Effects of Fatty Acid Concentration and Positional Specificity on Maize Triglyceride Structure

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ABSTRACT

The effects of fatty acid concentration and positional specificity on maize triglyceride structure were evaluated from the stereospecific analyses of triglycerides from 12 genotypes. The fatty acids at each position were influenced by the fatty acid concentration in the total triglyceride except for the saturates in the 2 position. The fatty acid concentration had the greatest effect on the fatty acid composition of position 3. The existence of positional specificity was evident from the nonrandom distribution of the fatty acids among the three positions of the triglycerides. The concentration and positional specificity effects could be separated in selected genotypes and their crosses. This indicated different genetic controls for each effect.

INTRODUCTION

Triglycerides isolated from kernels of inbred maize lines have been stereospecifically analyzed (1). Each position showed a distinct composition of fatty acids. However, for oleic acid and linoleic acid there was no consistent pattern of asymmetry among the maize strains. In some strains oleic was higher in position 3 than in 1; in others it was higher in 1 than in 3. Similar results were obtained for linoleic acid. The concentrations of these fatty acids in the total triglyceride seemed to have some influence on their distribution among the three positions of the triglycerides, but there also appeared to be positional specificity.

In this paper the effects of these two factors, concentration of fatty acids in the total triglyceride and positional specificity, have been examined. The range of fatty acid compositions in maize and the possible genetic combinations offer unique material for studying these effects.

MATERIALS AND METHODS

The source of materials and the methods employed in this paper are described in a companion report (1).

RESULTS

The concentration effect is defined as the influence of the amount of a particular fatty acid available for esterification. The effect of fatty acid concentration in the total triglyceride on each triglyceride position was assessed from stereospecific analyses of twelve maize strains. These strains exhibited a very wide range in fatty acid distributions (saturated acids 9.7-20.9%, oleic acid 17.0-53.9%, linoleic 32.6-68.3%).

In Figure 1 the concentrations of saturated acids (S), monoene (M) and diene (D) in the total triglyceride are individually correlated with their respective concentrations at the 1 position. Highly significant correlations of 0.86,

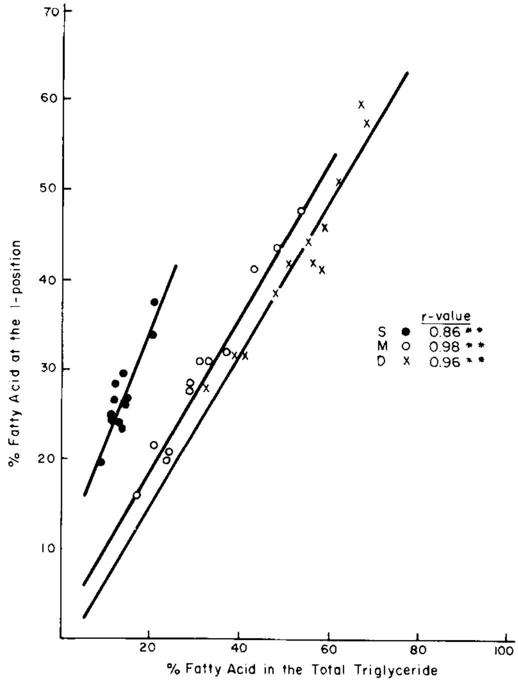

FIG 1. The concentration of S, M and D in the 1 position compared with total triglyceride. S, M and D denote saturated, monoene and diene fatty acids, respectively.

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TABLE I

Statistical Parameters for the Relationship Between Fatty Acids in Total Triglyceride and Fatty Acids at Each Position

Fatty acid	Position in triglyceride	$\bar{x}$	r[a]	a[c]	b[d]
16:0 + 18:0	1	26.6	0.86[b]	+ 9.95	1.19
	2	1.0	0.57	+ 0.11	0.07
	3	14.3	0.92[b]	- 10.30	1.76
18:1	1	29.9	0.98[b]	+ 1.36	0.88
	2	29.4	0.96[b]	+ 1.65	0.86
	3	38.0	0.96[b]	- 3.03	1.26
18:2	1	42.7	0.96[b]	- 1.48	0.83
	2	68.7	0.94[b]	+24.66	0.81
	3	46.0	0.95[b]	- 28.89	1.41

[a]r Correlation coefficient.
[b] Significant at the 1% level.
[c]a y intercept.
[d]b Regression coefficient.

0.98 and 0.96 were obtained for S, M and D, respectively (Table I).

Correlation coefficients were also calculated for M and D in the total and in the 2 position and found to be significant (r=.96 and .94, respectively) (Fig. 2 and Table I). When S in the total was correlated with S at the 2 position, a nonsignificant r value of 0.57 was obtained. Apparently, the concentration of S in the total triglyceride had little influence on the amount of S esterified at the 2 position. This is expected, because there was almost complete restriction of S from the 2 position.

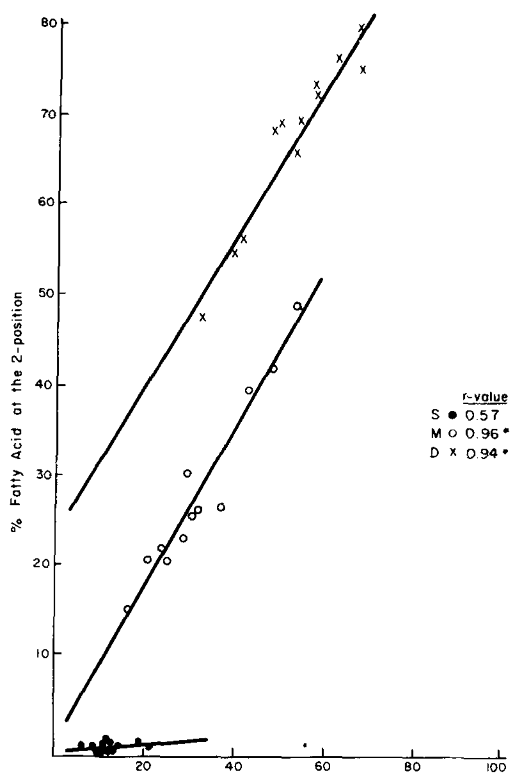

FIG 2. The concentration of S, M and D in the 2 position compared with total triglyceride.

The S, M and D fatty acids in the 3 position were all positively correlated with the total (Fig. 3 and Table I). This indicates that all three fatty acid types exert a significant concentration effect at the 3 position of the triglyceride.

A comparison of regression slope values for all three positions (Table I) suggests that the fatty acid composition at position 3 is the most strongly influenced by the concentration of S, M and D in the total triglycerides. We also observed that the least amount of positional specificity was exerted at this position (1).

In the maize triglycerides each fatty acid showed a concentration effect at each position except for the saturates at the 2 position. Strong specificity against S at this position resulted in a nonsignificant concentration effect. Concentration effects can only be present when the fatty acids exhibit partial or no positional specificity.

Positional specificity of fatty acid among the three positions of triglyceride could result from different rates of esterification. If these differences are major, then significant deviations from a "completely random" distribution would be found. All 12 maize strains showed a nonrandom distribution of fatty acids.

Thus, it is concluded that fatty acid distribution in maize triglycerides is the result of two major effects, concentration and positional specificity. If fatty acid synthesis and the positional specificity of esterification are under different genetic controls in maize, one may expect to find strains with differing positional specifications and the same fatty acid composition (positional specificity effect). Conversely, it may be possible to find strains with the same positional specificities and different fatty acid compositions (concentration effect).

Analyses of individual kernels from C105 for fatty acid composition revealed the presence of considerable variation in oleic and linoleic acids. It far exceeded the usual environmental variability of homozygous strains. Individuals showing extremes in linoleate content were selfed either one or two generations. Two homozygeous strains phenotypically identical except for a 6.4% difference in linoleate were isolated. The two strains were subjected to stereospecific positional analysis (Table II). The fatty acid distributions of all three positions were different, but the proportions of each fatty acid (notably oleic and linoleic) were similar at each position. Since proportion is an expression of positional specificity, it is concluded that these two strains of C105 were genetically similar for positional specificity.

A hybrid with C105 as the maternal parent was detected that had oleic and linoleic contents similar to those of Illinois High Oil (IHO).

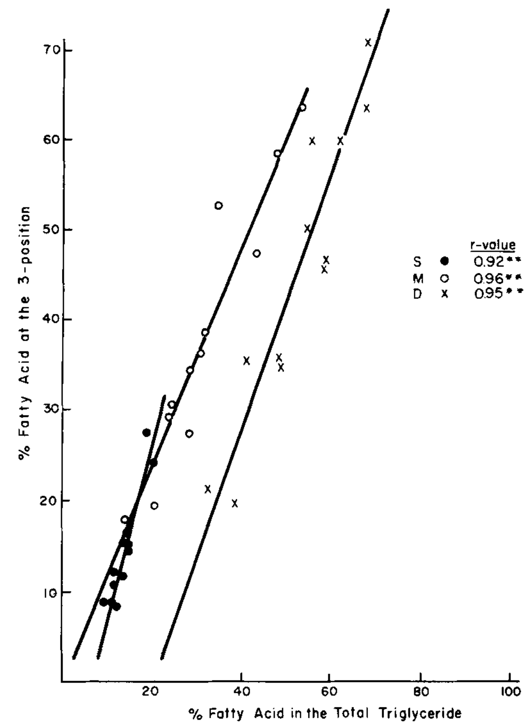

FIG 3. The concentration of S, M and D in the 3 position compared with total triglyceride.

TABLE II

Stereospecific Analyses of Triglyceride From Two Strains of C105

Compound or position	Fatty acid distribution, mole per cent[a]				
	16:0	18:0	18:1	18:2	18:3
Strain A					
Triglyceride	10.6	2.3	53.9	32.6	0.6
1	19.9	3.9	47.9	27.9	0.4
Per cent in 1[b]	62.6	56.5	29.6	28.5	22.2
2	0.6	---	50.0	48.9	0.4
Per cent in 2	1.9	---	30.9	50.0	22.2
3	11.3	3.0	63.8	21.0	1.0
Per cent in 3	35.5	43.5	39.5	21.5	55.6
Strain B					
Triglyceride	10.2	2.0	48.4	39.0	0.4
1	20.2	4.1	43.7	31.5	0.5
Per cent in 1	66.0	68.3	30.1	29.4	41.7
2	0.5	---	42.9	56.0	0.6
Per cent in 2	1.6	---	29.5	52.3	50.0
3	9.9	1.9	58.6	19.5	0.1
Per cent in 3	32.4	31.7	40.4	18.2	8.3

[a]Analyses done in triplicate.

[b]Per cent of fatty acid that is esterified at this position = $\frac{\text{\% of fatty acid in this position x 100}}{\text{\% of fatty acid in triglyceride x 3}}$

TABLE III

Stereospecific Analyses of Triglycerides From Two Maize Strains With Similar Fatty Acid Distributions

Compound or position	Fatty acid distribution, mole per cent				
	16:0	18:0	18:1	18:2	18:3
F_1[a]					
Triglyceride	8.6	1.8	37.6	51.3	0.8
1	21.4	3.9	35.0	39.1	0.6
Per cent at 1	82.9	72.2	31.0	25.4	33.3
2	0.9	0.1	33.2	65.2	0.6
Per cent at 2	3.5	1.9	29.4	42.4	33.3
3	3.8	1.4	44.6	49.6	0.6
Per cent at 3	14.7	25.9	39.5	32.2	33.3
IHO[b]					
Triglyceride	12.2	1.8	36.2	49.2	0.6
1	22.6	3.4	31.9	41.8	0.3
Per cent at 1	61.7	63.0	29.4	28.3	16.7
2	0.8	---	27.4	71.1	0.7
Per cent at 2	2.2	---	25.2	48.2	38.9
3	13.2	2.0	49.3	34.7	0.8
Per cent at 3	36.1	37.0	45.4	23.5	44.4

[a]F_1 hybrid with C105 as the maternal parent. Analysis done in duplicate.
[b]Analysis done in triplicate.

TABLE IV

Stereospecific Analyses of Reciprocal Crosses of Two Maize Inbreds

Strain	Compound or position	Fatty acid distribution, mole per cent				
		16:0	18:0	18:1	18:2	18:3
NY16 x C103[a]	Triglyceride	10.3	1.6	28.6	58.6	0.8
	1	22.3	3.9	27.4	45.5	0.9
	Per cent at 1	72.1	80.4	31.9	25.9	35.7
	2	0.9	0.3	24.2	73.9	0.8
	Per cent at 2	2.8	5.8	28.2	42.0	31.4
	3	7.8	0.7	34.3	56.5	0.8
	Per cent at 3	25.1	13.8	39.9	32.1	32.9
C103 x NY16	Triglyceride	10.3	1.7	32.1	55.1	0.8
	1	21.3	3.2	30.4	44.3	0.8
	Per cent at 1	68.8	65.1	31.5	26.8	30.1
	2	0.6	0.2	27.6	70.8	0.7
	Per cent at 2	2.0	4.4	28.7	42.9	28.9
	3	9.0	1.5	38.4	50.1	1.0
	Per cent at 3	29.2	30.5	39.8	30.3	41.0

[a]Analyses done in quadruplicate.

The stereospecific data (Table III) show the proportions of oleic and linoleic acids at positions 2 and 3 to be quite different in each strain. A far greater percentage of linoleate was at the 2 position of IHO as compared to the C105 F_1 hybrid. Further, the order of preference of linoleate between positions 1 and 3 was reversed in these two strains. Oleate had a stronger preference for the 3 position of IHO triglycerides as compared to the other strain. These positional differences were all significant. Thus, these two strains possess essentially the same concentrations of oleic and linoleic acids in their total triglycerides but differ in their positional specificities of esterification.

The triglycerides of the reciprocal crosses of two maize inbreds, NY16 and C103, were stereospecifically analyzed (Table IV). An LSD test was used to test the statistical significance of the data. The concentrations of oleic and linoleic acids in the total triglycerides of the two reciprocal crosses were significantly different at the 5% level. Linoleic acid was higher in NY16 x C103 than in C103 x NY16, while oleic acid was higher in C103 x NY16. However, the proportions of these fatty acids at all three positions were not significantly different. The two reciprocal crosses show identical positional specificities.

The effect of fatty acid concentration was clearly shown when the triglyceride species of the reciprocal crosses (Table V) were isolated by silver nitrate thin layer chromatography (2). The percentages of the triglyceride species which contained two or three moles of linoleic acid were higher in NY16 x C103 than in C103 x NY16. In contrast, those species containing two or three moles of oleic acid were higher in C103 x NY16. Although the positional specificities of the two crosses were identical, the fatty acid composition of the total triglyceride had a very strong influence on the amounts of the various triglyceride species.

TABLE V

Concentrations of Triglyceride Species of Reciprocal Crosses of Two Maize Inbreds

	Per cent of total triglyceride	
Species	NY16 x C103[b]	C103 x NY16
S_2M[a]	1.1 ± 0.3	1.2 ± 0.3
SM_2	4.6 ± 1.3	4.6 ± 0.2
S_2D	2.4 ± 0.4	2.0 ± 0.3
M_3	3.5 ± 0.2	5.7 ± 0.1
SMD	12.2 ± 0.2	12.2 ± 0.2
M_2D	12.9 ± 0.2	16.3 ± 0.2
SD_2	13.9 ± 0.5	11.8 ± 0.8
MD_2	26.4 ± 1.4	26.5 ± 1.7
D_3, D_2T	22.5 ± 0.9	19.6 ± 1.0

[a]Abbreviations: S, saturated; M, monoene; D, diene; T, triene fatty acids.
[b]Mean of four samples ± standard deviation.

DISCUSSION

Several conclusions can be drawn from positional specificity and concentration effects in maize triglycerides. In the 12 strains studied the proportions of oleate and linoleate were most constant in the 1 position. In other words, positional specificity at the 1 position was very uniform over a wide range of oleate and linoleate composition. All the fatty acids exhibited only partial specificities at all three positions with the exception of the saturated acids at position 2. Thus, with one exception, each position showed a sizeable concentration effect for each fatty acid. The concentration effects for oleate and linoleate were very similar at positions 1 and 2 of the triglycerides (similar regression line slopes, Fig. 1 and 2 and Table I). The fatty acids at the 3 position were the most strongly influenced by the content of saturated acids, oleate and linoleate in the total triglyceride.

The specific location of fatty acids in the triglyceride molecules of vegetable oils is important for several reasons. First, the placement of the fatty acids in the triglycerides affects their stability toward oxidation. When Sahasrabudhe and Farn (3) heated corn oil, they found that the fatty acids in the 1 and 3 positions were more susceptible to oxidation than those in the 2 position. Raghuveer and Hammond (4) suggested that concentrating the unsaturates at the 2 position may orient the triglyceride molecule to make it more stable against autoxidation.

High specificity for polyunsaturated acids at the 2 position is also desirable from a nutritional point of view. From 72% to 80% of the dietary triglycerides are absorbed into the intestinal wall as 2-monoglycerides (5). The free fatty acids are either metabolized or reesterified. The monoglycerides are converted to triglycerides and polar lipids. Since polyunsaturated fatty acids are essential in the diet, esterification of these acids at the 2 position would ensure their conservation during digestion.

If we better understood both the biochemical regulation of fatty acid synthesis and the mechanisms for placement of these acids in the triglyceride molecules of plants, triglycerides could be produced for specific purposes.

ACKNOWLEDGMENTS

Supported in part by a grant, Plant Science Research

Division, ARS, USDA Cooperative Agreement 12-14-100-8457-(34). Lorraine Bollinger gave technical assistance.

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[Revised manuscript received April 5, 1971]