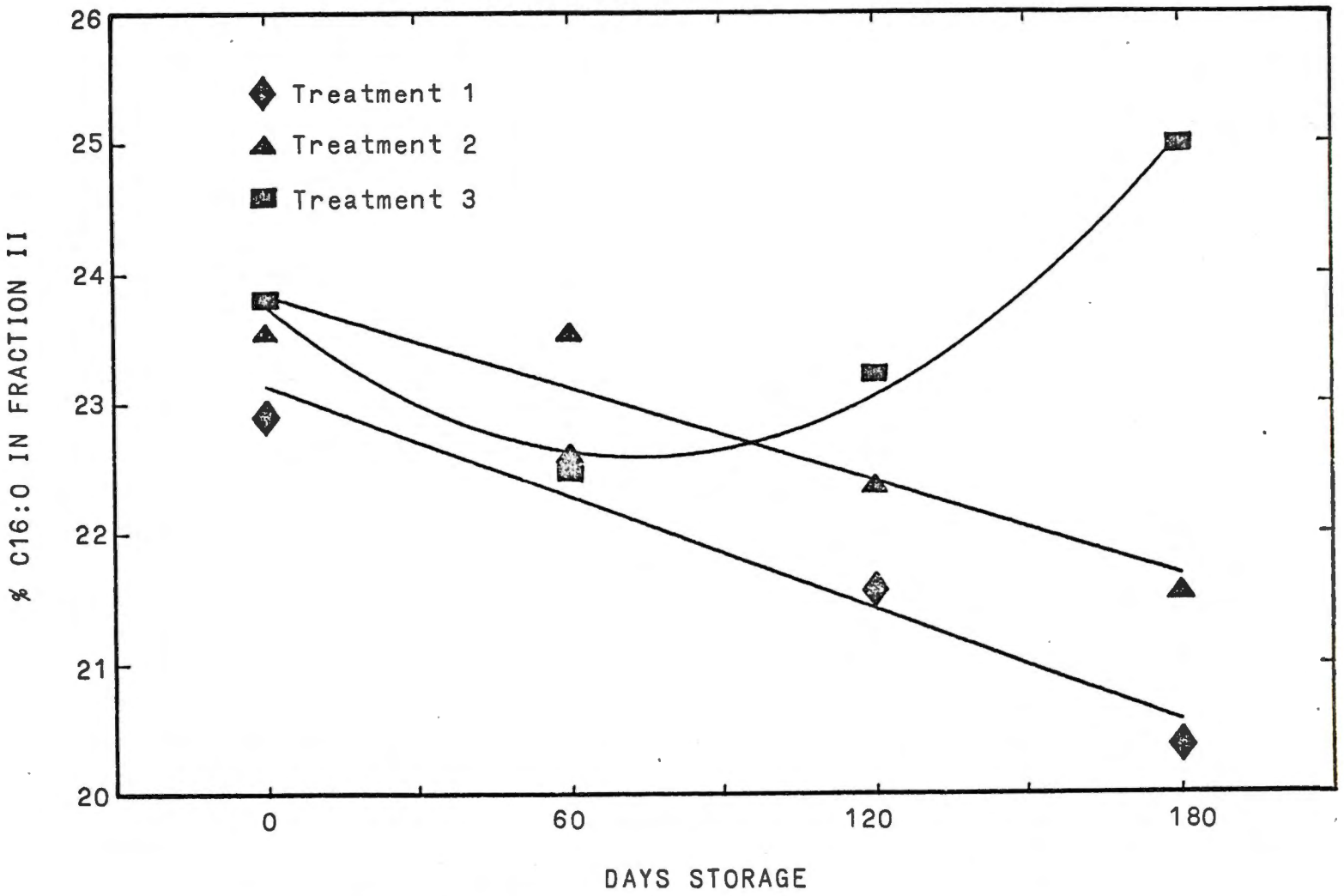


FIGURE 17. The mean relative percent of palmitic acid in Fraction II for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

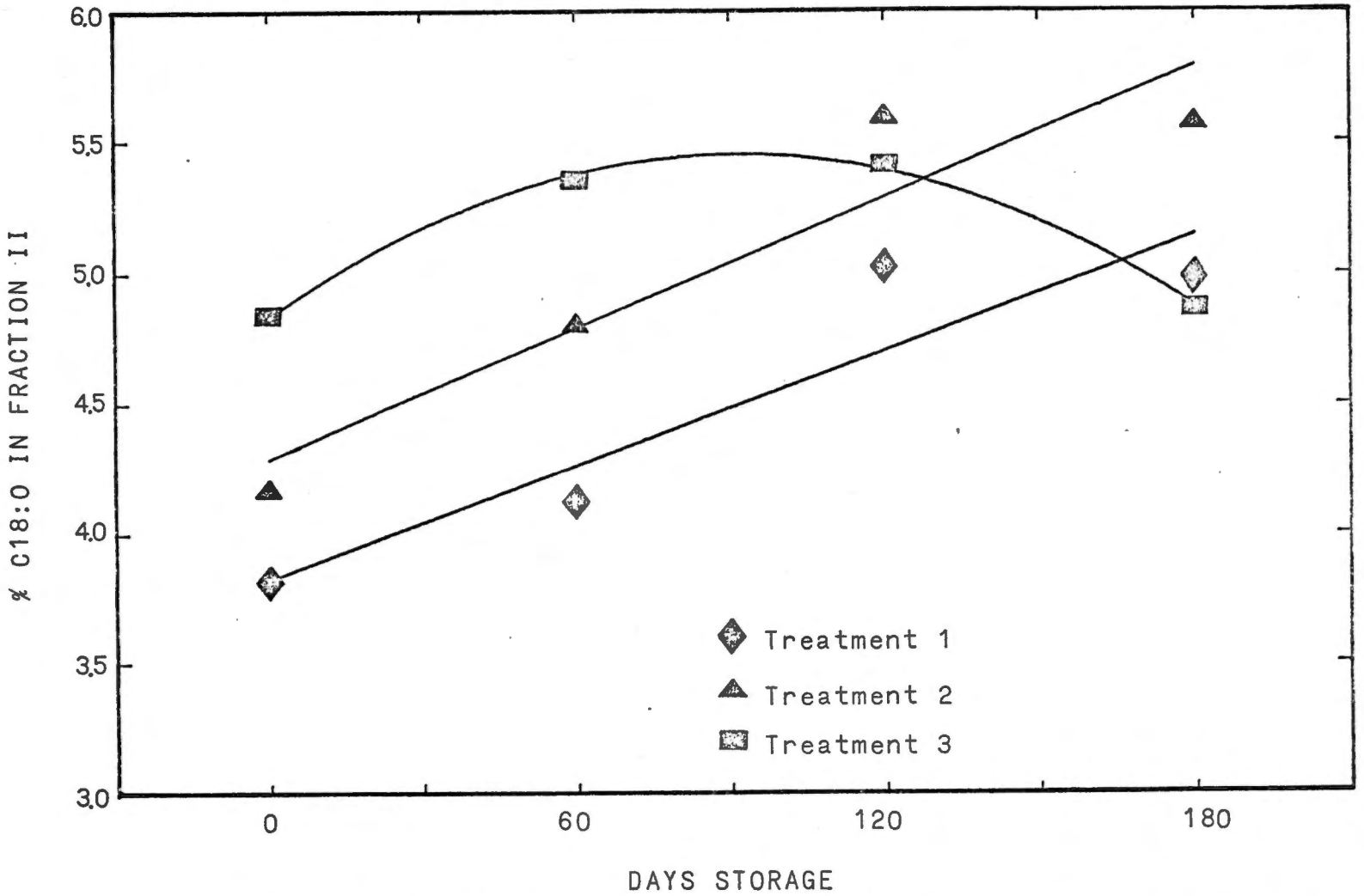


FIGURE 18. The mean relative percent of stearic acid in Fraction II for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

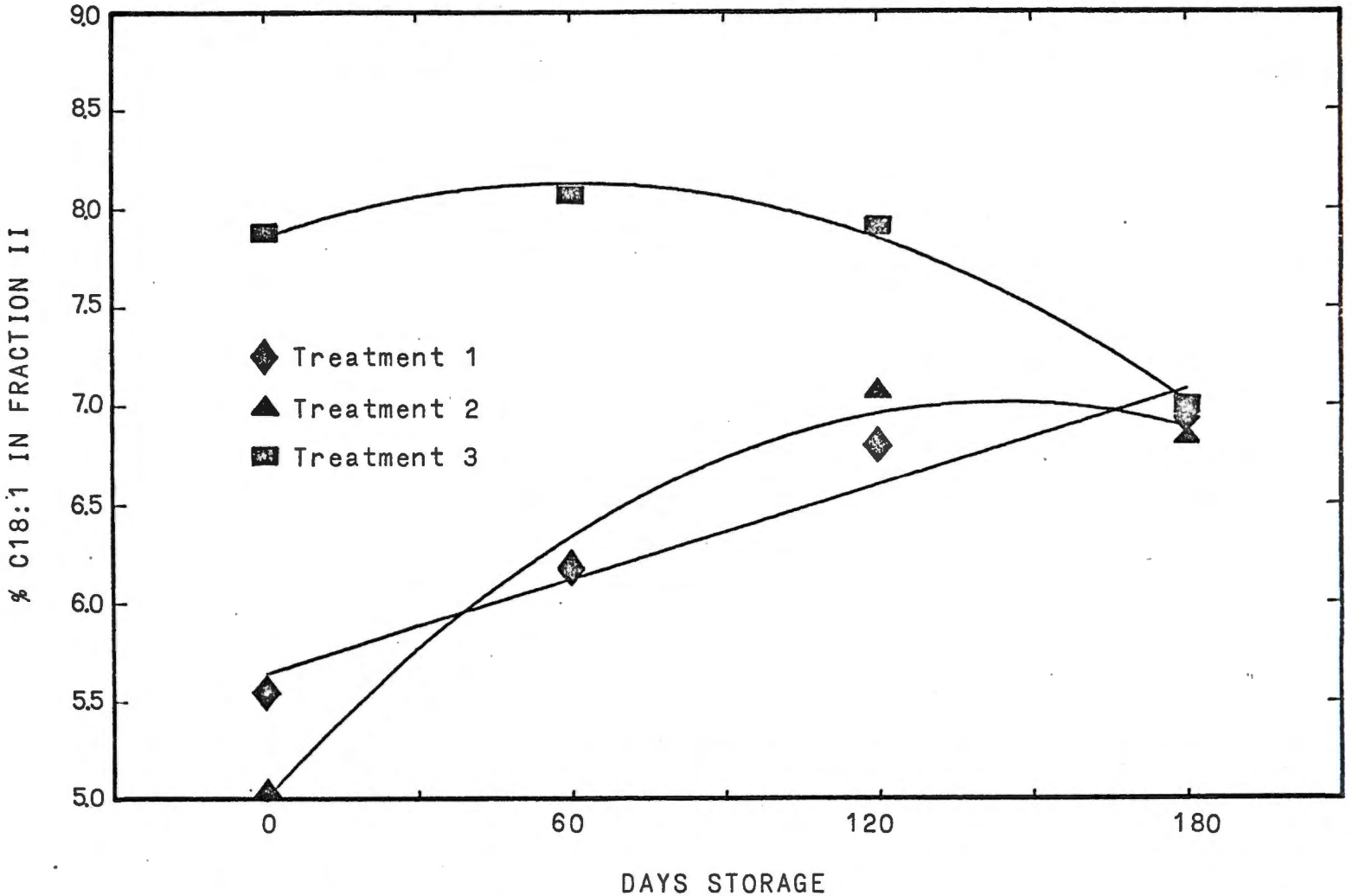


FIGURE 19. The mean relative percent of oleic acid in Fraction II for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

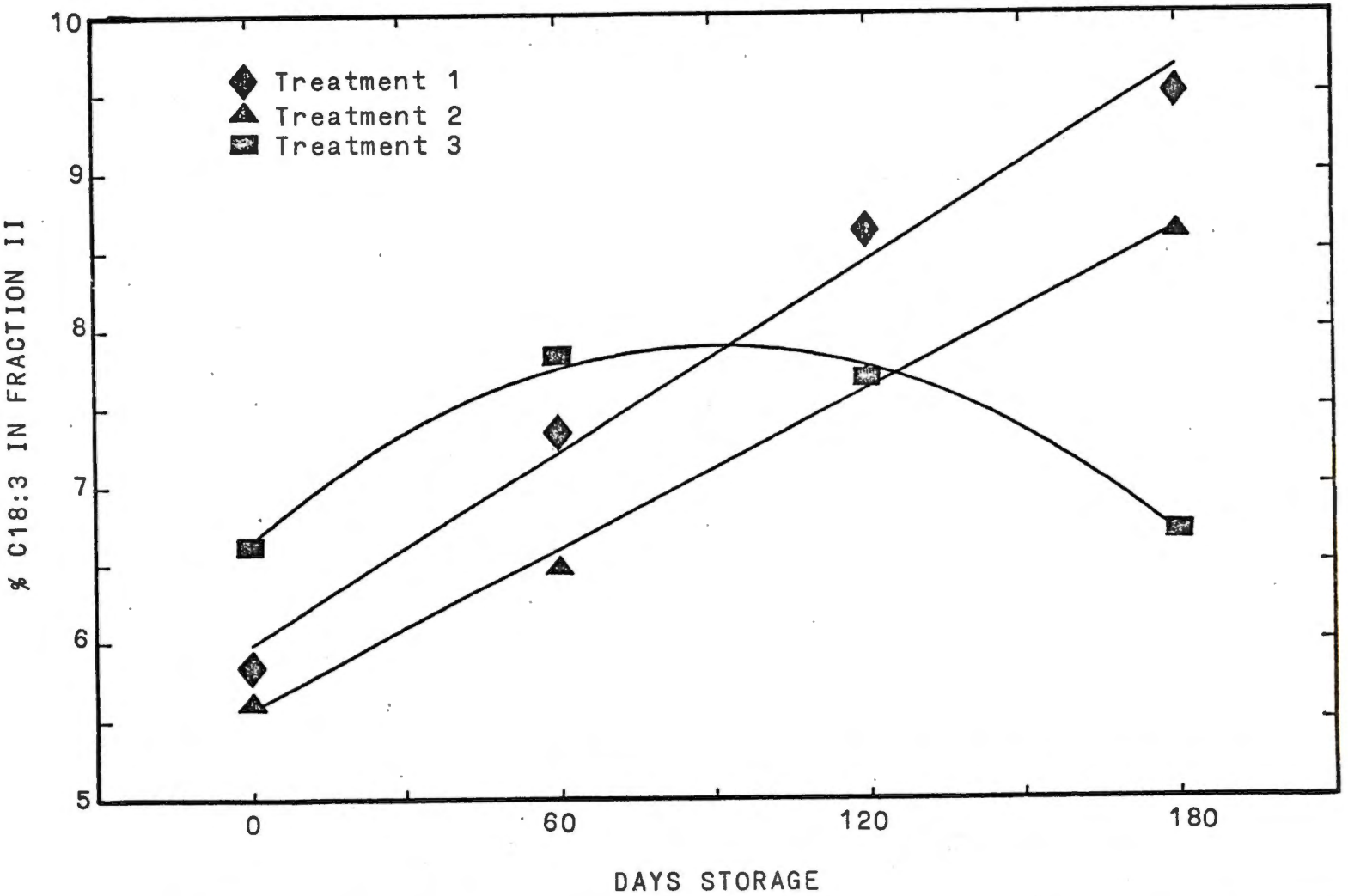


FIGURE 20. The mean relative percent of linolenic acid in Fraction II for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

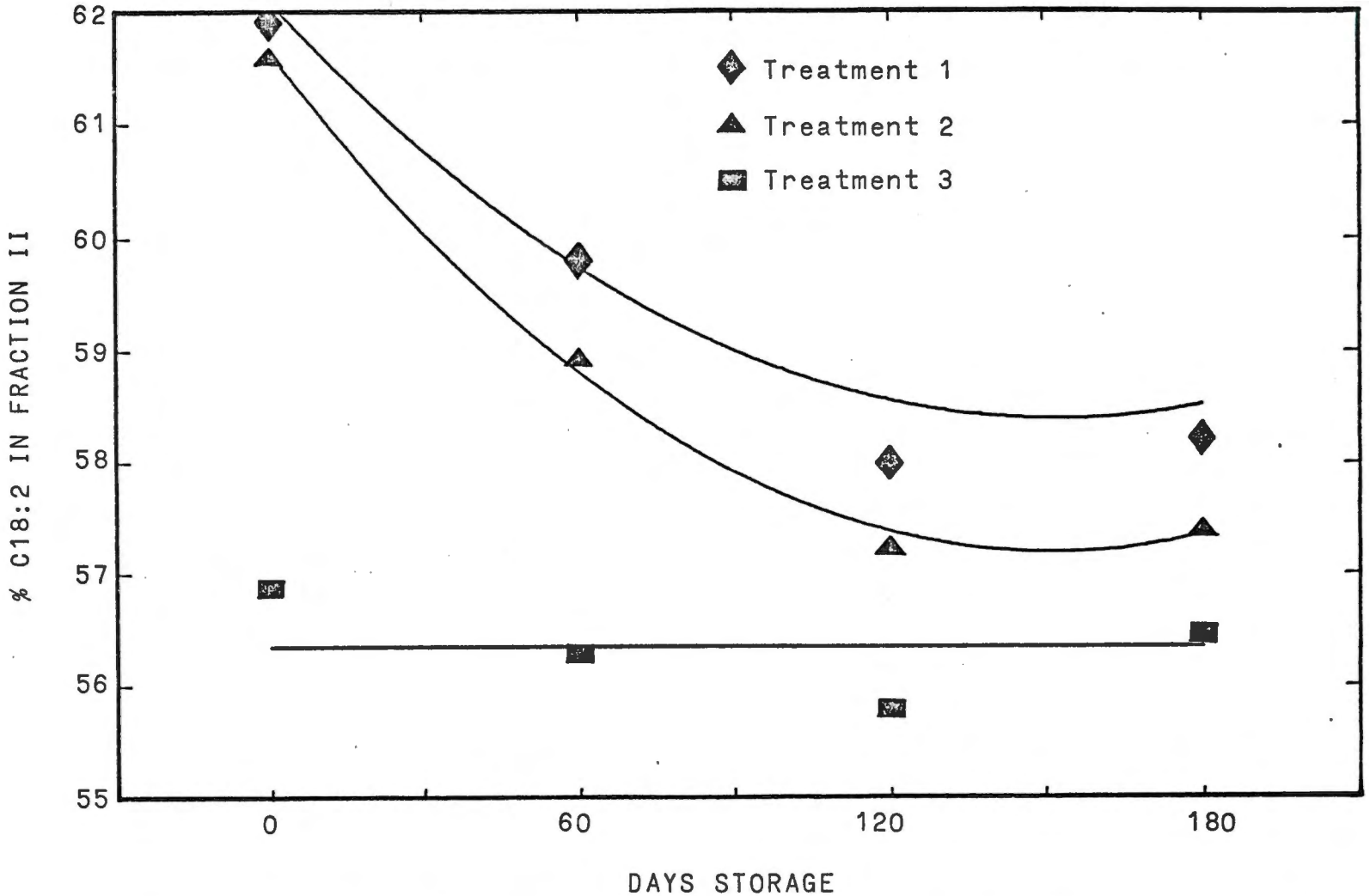


FIGURE 21. The mean relative percent of linoleic acid in Fraction II for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

19, and 20, respectively). A decrease in the relative percent of palmitic acid was also observed for Treatments 1 and 2 (Figure 17).

Only a small net change in the relative fatty acid distribution was found in the lipids in Fraction II of Treatment 3. The relative percent of stearic acid, oleic acid, and linolenic acid of Treatment 3 increased approaching a maximum and then started decreasing (Figures 18, 19, and 21, respectively). The relative percent of palmitic acid of Treatment 3 decreased approaching a minimum and then started increasing (Figure 17). Linoleic acid, as stated earlier, showed no statistical change in Treatment 3 over time.

X. FATTY ACID DISTRIBUTION IN FRACTION III

As in Fraction II, the relative percent of all five fatty acids in Fraction III had significant mean squares for time and all but linolenic acid had significant mean squares for treatment (Appendix B). The relative percent of stearic acid, oleic acid, and linoleic acid in Fraction III had significant treatment x time interactions (Figures 22 through 24, and Table 4, Appendix B).

In Fraction III, as in Fraction I and II, the relative percent of linoleic acid decreased in Treatments 1 and 2 while the relative percent of linoleic acid in Treatment 3 remained constant (Figure 24). The relative percents of stearic acid and oleic acid in Treatment 3 (Figures 22 and 23) remained constant over time while stearic acid and oleic acid (Figures 22 and 23) in Treatments 1 and 2 increased approaching a maximum and then started decreasing.

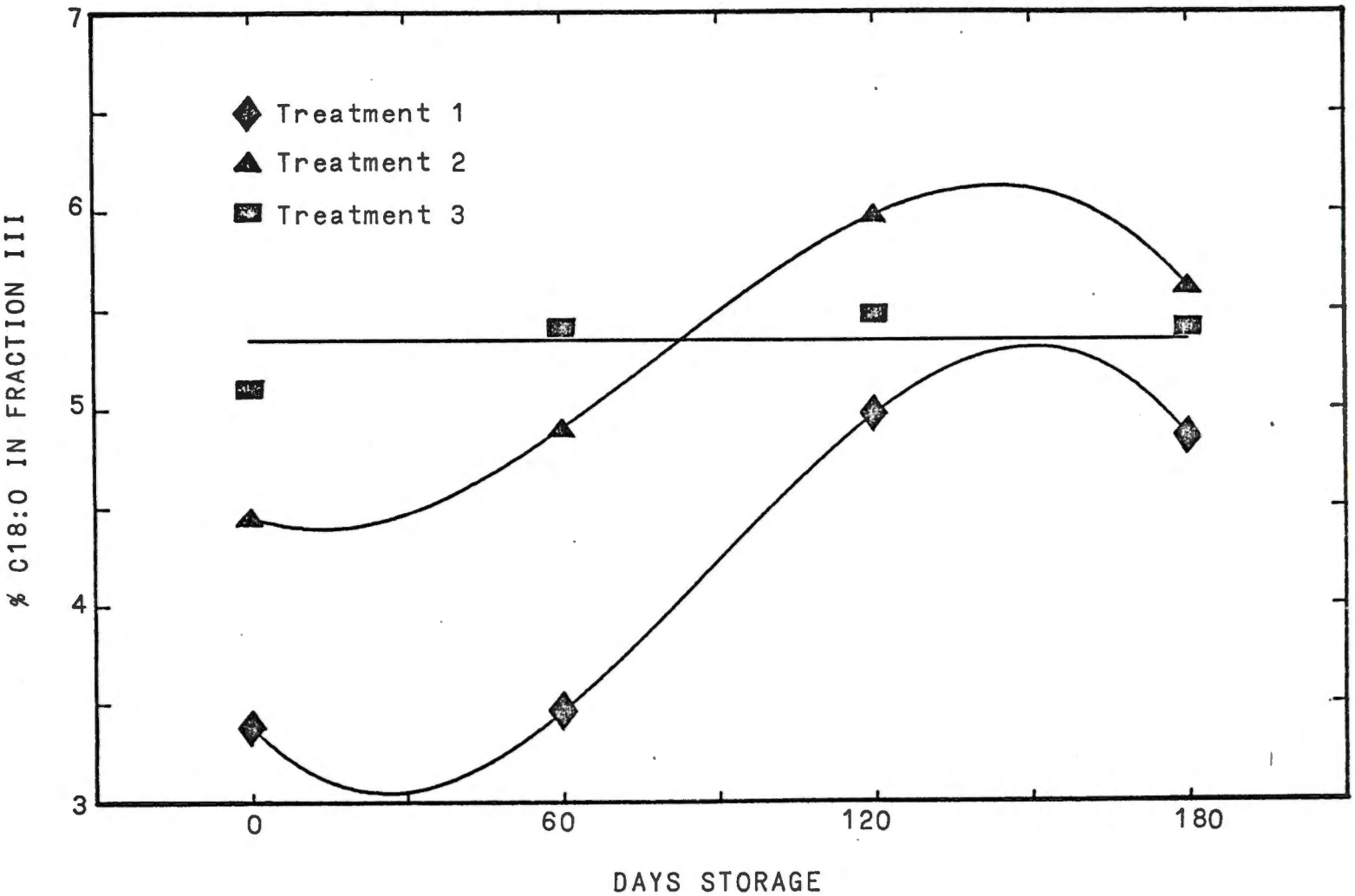


FIGURE 22. The mean relative percent of stearic acid in Fraction III for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

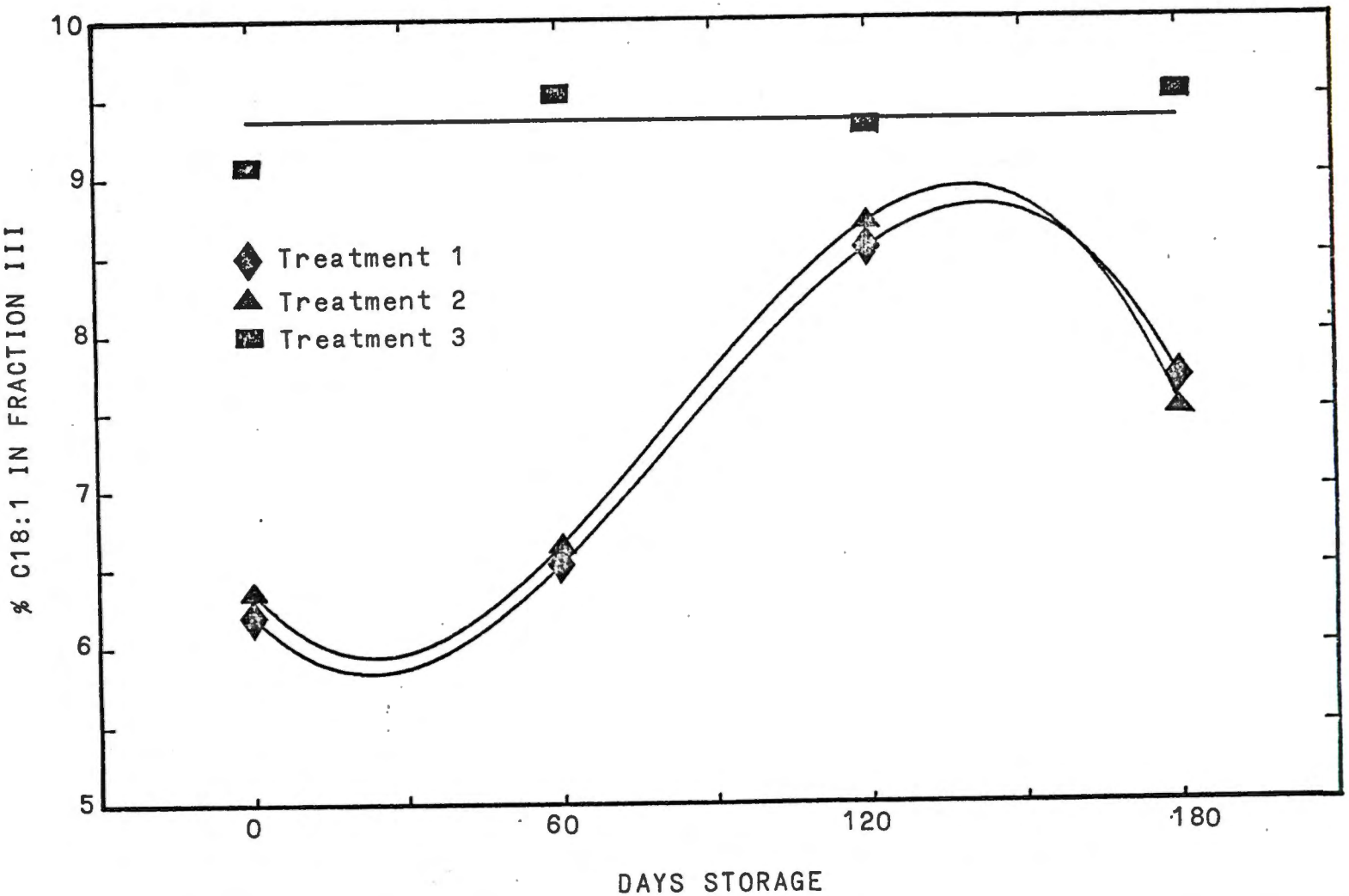


FIGURE 23. The mean relative percent of oleic acid in Fraction III for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

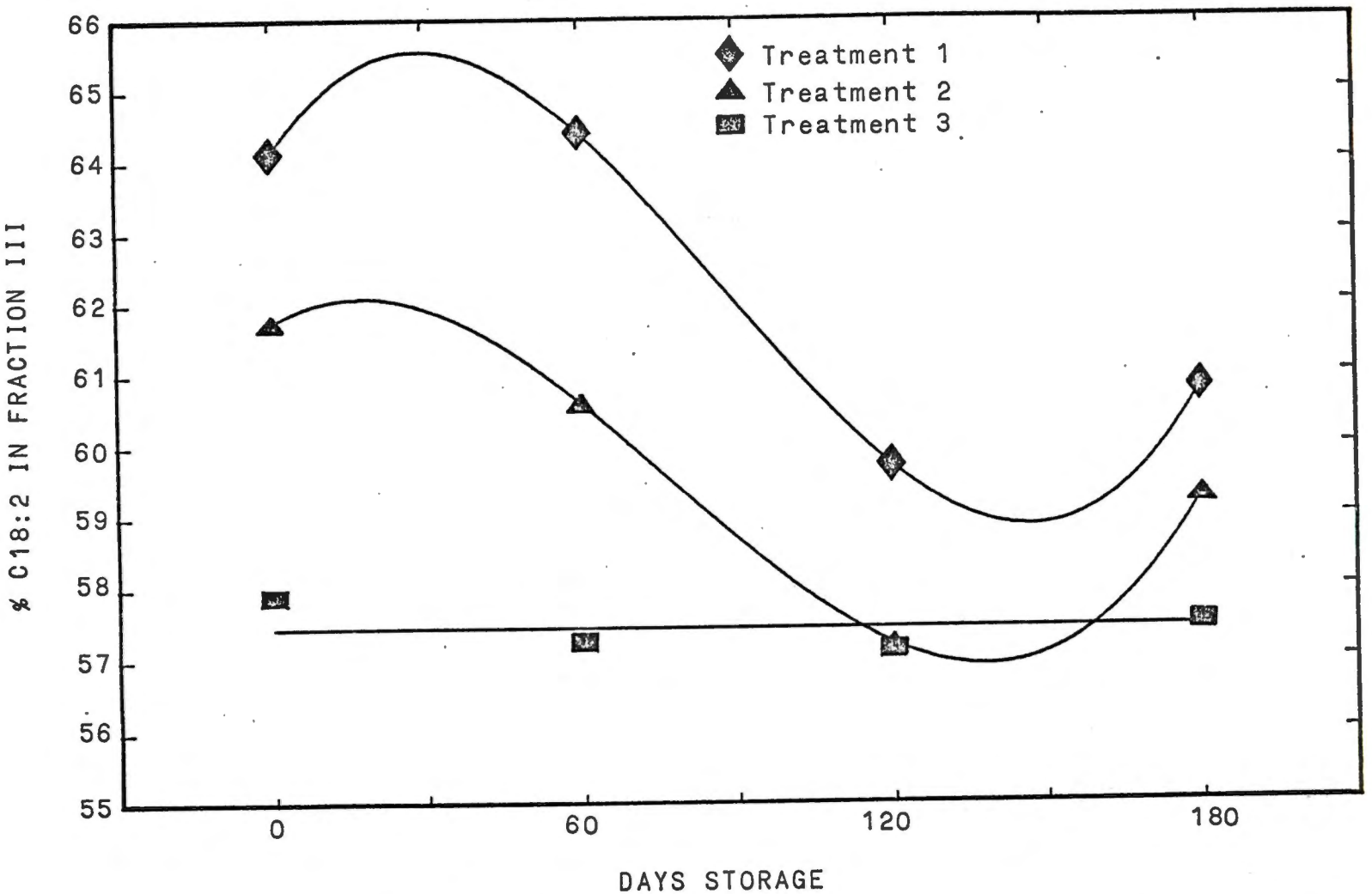


FIGURE 24. The mean relative percent of linoleic acid in Fraction III for each treatment of defatted soy-flour plotted as a function of storage time. Each point represents the mean of five replicates. Treatment 1 = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

Analysis of treatment by the Student-Newman-Keuls test showed that the relative percent of palmitic acid was significantly less in Treatment 1 than in Treatments 2 or 3 (Table 2, page 24). An average of the relative percent of palmitic acid across treatments showed a linear decrease within the range of the data (Figure 25). The average relative percent of linolenic acid across treatments showed a linear increase (Figure 26).

Apparently, the most important change in the fatty acid composition in the silicic acid fractions (I, II, or III) was with linoleic acid (Figures 15, 21, and 24, pages 42, 49, and 53, respectively). In each fraction, the relative percent of linoleic acid decreased significantly in Treatments 1 and 2. Since about as much decrease in the relative percent of linoleic acid occurred with the lipoxygenase inactive Treatment 1 as with the low lipoxygenase active Treatment 2, the decrease was probably due to autoxidation of the linoleic acid. The lipoxygenase active flour, Treatment 3, showed no decrease in the relative percent of linoleic acid in any of the fractions. The lower initial level of the relative percent of linoleic acid in Treatment 3 compared with Treatments 1 or 2 for all silicic acid fractions suggests that linoleic acid reached a level at which it was no longer available for oxidation catalyzed by lipoxygenase. Lipoxygenase action during processing or during the 92 days between processing and the beginning of storage possibly resulted in the lower initial level of linoleic acid in Treatment 3. Since autoxidation appeared to reduce the amount of linoleic acid in Treatments 1 and 2 but not in Treatment 3, the lower initial level of linoleic acid in Treatment 3 seemed to be

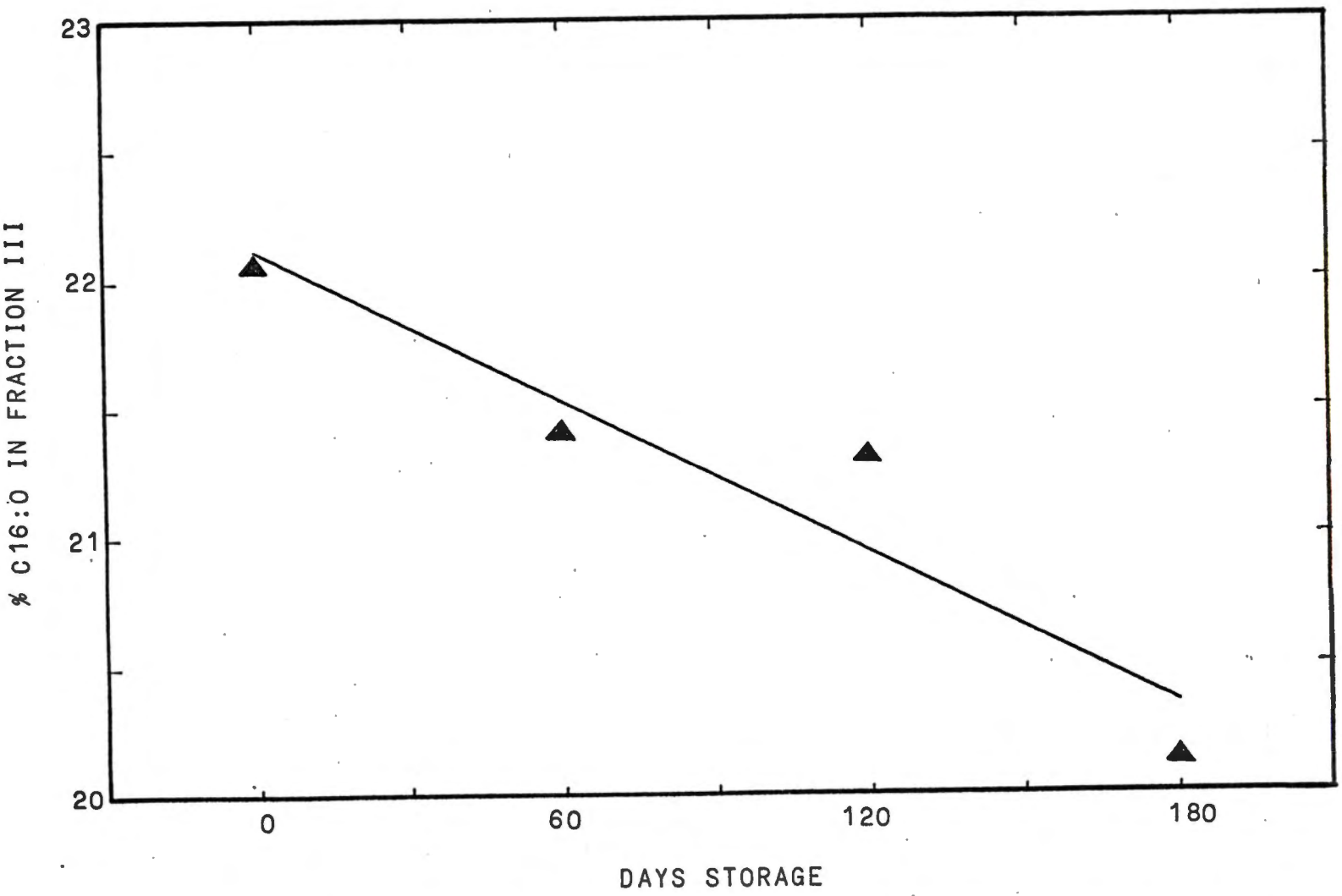


FIGURE 25. The mean relative percent of palmitic acid in Fraction III of three treatments of defatted soy-flour plotted as a function of storage time. Each point represents the mean of three treatments x five replicates.

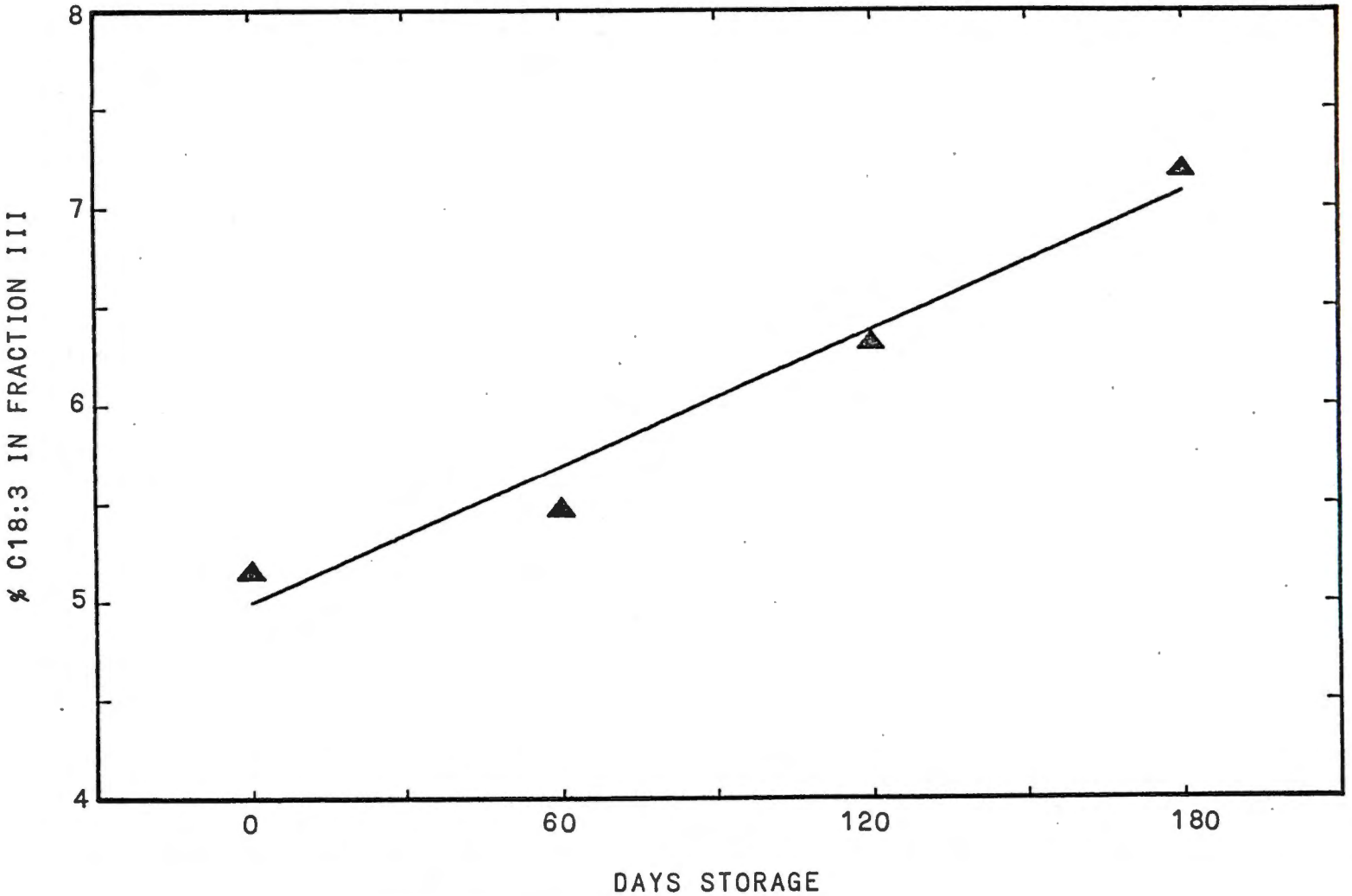


FIGURE 26. The mean relative percent of linolenic acid in Fraction III of three treatments of defatted soy-flours plotted as a function of storage time. Each point represents the mean of three treatments x five replicates.

not available for autoxidation, either. It is not known whether the low level itself, the order of esterification in the lipids, or if some other reason is responsible for this apparent non-availability.

Small decreases in the relative percent of linolenic acid in Fraction I and II of Treatment 3 possibly were due to lipoxygenase action (Figures 16 and 21, pages 43 and 49, respectively).

Decreases in the relative percent of palmitic acid were observed in Fraction I of Treatment 1 (Figure 12, page 39), in Fraction II of Treatments 1 and 2 (Figure 17, page 45), and in the average of all treatments over time in Fraction III (Figure 25, page 55). No reasons can be given at this time for these observations.

The autoxidation of linoleic acid in all three silicic acid lipid fractions of Treatments 1 and 2 did not result in any increase in TBA number during storage (Figure 3, page 26). Apparently, autoxidation of linoleic acid in residual lipids of soybean flour did not give rise to TBA reactive substances. At the present time, it is not possible to say whether this oxidation resulted in build-up of soy off-flavors. A sensory evaluation of three replications of the three treatments has been completed, but the author did not do this evaluation and it has yet to be statistically analyzed.

The fact that linoleic acid decreased in all three lipid fractions for Treatments 1 and 2 indicates that autoxidation of linoleic acid will occur no matter with which lipid it is esterified. It cannot be concluded that lipoxygenase can catalyze oxidation of linoleic acid when it is esterified to any lipid. However, the significantly lower level of

linoleic acid in all three lipid fractions for Treatment 3 compared with Treatments 1 and 2 may infer this.

Also, the fact that Fraction III contains mainly phosphatidyl choline (Figure 11, page 37), infers that lipoxygenase can catalyze the oxidation of linoleic acid while it is esterified to the phosphatidyl choline molecule. More investigation is needed to confirm this possibility since the oxidation of lecithin as well as other soy lipids has been implicated in production of off-flavors in soy protein products (44).

CHAPTER V

SUMMARY

Five replicates of three defatted soy-flours (fully toasted, white and enzyme active) were stored for 0, 60, 120, and 180 at 23°C. At each storage time, samples were removed from storage and analyzed for lipoxygenase activity, nitrogen solubility index, thiobarbituric acid number, and percent extractable lipid material. Lipid extracts were fractionated by silicic acid column chromatography by eluting with 300 ml portions of chloroform-methanol 20:1, chloroform-methanol 1:1, and methanol. Lipids in each fraction were separated by thin layer chromatography and qualitatively analyzed by specific detection tests and comparison of R_f values with R_f values of known standards. Methyl esters of the fatty acids in each fraction were prepared and the relative percent of each fatty acid (palmitic, stearic, oleic, linoleic, and linolenic) was determined by a gas liquid chromatographic procedure.

Both enzyme active and white flours had lipoxygenase activity; the enzyme active flour had the highest activity. Lipoxygenase activity of the enzyme active flour decreased at an increasing rate during storage. TBA number decreased during storage and thus was of little value in measuring amount of oxidation. The average percent of extractable lipid material decreased from approximately 3.0 to 2.8 percent during storage. Fraction I, containing about 30 percent of the lipid, consisted mainly of glycerides, glycolipids, and steroids. Fraction II, containing

about 57 percent of the lipid, consisted mainly of phospholipids (phosphatidyl inositol, lecithin, and cephalin), glycolipids, and steroids. Fraction III was almost all lecithin and comprised the last 14 percent of the lipid. Of significance in the fatty acid analysis, the relative percent of linoleic acid in all three fractions of lipid extracted from the fully toasted and white flour decreased significantly during storage while the relative percent of linoleic acid in all three fractions of lipid extracted from the enzyme active flour did not change. A considerably lower initial level of linoleic acid was found in all three fractions of lipid extracted from the enzyme active flour than in the fully toasted and white flours.

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APPENDICES

APPENDIX A

TABLE 3

POLYNOMIAL EQUATIONS OF DEPENDENT VARIABLES AS A FUNCTION OF STORAGE TIME

Variable	Treatment[a]	Equation
Lipoxygenase Activity	1	$y=0$
	2	$y=0.0377$
	3	$y=5.3462+0.4172T-0.1958T^2$
Nitrogen Solubility Index	1	$y=21.4374$
	2	$y=74.4560-5.8733T+0.8401T^2$
	3	$y=80.9283+4.0407T-0.6639T^2$
TBA No.	1	$y=3.8453+0.1282T-0.4369T^2+0.0949T^3$
	2	$y=5.4411-0.2049T$
	3	$y=7.7493-2.2554T+0.3574T^2$
% Lipid Dry Matter Basis	1,2,3	$y=3.027-0.0662T$
% Lipid in Fraction I	1,2,3	$y=32.30-3.21T+0.71T^2$
% Lipid in Fraction II	1,2,3	$y=63.3473-11.588T+5.9987T^2-0.9067T^3$
Fatty Acids Fraction I		
% C16:0	1	$y=15.264-0.3036T$
	2	$y=16.3205$
	3	$y=15.898+0.3728T$
% C18:0	1	$y=2.314+0.4928T$
	2	$y=-3.128+9.1037T-3.6870T^2+0.4753T^3$
	3	$y=3.7255+1.0955T-0.1885T^2$
% C18:1	1	$y=15.649+0.4168T$
	2	$y=7.902+9.034T-3.513T^2+0.437T^3$
	3	$y=23.522-7.735T+3.564T^2-0.493T^3$
% C18:2	1	$y=63.1715-4.3729T+0.5535T^2$
	2	$y=72.948-18.3937T+6.6260T^2-0.7883T^3$
	3	$y=52.616$
% C18:3	1	$y=4.3885+2.9923T-0.3985T^2$
	2	$y=6.23+0.6332T$
	3	$y=7.671-0.2972T$
Fatty Acids Fraction II		
% C16:0	1	$y=23.991-0.8566T$
	2	$y=24.551-0.7166T$
	3	$y=26.431-3.458T+0.778T^2$

TABLE 3 (continued)

Variable	Treatment[a]	Equation
% C18:0	1	$y=3.384+0.4412T$
	2	$y=3.793+0.5T$
	3	$y=3.7595+1.3405T-0.2655T^2$
% C18:1	1	$y=5.165+0.4798T$
	2	$y=3.002+2.3674T-0.349T^2$
	3	$y=7.0315+1.1027T-0.2765T^2$
% C18:2	1	$y=65.6040-4.1904T+0.58T^2$
	2	$y=65.893-4.9534T+0.705T^2$
	3	$y=56.3495$
% C18:3	1	$y=4.761+1.2276T$
	2	$y=4.555+1.0224T$
	3	$y=4.4335+2.7589T-0.5495T^2$
Fatty Acids Fraction III		
% C16:0	1,2,3	$y=22.7053-0.5877T$
% C18:0	1	$y=7.802-7.692T+3.783T^2-0.511T^3$
	2	$y=6.708-4.2973T+2.389T^2-0.3457T^3$
	3	$y=5.3515$
% C18:1	1	$y=12.114-10.563T+5.405T^2-0.76T^3$
	2	$y=12.878-11.6107T+5.929T^2-0.8403T^3$
	3	$y=9.3635$
% C18:2	1	$y=48.148+27.3887T-13.2010T^2+1.7863T^3$
	2	$y=52.914+16.377T-8.836T^2+1.285T^3$
	3	$y=57.4445$
% C18:3	1,2,3	$y=4.3047+0.6943T$

[a]Treatment = fully toasted flour, Treatment 2 = white flour, and Treatment 3 = enzyme active flour.

APPENDIX B

TABLE 4

MEAN SQUARES FOR EACH DEPENDENT VARIABLE

Variables	Source of Variation		
	Treatment df 2	Time df 3	Treatment x Time df 6
	Mean Squares		
Lipoxygenase Activity	160.2126**	0.9748**	0.9570**
Nitrogen Solubility Index	21887.9881**	3.7521	16.7606
TBA No.	8.8929**	1.2188**	0.3530**
% Lipid Dry Matter Basis	1.0432*	0.1330*	0.0589
% Lipid in Fraction I	444.2554**	13.9185*	2.6955
% Lipid in Fraction II	320.2490**	25.3789**	5.3191
% Lipid in Fraction III	62.0474**	3.1000	3.2216
% C16:0 in Fraction I	29.8709	0.0580	1.3366**
% C18:0 in Fraction I	12.5864**	4.6288**	0.7121**
% C18:1 in Fraction I	62.2514**	2.5462**	1.9363**
% C18:2 in Fraction I	111.1611**	36.4412**	8.6383**
% C18:3 in Fraction I	19.1168**	5.9648**	4.0106**
% C16:0 in Fraction II	15.6950	3.9360*	6.4664**
% C18:0 in Fraction II	2.3835	3.3316**	0.6823*
% C18:1 in Fraction II	12.7287*	3.2049**	2.0519**
% C18:2 in Fraction II	54.1384*	29.3412**	4.2123
% C18:3 in Fraction II	3.0389	15.2733**	4.1192**
% C16:0 in Fraction III	22.8314*	9.6488**	1.4783
% C18:0 in Fraction III	8.5216**	4.6431**	0.8094**
% C18:1 in Fraction III	29.0384**	8.0810**	1.8246**
% C18:2 in Fraction III	116.5935**	32.6332**	7.2055**
% C18:3 in Fraction III	2.8076	12.4995**	0.8918

*Significant difference at the 5 percent level.

**Significant difference at the 1 percent level.

APPENDIX C

TABLE 5

ERROR MEAN SQUARES FOR EACH DEPENDENT VARIABLE

Variables	Source of Variation		
	Treatment df 2	Time df 3	Treatment x Time df 6
	Error Mean Square		
Lipoxygenase Activity	16.2747	0.1168	0.1168
Nitrogen Solubility Index	12.3518	7.3842	7.3842
TBA No.	0.1131	0.0569	0.0569
% Lipid Dry Matter Basis	0.2195	0.0321	0.0321
% Lipid Fraction I	59.5238	3.4542	3.4542
% Lipid Fraction II	31.6740	4.6381	4.6381
% Lipid Fraction III	8.3086	2.5148	2.5148
% C16:0 in Fraction I	16.4640	0.2336	0.2336
% C18:0 in Fraction I	1.3443	0.1567	0.1567
% C18:1 in Fraction I	5.2798	0.3331	0.3331
% C18:2 in Fraction I	7.9602	1.3608	1.3608
% C18:3 in Fraction I	1.1541	0.3757	0.3757
% C16:0 in Fraction II	4.0960	1.0363	1.0363
% C18:0 in Fraction II	0.9828	0.2557	0.2557
% C18:1 in Fraction II	2.3295	0.3650	0.3650
% C18:2 in Fraction II	10.1695	2.2174	2.2174
% C18:3 in Fraction II	1.1663	0.7460	0.7460
% C16:0 in Fraction III	4.0704	1.2611	1.2611
% C18:0 in Fraction III	0.9095	0.1721	0.1721
% C18:1 in Fraction III	3.2636	0.2754	0.2754
% C18:2 in Fraction III	6.5027	1.7176	1.7176
% C18:3 in Fraction III	1.6594	0.7548	0.7548

VITA

Richard Edward Moyers was born February 10, 1952, to George P. and Thelma B. Moyers in Fayetteville, Tennessee. He graduated from Fayetteville Central High School in May of 1970, and entered The University of Tennessee later that fall. In June, 1974, he received a Bachelor of Science degree in Agriculture with a major in Dairy Manufacturing. He was married to the former Miss Pamela Sue Winter in Alexandria, Virginia, in August of 1974. Later that August, he enrolled in the Graduate School of The University of Tennessee, Knoxville, and since that time, has been working towards a Master of Science degree in Agriculture with a major in Food Technology and Science. While working on his graduate degree he has held the position of graduate research assistant in the Department of Food Technology and Science. He is a member of the Institute of Food Technologists, Alpha Zeta, Phi Kappa Phi, and Gamma Sigma Delta.