Sodium taurocholate, manganese sulphate, KH_2PO_4, sodium acetate, etc., accelerate the activity of the mold lipase.

The change in activity of acetone-dried sample of mold lipase with time is negligible.

The synthetic activity of the mold lipase is higher than that of seed and cake lipases.

A definite relation between the vitamin contents and the lipolytic activity of the molds could not be found.

Peanut cake medium appears to be a good source to grow the lipolytic molds on a large scale.

Acknowledgment

Our thanks are due to the Indian Council of Medical Research for having awarded a Research Fellowship to one[2] of us for carrying out this investigation.

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[2] C. V. Ramakrishnan.

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Fatty Acid Composition of Hydrogenated Linseed Oil

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The development in recent years in the fields of spectrophotometric analysis has led to improved methods for the determination of linoleic and linolenic acids in fats. Applying the method of Mitchell, Kraybill, and Zscheile (1), a number of investigators have examined the catalytic hydrogenation of unsaturated oils.

On investigating the composition of hydrogenated linseed oil, Lemon (2) found an isomer of linoleic acid which did not undergo conjugation upon treatment with alkali. This acid was believed to be 9,15-octadecadienoic acid and assumed to be produced by the selective hydrogenation of the central double bond of linolenic acid. Subsequently Mattil (3) and Filer (4) ascertained the presence of 9,15-octadecadienoic acid in hydrogenated soybean oil. The acid is named isolinoleic acid. Fischer, O'Connor, and Dollear (5) stated that iso-linoleic acid is not produced during hydrogenation of cottonseed and peanut oils. Bailey and Fischer (6) reported the relative reactivities toward hydrogenation of oleic, iso-linoleic, linoleic, and linolenic acids. They found iso-linoleic acid to be hydrogenated only about three times as fast as oleic acid whereas linoleic and linolenic acid are hydrogenated respectively 20 and 40 times as fast as oleic acid. Later Bailey (7) made a more complete analysis of the reaction kinetics through a mathematical approach. The reactions occurring in the hydrogenation of linseed oil are shown diagrammatically in Figure 1.

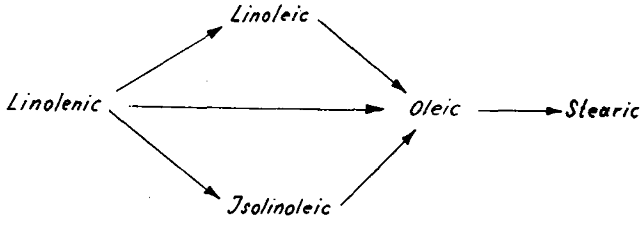


FIG. 1.

Rebello and Daubert (8) hydrogenated methyl linolenate and isolated a fraction rich in methyl isolinoleate which was found to be a mixture of at least three isomers, the 8,14-, 9,15-, and 10,14-isolinoleic acids. Thompson (9) hydrogenated a mixture of 50% tung oil and 50% linseed oil. Reactivity ratios for the various fatty acids were calculated, and it was found that during the initial stage of hydrogenation the elaostearin was about 20 times more reactive than normal linolenin.

Our present investigation will prove that during hydrogenation of linseed oil comparatively great amounts of conjugated linoleic acid are formed. The reactions pictured in Figure 1 are apparently only part of those involved, and in our opinion the hydrogenation of linolenic acid is more likely to proceed as shown in Figure 2.

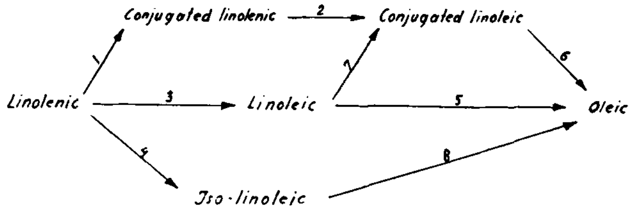


FIG. 2.

The Isomerization Effect of Ni-catalyst. Among several catalysts reported to be effective in conjugating unsaturated fatty acids, nickel catalysts are well known. Radlove, Teeter, Bond, Cowan, and Kass (10) examined the conjugation effect of nickel catalysts upon soybean oil and linseed oil and found that about 35% of the di- and tri-unsaturated acids were converted to conjugated forms. Mattil (3) observed a slight conjugation when heating soybean oil with a nickel catalyst at 200°C. Blekkingh (11) reports that a nickel hydrogenation catalyst in contact with hydrogen has the property of shifting the double bonds in unsaturated fatty acids.

In investigations regarding the kinetics of the conjugation effect of nickel catalysts the temperature is found to have a very great influence on the reaction rate of the isomerization (10). The present investigation is concerned with catalytic hydrogenations at different temperatures, and it is shown that great differences in conjugation are due to these changes of temperature.

Experimental

The fat used was fresh refined linseed oil. Hydrogenations were carried out on a laboratory scale with 0.2% Ni supported on Kieselguhr. Three series at 160°C. and 1 atm. pressure and three series at 235°C. and 1 atm. pressure were carried out. The isomerization effect of the catalyst was determined by heating the oils at 160° and 235°C. respectively, with Ni-catalyst, 0.2% Ni, in CO_2 atm. and with stirring.

Methods of Analysis and Calculation of Results. Iodine values were determined by the Wijs method. Normal linolenic and linoleic acids were determined by the spectrophotometric method of Mitchell, *et al.* (1). Conjugated acids were determined spectrophotometrically without alkali isomerization using n-hexane as solvent.

The following specific extinctions were used as reference values:

	233 mμ	268 mμ
Alkali-isomerized linoleic acid	86.0	0.77
Alkali-isomerized linolenic acid	60.4	53.5
Conjugated linoleic acid in n-hexane	115.0	
Conjugated linolenic acid in n-hexane		205

Saturated acids were determined by the Bertram oxidation method. The figures for isolinolein were calculated from the iodine values and the contents of linolenin, linolein, and saturated glycerides. Assuming the contents of isolinolein to be x and the contents of olein to be y, we shall have the following equations:

I. $x + y = 100 \div (\%\ \text{linolenin} + \%\ \text{linolein} + \%\ \text{saturated}) = S$

II. $\frac{x \cdot 173.2}{100} + \frac{y \cdot 85.9}{100} = d.$

where d stands for the difference between the iodine value of the sample and the theoretical iodine value of the linolenin and linolein present.

From the equations we get:

$$x = \frac{100\,d - 85.9 \cdot S}{87.3}$$

$$y = S - \frac{100\,d - 85.9 \cdot S}{87.3}$$

All the data for the composition of the oils are expressed as triglycerides. The conversion constant used between fatty acids and triglycerides is 1.045.

TABLE I

Temp.	Isomerization time	K 233	K 268
160°C.	15 min.	0.58	0.25
160°C.	30 min.	0.75	0.38
235°C.	15 min.	2.90	1.67
235°C.	30 min.	3.60	2.25

Results

A. *Isomerization Effect.* The specific extinctions from the isomerization series are given in Table I.

In Figure 3 the absorption spectra of the isomerization experiments are plotted.

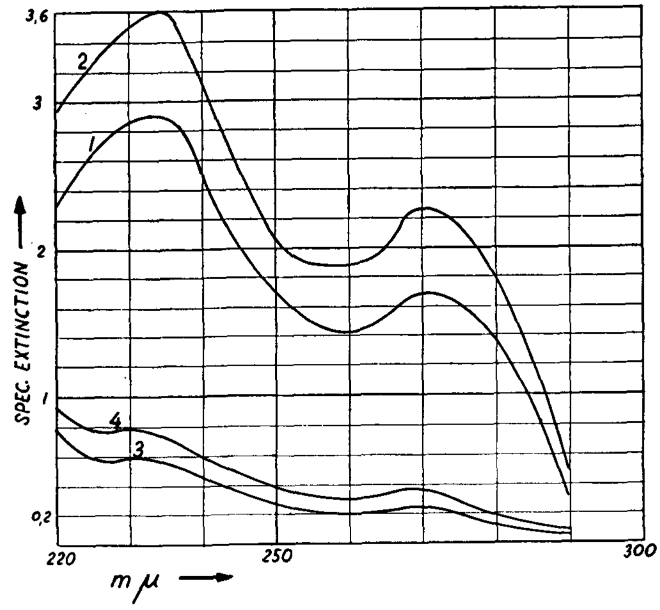


FIG. 3. Isomerization of linseed oil with 0.2% Ni-catalyst.
Curve 1: Isomerized 15 min. at 235°C.
Curve 2: Isomerized 30 min. at 235°C.
Curve 3: Isomerized 15 min. at 160°C.
Curve 4: Isomerized 30 min. at 160°C.

B. *Composition of the Hydrogenated Oils.* The reproducibility of the experiments in the series proved to be very good, and we therefore find it sufficient to give only the mean values from each. The results are shown in Table II and plotted graphically in Figures 4 and 5. In Figure 6 are plotted the absorption spectra of some of the determinations made in hexane.

Discussion

The results obtained show that the isomerization effect of the catalyst is responsible for relatively large

TABLE II

Temp.	Iodine value	Time of hardening	Normal linolenic	Conjugated linolenic	Normal linoleic	Conjugated linoleic	Iso-linoleic	Oleic total	Saturated
°C.	Wijs	min.	Composition of glycerides, %						
Temp. 235°C.	185	0 min.	51.5	0	18.5	0	0	22.4	7.6
	170.8	12 —	40.1	1.2	14.4	8.0	0	28.7	7.6
	156.7	25 —	30.7	1.4	10.6	13.0	0.5	36.0	7.8
	146.8	36 —	25.2	1.1	9.6	13.7	1.4	41.0	8.0
	134.4	51 —	18.0	0.5	10.2	12.8	3.4	47.0	8.1
	123.5	65 —	11.5	0.3	10.8	10.8	6.2	52.1	8.3
	114.3	81 —	6.7	Traces	9.8	8.2	9.2	57.5	9.6
	97.5	120 —	1.2	0	4.3	2.2	14.7	66.8	10.8
	87.2	142 —	Traces	0	1.3	Traces	15.4	67.8	15.5
	73.2	174 —	0	0	0	0	11.0	63.0	26.0
Temp. 160°C.	185	0 min.	51.5	0	18.5	0	0	22.4	7.6
	165.8	32 —	38.5	0.2	15.8	2.0	3.5	32.4	7.6
	144.5	70 —	25.4	0.2	12.2	3.6	9.0	40.1	9.5
	130.6	105 —	17.6	0.1	10.0	3.6	13.3	44.0	11.4
	106.1	180 —	4.0	0	5.1	1.2	23.1	51.4	15.2
	96.3	205 —	1.0	0	2.0	0.4	24.2	54.8	17.6
	85	252 —	Traces	0	0.5	0	21.0	55.4	23.1
	72.2	300 —	0	0	0	0	15.4	53.0	31.6

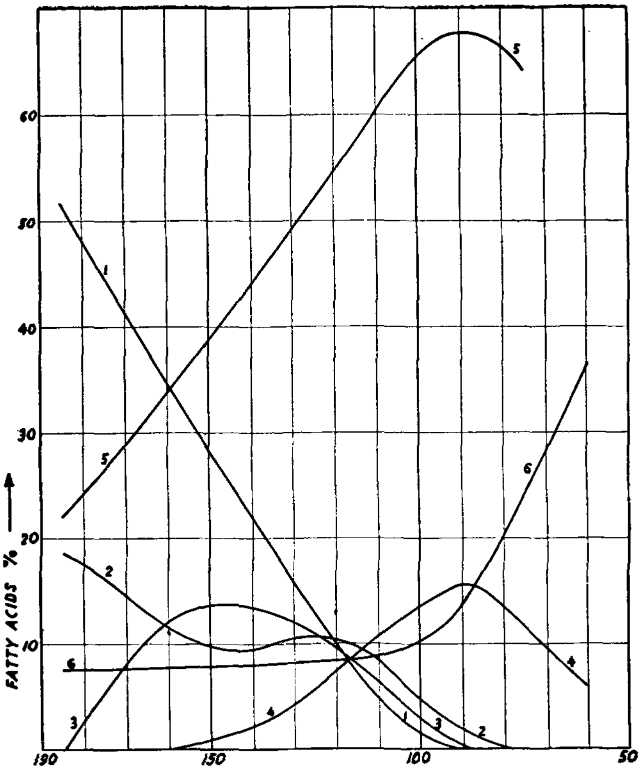


FIG. 4. Hydrogenation of linseed oil at 235°C.

Curve 1: Total linolenic.
Curve 2: Normal linoleic.
Curve 3: Conjugated linoleic.
Curve 4: Iso-linoleic.
Curve 5: Oleic.
Curve 6: Saturated.

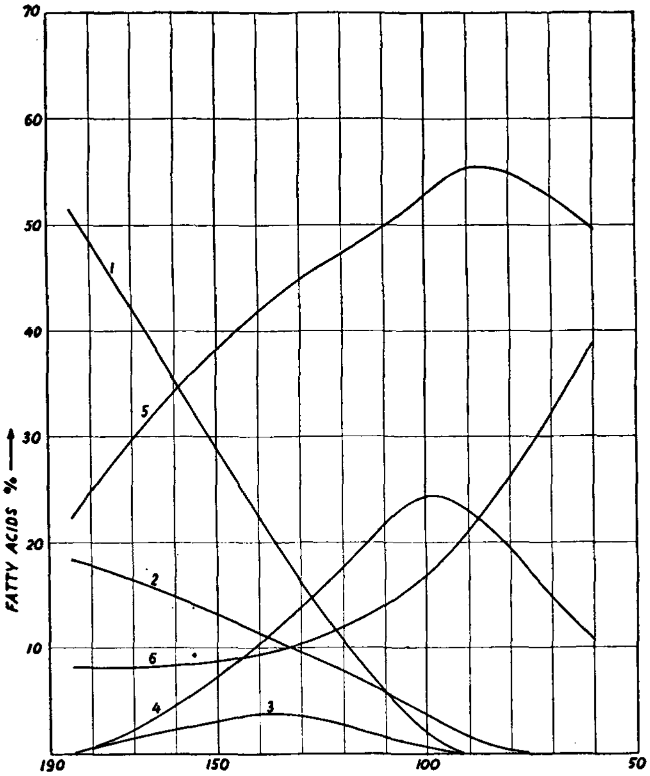


FIG. 5. Hydrogenation of linseed oil at 160°C.

Curve 1: Total linolenic.
Curve 2: Normal linoleic.
Curve 3: Conjugated linoleic.
Curve 4: Iso-linoleic.
Curve 5: Oleic.
Curve 6: Saturated.

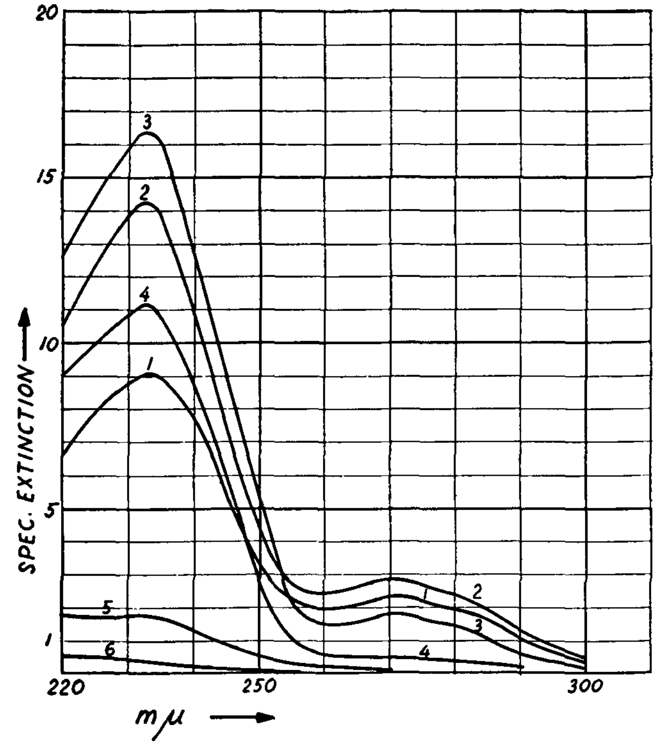


FIG. 6. Absorption spectra of hydrogenated linseed oil without alkali isomerization. The hydrogenation carried out at 235°C.

Curve 1: Iodine value: 170.5
Curve 2: Iodine value: 163.0
Curve 3: Iodine value: 143.0
Curve 4: Iodine value: 118.3
Curve 6: Iodine-value: 96.3
Curve 6: Refined linseed oil.

amounts of conjugated acids formed during the hydrogenation. The curves in Figure 3 show the isomerization effect, and, comparing Figure 3 and Figure 6 it seems probable that the hydrogen during the hardening serves as an additional activator for the isomerization.

The large amounts of conjugated linoleic acid formed especially at 235°C. indicate that this acid must be formed by hydrogenation of conjugated linolenic acid. At the same time an isomerization of normal linoleic to conjugated linoleic acid will probably occur. The last reaction however seems by itself to be responsible for only a small part of the conjugated acid which is formed. No iso-linoleic and apparently no normal linoleic acid is produced from linolenic acid during the first minutes in the series at 235°C. Possibly all the linolenic acid is hydrogenated to conjugated linoleic acid. Later, when the iodine value has dropped to about 155, iso-linoleic as well as normal linoleic acid is formed by hydrogenation of linolenic acid.

The curves in Figure 4 show that the conjugated linoleic acid has a comparatively great reactivity toward hydrogenations. When we know that:

> amount of conjugated linoleic acid in a sample = formed conjugated linoleic acid minus hydrogenated conjugated linoleic acid,

it is obvious that a great deal of the reactions passes over the conjugated linoleic acid. The results show that the conjugated linolenic acid reacts almost spontaneously with the hydrogen. The investigation of Thompson (9), who hydrogenated an equal mixture of tung oil and linseed oil, confirms this conclusion.

In the series at 160°C., on the contrary, iso-linoleic acid is formed at once and to a comparatively greater extent. At the same time we have a smaller amount of conjugated linoleic acid. Figure 2 gives a diagrammatic illustration of the different reactions. At 235°C. reactions 1, 2, and 6 seem to be predominating. At lower temperatures 3 and 4 will predominate.

It is well known that high temperature promotes the selectivity of the nickel-hydrogenation catalyst. The present investigation indicates that the dependence of the conjugation on the temperature may be an explanation. High temperature during hydrogenation causes high isomerization and increased reactivity toward hydrogenation.

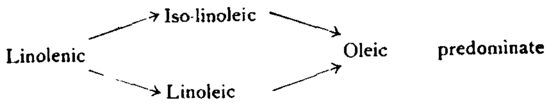
We recognize that by the calculation of composition by difference (see Table II) a relatively small error can greatly influence the final results. We know however that our experimental data are of sufficient accuracy for the purposes of discussion in this paper.

Summary

The fatty acid composition of hydrogenated linseed oil has been calculated from the iodine value, ultraviolet absorption with and without alkali isomerization, and the contents of saturated acids determined by the Bertram oxidation method. The hydrogenations are carried out at 160°C. and 235°C. The isomerization effect of the catalyst at the two temperatures is shown by heating the oils with 0.2% Ni-catalyst at 160°C. and 235°C. in CO_2-atm. The analyses show:

1. At 235°C. the conjugation of the double bonds is very large compared with 160°C.
2. The hydrogen seems to serve as an additional activator for the isomerization.
3. By hydrogenation at 235°C. 14% conjugated linoleic acid is found in the hydrogenated fat. By 160°C. only 3.6% of the acid is found. The contents of conjugated linoleic acid are due to the isomerization effect of the catalyst.
4. By 235°C. the reactions: linolenic ⟶ conjugated linolenic ⟶ conjugated linoleic ⟶ oleic, predominate.

By 160° C the reactions:



5. A theory is advanced that the temperature influences the selectivity through the conjugation of the double bonds.

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Report of the Seed and Meal Analysis Committee, 1951-1952

THE current work of the Seed and Meal Analysis Committee is conducted by five subcommittees. They are: Screen Test for Soyflour; Analysis of Copra and Copra Meal; Analysis of Flaxseed and Linseed Meal; Analysis of Tung Fruit and Meal; and Analysis of Castor Beans and Pomace. Reports and recommendations of the last two subcommittees are as follows:

Analysis of Tung Fruit and Meal

DURING the past three years the present tentative methods for the sampling and analysis of tung fruit have been studied collaboratively. In addition, the forced draft oven method (Ad 2-48) has been compared with a vacuum oven method (A.O.A.C., 7th Edition, 1950, Moisture, 22.3, maintaining a vacuum of less than 50 mm. of mercury and heating for 2.5 to 3.0 hours), and Bidwell-Sterling distillation method (A.O.A.C., 22.4, using ground glass jointed apparatus). The results obtained by the members of the committee for samples of hulled tung fruit, tung kernels, and tung hulls by use of the A.O.C.S. method showed good agreement and were slightly lower than those obtained using the A.O.A.C. methods on the same samples. The moisture obtained by the distillation averaged 0.2% in units higher and those obtained by the vacuum oven method 0.4% in units higher than those obtained using the present tentative method. The present tentative method (Ad 2-48) appears satisfactory for evaluating samples for commercial transactions and control analysis at tung mills.

Good agreement has been found between the results for the analysis for the oil content by the whole fruit method (Ad 3-48) and those obtained by use of the physical analysis method (Ad 4-48) and the kernel method (Ad 5-48). The average oil content of six samples was 19.98% and 20.02%, respectively. The analysis of samples of hulled tung fruit for oil content by the kernel method (Ad 5-48) showed considerable variation in reported results, with standard deviations varying from ± 0.7 to ± 2.7% and an average of 1.5% oil.

As growers are marketing hulled tung fruit in increasing quantities, interest was directed toward developing a method for their analysis. An adaptation of the whole fruit methods for moisture (Ad 2-48) and oil (Ad 3-48) was investigated. It involved adding an equal weight of ground tung fruit hull to the Wiley-ground hulled tung fruit. The results of the initial analysis of six samples by the six members of the subcommittee were rather disappointing as the standard deviation of the results was ± 2.3% oil. In view of the excellent results by use of the whole fruit method (Ad 3-48) it appeared probable that faulty sampling techniques were responsible for this variation.

Studies conducted in the laboratory of one of the committeemen on the new procedure for the analysis of hulled tung fruit indicated the necessity of carefully riffling the Wiley-ground hulled fruit and the Bauer-ground mixture of hulled fruit and hulls to obtain uniform aliquot portions. It was also indicated that the aliquot to be used as the sample for analysis