It is perhaps significant that a given weight of fresh flax seed tissue can incorporate in a given time almost twice as much acetate as the same weight of safflower seed tissue.

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[Received September 12, 1960]

Lipid Changes in Maturing Oil-Bearing Plants. II. Changes in Fatty Acid Composition of Flax and Safflower Seed Oils[1,2]

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Changes in the fatty acid composition of the oil in flax and safflower seed that occur during the seed-ripening period have been measured. Concentrations of lipid or of specific fatty acid, expressed on a weight-per-seed basis, have been plotted as functions of days after fertilization and of percentage of oil development. Relations between these two independent variables have been established, and limitations to the usefulness of the latter variable have been pointed out. Days after fertilization proved to be the more useful abscissa.

Nonpolar solvents were used to remove free lipid from the tissue, and the total extractable matter was separated into true lipid and nonlipid components. With both flax and safflower, weight of true free lipid per seed and total unsaturation increased during the same period of growth. Nonlipid extractable matter was an inverse function of the extent of development.

In developing flax seed, oleic, linoleic, and linolenic acids all increased continuously; oil in immature seed however was more saturated than oil in more mature seed. Nevertheless the ratio of linolenic acid to linoleic acid that characterizes linseed oil was established by the 20th day after fertilization during a normal growing season.

In developing safflower seed, oleic acid concentration increased slowly during the first 30 days after fertilization and then appeared to level off in some cases as maturity was approached. Initially linoleic acid was present in almost the same amount as oleic acid, but by the 20th day after fertilization its concentration was three times that of oleic acid. This ratio of linoleic to oleic acid tended to increase steadily during the latter part of seed development.

THE FIRST PAPER of this series (1) described overall changes in the lipid synthesis patterns of flax and safflower seed. The true free lipid in the seed was shown to become more unsaturated during the period of greatest increase in oil content, and the acetate-incorporating ability of the tissues was shown to increas during this same interval. A detailed study of the changes in fatty acid composition of the seed oils was therefore undertaken to provide further information on the fat-synthesis program of these two very different genera.

Studies of this type have been made before. Simmons and Quackenbush followed fatty acid deposition in soybeans of increasing extents of maturity (2). They found that, although the amounts of unsaturated fatty acid per bean increased, the iodine value of the oil decreased. Crombie (3) has described fatty acid formation in the maturing kernel of the West African palm. The kernel oil is however of a limited degree of unsaturation. More recently Vidal (4) and Kartha (5) have both studied characteristics of oils formed at various stages of development, but their data are few. The investigations of Painter in 1942 (6) and by McGregor and co-workers in 1939 (7) still remain the most complete studies of changes in linseed oil as the seeds increase in maturity. Since then however new methods of analysis and new ideas on fat biosynthesis have made this present study desirable. Moreover no information was available on the fatty acid synthesis pattern in maturing safflower seed.

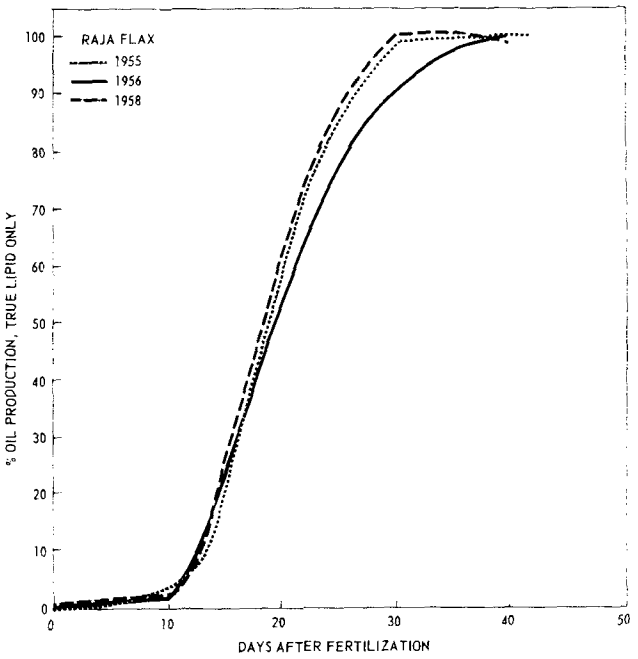

FIG. 1. Relation between % oil production and days after fertilization, Raja flax.

Materials and Methods

Oils used in this investigation were from the same Raja and Rocket flax seed and Indian safflower seed

[1] Contribution No. 45 from the Genetics and Plant Breeding Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.
[2] Presented at the Fall Meeting, American Oil Chemists' Society, New York, October 17–19, 1960.

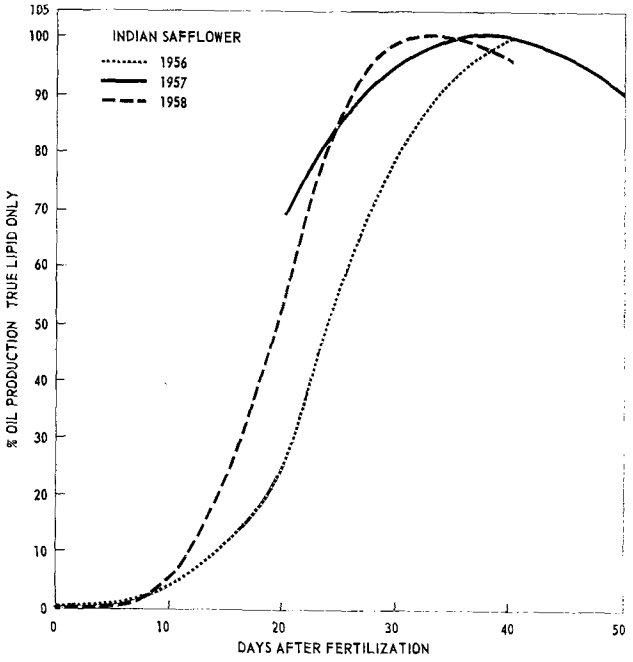

FIG. 2. Relation between % oil production and days after fertilization, Indian safflower.

that had been collected for the previous study (1).

The methods of flower tagging, seed collection, and oil extraction were the same as before (1). Iodine value was determined by the Hiscox modification of the Wijs method (8), and the concentration of polyunsaturated fatty acids was measured by Vandenheuvel's procedure for alkali isomerization (9).

The oil content or quantity of a specific fatty acid was expressed as mg. per 100 seeds. These data were then plotted as functions of days after fertilization or of percentage of oil development. This latter independent variable is calculated by expressing mg. of oil, or specific fatty acid, per 100 seeds at a given date as a percentage of the maximum concentration of oil or specific fatty acid.

Results and Discussion

The relation between days after fertilization and percentage of oil development was established for both flax and safflower seed grown between 1955 and 1958 (Figures 1 and 2). In all cases sigmoid curves were obtained. The variable, "days after fertilization," reflects differences in climate between years whereas percentage of oil development is not a function of the weather. As shown in the previous study (1), flax reflects adverse growing conditions less than safflower, and this is shown by the greater affinity of the curves for flax.

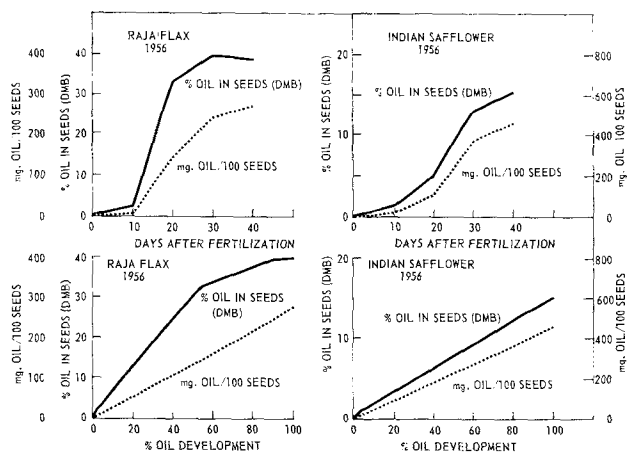

FIG. 3. Effect of mode of representation on form of curve.

The different modes of presentation of the data were next investigated; typical relations are shown in Figure 3. When the oil content, for example, is plotted as a function of each of the variables, the effect of using "Percentage of Oil Development" is to spread out the early data. The over-all shape of the curves is unchanged however.

Oil development is calculated from oil concentration data, *i.e.*, mg. oil per 100 seeds. Consequently a plot of mg. oil per 100 seeds as a function of percentage of oil development can only yield a straight line unless the data are biased by some external influence. Consequently this type of plot was used only to check the quality of the data, which were then plotted as oil or fatty acid concentrations as functions of days after fertilization.

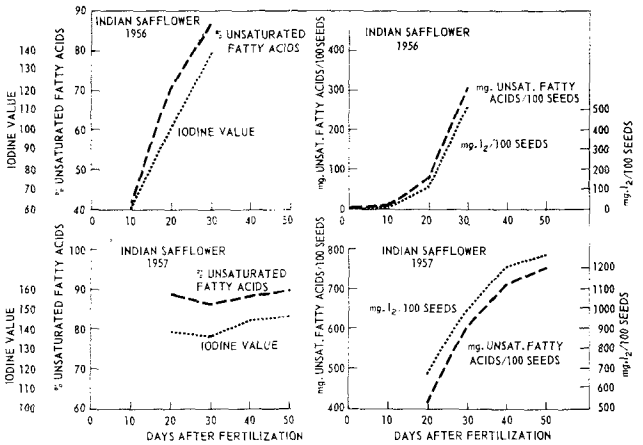

FIG. 4. Time course of unsaturation in oil from Indian safflower seed.

Typical data for developing safflower seed are listed in Table I. These and other data are plotted in Figure 4, where the effect of mode of representation is shown, and in Figure 5, where the absence of a constant linoleic:oleic acid ratio is demonstrated. Total unsaturation in safflower oil was found to increase continuously, but the rate of formation of oleic acid was always less than that of linoleic acid. Consequently the ratio of the concentrations of linoleic and oleic acids varied continuously. Linolenic acid was detected in concentrations $<1\%$ in the 10- and 20-day samples. It was not found in the more mature samples.

The effect of climate on lipid development in flax and safflower seed was described in general terms in the first paper of this series (1). A more complete picture of this effect is given in Figures 4 and 5. The more favorable summers of 1957 and 1958 resulted in levels of unsaturation that were nearly twice as high as that achieved during the cold, wet summer of 1956. Conditions in 1958 were less favorable for safflower development than those in 1957. This is reflected by the lower concentrations of both oleic and linoleic acids.

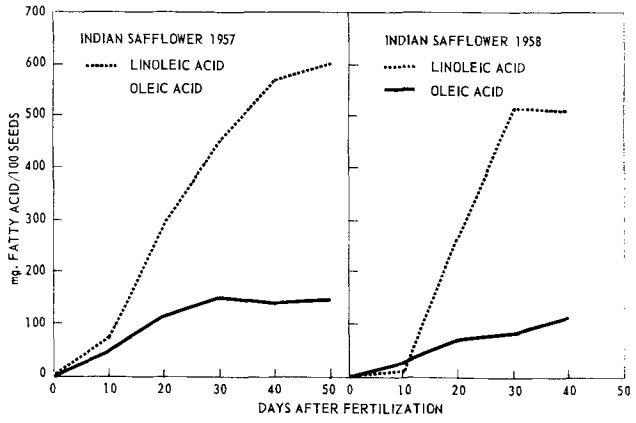

FIG. 5. Concentration of individual unsaturated fatty acids in oil from Indian safflower seed as function of days after fertilization.

Typical data for developing flax seed (Table II and Figures 6 and 7) demonstrate the time course of the unsaturation in the oil. Because response to climate of the early- and late-ripening varieties of flax is different and the general development of unsaturation

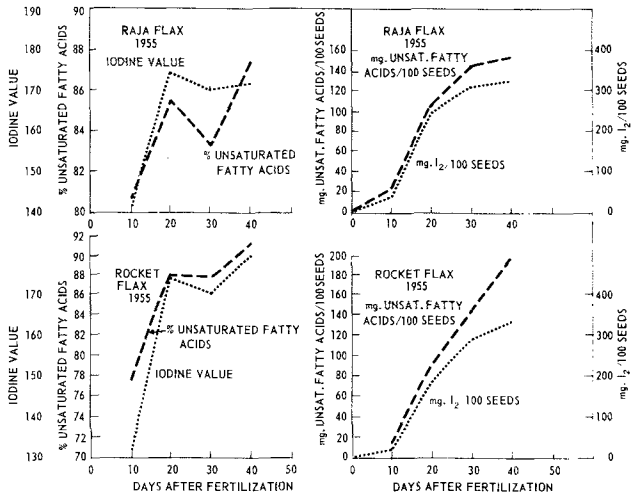

FIG. 6. Time course of unsaturation in oil from Raja and Rocket flax seed.

is also dependent on environment, a discussion of these data without reference to variety and environment would be pointless.

During the short, hot growing season of 1955 early-ripening Raja flax developed much less linolenic acid than it did in 1956. The ratio of linolenic acid to linoleic acid in both years however remained close to 2:1 after the 20th day. In contrast, late-ripening Rocket flax had a linolenic-to-linoleic-acid concentration ratio of 1.5:1. It was much less affected by climatic changes.

TABLE I

Indian Safflower, 1957

Days after fertilization	Wt. 100 seeds	% Moisture	% True lipid	Iodine value	mg./100 Seeds				
					Oil	Iodine	Oleic acid	Linoleic acid	Linolenic acid
20	4.805	32.4	15.0	138.3	486	671	114.9	297.7	1.0
30	4.772	24.0	20.2	136.5	732	996	151.4	453.8	....
40	4.480	14.3	21.9	144.1	839	1211	140.5	571.1	....
50	4.424	10.7	22.1	146.3	872	1272	148.8	602.4	....

TABLE II

Raja Flax, 1956

Days after fertilization	Wt. 100 seeds	% Moisture	% True lipid	Iodine value	mg./100 Seeds				
					Oil	Iodine	Oleic acid	Linoleic acid	Linolenic acid
10	0.996	74.0	4.86	122.9	11	14	3.55	2.07	2.18
20	0.989	55.0	33.3	172.8	148	256	31.9	27.3	60.9
30	0.906	38.5	39.8	180.8	244	441	40.9	46.7	110.3
40	0.789	10.2	38.5	181.0	272	492	60.7	51.8	118.4

A further varietal difference is that the concentrations of linoleic and oleic acids in Raja seed were approximately equal whereas the amount of oleic acid in Rocket seed was always greater than the amount of linoleic acid.

Data obtained in 1957 and 1958 confirm the relations described for longer growing periods.

As the average flax or safflower seed matures, the quantity of oil increases and the degree of unsaturation also increases. The observed increase in unsaturation could be due to serial desaturation, accompanied by synthesis of the least unsaturated component of the desaturation chain, or to *de novo* synthesis of the individual fatty acids at rates sufficient to produce the observed changes in composition, or to a combination of these two systems. Inclusion of concurrent lipid catabolism merely introduces the requirement of greater speed of synthesis.

Kartha (5) has employed a formula to show that *de novo* synthesis of fatty acids in developing niger seed oil must be supplemented by desaturation of fatty acids. The derivation of this relation is questionable however, and, when applied to data for safflower and flax (*loc. cit.*, 6), the relation fails.

Alternatively the data may be treated in the follow-

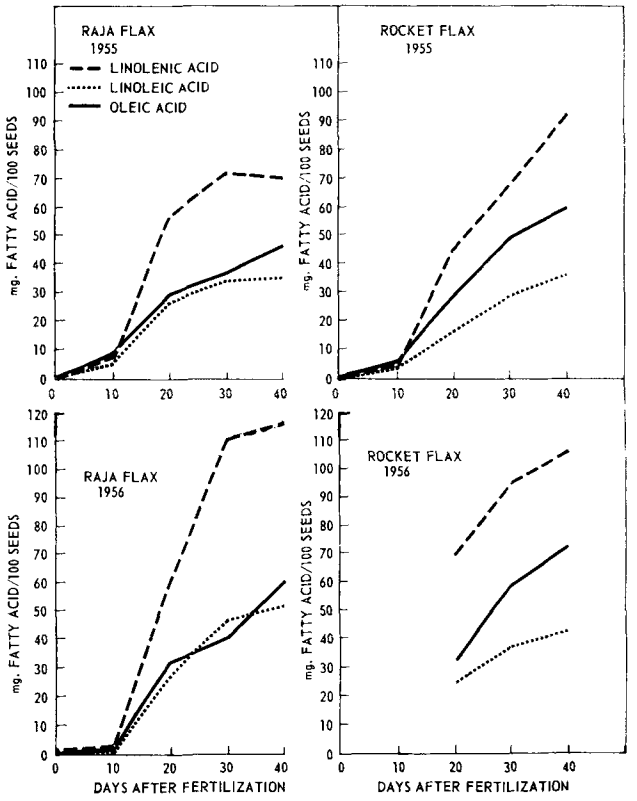

FIG. 7. Concentration of individual unsaturated fatty acids in oil from Raja and Rocket seed as function of days after fertilization.

ing way. If, for a given time-interval, the measured increase in iodine absorbed by the oil in 100 seeds is divided by the appropriate increase in weight of oil, the quotient can be considered the iodine value of the new oil. When the safflower data were tested in this way, the iodine value of the new oil ranged from 133 to 207 with a mean of 155 for 14 items and with the highest values occurring between the thirtieth and fortieth days. An iodine value greater than 181, in the presence of only dienoic fatty acids, suggests the operation of an alternative to *de novo* synthesis. The iodine value of 207 was however an isolated instance. With flax, the iodine value of the new oil, calculated in this manner, ranged from 176 to 255, with a mean of 190 for 18 items. Again the highest values occurred toward the mid-point of development. The presence of linolenic acid in flax obviates the necessity of invoking the desaturation hypothesis.

The dynamic state of lipids in plant tissue means that any observed change in unsaturation is, of necessity, the result of concurrent anabolism and catabolism. It is therefore incorrect to speculate on the occurrence of desaturation or to attribute changes in iodine value to *de novo* synthesis without knowledge of the turnover numbers of the various fatty acids at progressive stages of development.

Acknowledgment

The authors wish to thank the Analytical Research Service Unit for performing the iodine value and polyunsaturated fatty acid determinations.

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[Received November 15, 1960]

Reactions of Ozone. V.[1] A New Method of Determining Unsaturation Values of Fatty Acids and Oils by Ozone[2]

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FOR YEARS a simple, reliable, quantitative method for determining olefinic unsaturation has been sought. Existing methods such as bromination, iodination, catalytic hydrogenation, and peroxide reactions have their specific limitations as discussed by numerous investigators (1,2,5,6). However, for many olefinic compounds, ozone is an excellent titrimetric reagent (5). Boer and Kooyman (3) have shown that ozonization could well supplant these other methods. Their procedure can be simplified by using commercially-available apparatus. The analysis is based on determining the time required to titrate a sample of olefin with a stream of ozonized air containing a known amount of ozone. Three things are necessary: a constant stream of ozonized air, complete absorption of the ozone, and precise recognition of an end-point.

Experimental

A schematic diagram of the equipment is shown in Figure 1. Clean dry air is supplied to the Model T-23 laboratory ozonator.[3] Using 0.02 c.f.m. of air, the applied voltage[4] is adjusted to *ca.* 90 V., to give 15 to 20 mg. of ozone per minute. Precise control of gas flow is assured with the stainless steel needle[5] valve and the external flow meter.[6]

Ozone production is determined by absorbing the ozone in neutral 2% KI solution for a measured 3 min. After acidification, the liberated iodine is titrated with 0.1 N thiosulfate. The ozone rate is calculated in mg./min. The ozone production should be rechecked periodically, before and after each series of determinations. At this low production rate it is not necessary to have a glass frit on the gas-washing bottle, but a tube drawn to *ca.* 1 mm. i.d. capillary disperses the gas sufficiently to give complete absorption. There is very little back pressure in the system without a frit, making it easier to maintain the same flow rate for determining the ozone production and for determining the unsaturation in the sample. Complete absorption of ozone for a particular modified dispersing tube can be checked by placing a second regular absorber in series.

Complete absorption of ozone is obtained in a "Mini Lab"[7] gas-liquid reactor. The stirring speed (*ca.* 2,500 r.p.m.) is adjusted to raise the liquid level to 1 cm. from the bottom of the glass joint while the flask is cooled to −50°C. Care should be taken to avoid forcing liquid into the ground glass joint at the top of the reactor.

To carry out a determination, a sample containing 6 to 7 milli-equivalents of double bond is weighed accurately into the reaction flask and dissolved in 35 ml. of chloroform. The solution is held at −45 to −55°C., using a dry-ice acetone bath. After the ozone production rate is determined, the ozone stream

[1] For preceding paper in this series see Ref. 4.
[2] Presented at the 50th meeting, American Oil Chemists' Society, New Orleans, La., April 22, 1959.
[3] The Welsbach Corporation, Philadelphia, Pa.
[4] The ozonator operates on standard 115-volt, 60-cycle A.C. Line voltage variations greater than 1% will adversely affect ozone production; in these cases a constant voltage supply is recommended.
[5] Hoke Inc., Cresskill, N.J., No. 332.
[6] Emil Greiner Company, New York. Cat. No. 69144B is used with the ss float.
[7] Ace Glass Inc., Vineland, N.J.