Component Triacylglycerols of Six Seed Oils of Malvaceae

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The component triacylglycerols of six seed oils of the Malvaceae family—*Gossypium barbadense* (Egyptian cotton), *Hibiscus cannabinus* (kenaf), *Hibiscus sabdarifa* (roselle), two varieties of *Hibiscus esculentas* (okra) and *Althea rosea* (ketmia, hollyhock or Egyptian hemp)—have been determined by using the lipase hydrolysis technique. The oils were found to contain triacylglycerols belonging to trisaturated (1.0–2.1), disaturated-monounsaturated (12.3–20.9), monosaturated-diunsaturated (42.3–46.6) and triunsaturated (30.1-44.2) types of triacylglycerols.

KEY WORDS: Composition, fatty acids, lipase, Malvaceae, oils, seeds, triacylglycerols.

According to an early report of Hilditch and Williams (1), many seed oils of mallow *(Hibiscus)* species are practically identical to cottonseed oil, and thus attract attention as being suitable for extended cultivation. This study is an extension of the work being done by many authors, to evaluate local seeds as a potential source for vegetable oil with special emphasis on their glyceride structure (2–4; and H.M. El-Khalafy, M.M.M. El-Mallah and H.M. Fadel, personal communication).

The main fatty acids of many species of the Malvaceae family are palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids (5–13). On the other hand, only few articles dealt with the structure of triacylglycerols of this family. Cottonseed oil, a typical high linoleic acid oil, has been examined for its triglycerides by Jurriens and Kroesen (12), Vereshchagin (13) and Van der Wal (14). Also, okra seed oil has been investigated for its triglycerides by Gunstone (15) and Osman (11).

MATERIALS AND METHODS

Lipid extraction. Seeds of Egyptian cotton, kenaf, roselle, okra (two varieties) and ketmia (Eyptian hemp) were purchased from a local seed and herb store. The crushed seeds were extracted by n-hexane at room temperature. The oil was obtained by evaporation under a stream of purified nitrogen.

Preparation of triacylglycerols. The neutral triacylglycerols (TAGs) were obtained by separation of lipid classes of the oil on a silicic acid column using stepwise elution (16). The obtained triacylglycerols were further purified by preparative TLC (17). Then the mixed fatty acids of the neutral triacylglycerols were prepared via saponification (18).

Pancreatic lipase hydrolysis. Lipolysis of the neutral triacylglycerols (TAGs) was done by the method of Mattson and Volpenhein (19). For each sample the time necessary to produce 2/3 hydrolysates was determined by preliminary experiments. The isolated hydrolysates were then separated by preparative TLC. The methyl esters of the separated fatty acids of the 2-monoacylglycerols (2-MAGs), as well as those of the original TAGs, were prepared by HCl/Methanol (20).

Determination of the fatty acids. The methyl esters of the 2-MAGs and those of the original TAGs were analyzed, each in duplicate, by gas liquid chromatography. Peak areas were measured by triangulation. All compositions were converted to molar percentages of the fatty acids in the calculated 1,3-position, and in that of the isolated 2-position. Then the component triacylglycerols were computed according to Coleman (21), Van der Wal (14), Mattson *et al.* (22,23) and Gunstone (15).

RESULTS AND DISCUSSIONS

Fatty acids in TAGs and 2-MAGs. Fatty acid composition of the whole triacylglycerols (TAGs) and that of the 2-monacylglycerols (2-MAGs) in mole % in the six Malvaceae seed oils are shown in Table 1. Meanwhile, the total saturated and unsaturated fatty acids of TAGs and 2-MAGS of each oil are shown in Table 2.

The main fatty acids in TAGs are palmitic (21.1–30.7%), oleic (19.7–38.5%) and linoleic (34.9–52.0%). Stearic acid is present only in small amounts (1.0–2.8%). Fatty acid composition of *Gossypium barbadense* (Egyptian cotton) (Table 1) was in agreement with that of the literature data. Oleic/linoleic ratio was about 20:50 and palmitic acid was around 25, while stearic acid was between 2 and 3 (7,8,23). On the other hand, the literature data for roselle-seed oil showed wide variations—C16:0 ranged from 15.0% (9) to 35.2% (25), for two native Indian varieties. Meanwhile, C16:0 in the variety studied was 24.7%, which is close to that of the Sudanese variety, 23.6% (10). Consequently, the ratio of C18:1 and C18:2 showed wide variations—29.6:49.0 (9) to 34.0:14.6 (24) and 25.3-32.5 (10), all compared to that of the studied roselle oil, 37.6:34.9 (all values in percents). Thus, it is clear that even the sums of the unsaturated oleic and linoleic acids were not similar. Also, stearic acid values were different and reached to 5.8% (10) in roselle oil, and linolenic acid was found occasionally, but in small amounts.

Although Hilditch and Williams concluded that the fatty acid composition of okra seed oil is similar to that of cottonseed oil, the data published in their book varied even within the same species and with the two okra varieties studied (Table 1). Again, the discrepancies are not less than those of roselle oil, except that the sum of unsaturated oleic and linoleic acids showed little variation and ranged between 62.0% for the Canadian variety and about 70% for those of the tropics and subtropics (1). The sum of these two acids in the two okra varieties studied (Table 2) was ca. 77% for *Hibiscus esculentas* var. *baladi* and 68% for *H. esculentas* var. *romi,* as compared to that grown in Sudan (ca. 70%) (1). The same data showed that the total unsaturated acids for kenaf oil was ca. 71%, while that of ketmia varied from 89 to 71%, as compared to the values of the varieties studied, which was ca. 70% for both oils (Table 2).

One can conclude that the component fatty acids in the Malvaceae seed oils are similar, but with a wide range of proportions. This fact has been supported by the work of El-Nockrashy *et al.* (8) on 25 species of *Gossypium,* and

TABLE 1

Fatty Acid Composition of Triacylglycerol, 2-Monoacylglycerol and Proportion of Each Fatty Acid in 2-Position

	Cotton			Kenaf			Roselle			Okra[a]			Okra[b]			Ketmia (Hollyhock)		
	TAG[c]	2-MAG[d]	% Mono-[e]	TAG	2-MAG	% Mono-	TAG	2-MAG	% Mono-	TAG	2-MAG	% Mono-	TAG	2-MAG	% Mono-	TAG	2-MAG	% Mono-
Palmitic	25.7	10.9	14	29.0	12.0	14	24.7	11.5	15	21.1	10.9	17	30.7	12.7	13	27.9	12.7	15
Stearic	2.6	0.0	—	1.2	0.0	—	2.8	0.0	—	2.2	0.0	—	1.0	0.0	—	1.0	0.0	—
Oleic	19.7	24.1	40	32.0	24.2	25	37.6	50.8	45	38.5	53.1	46	31.9	52.7	55	30.1	44.7	50
Linoleic	52.0	65.0	42	37.8	63.8	56	34.9	37.7	36	38.2	36.0	32	36.4	34.6	31	41.0	42.6	34

[a]*Hibiscus esculentus* var. *baladi.*
[b]*Hibiscus esculentus* var. *romi.*
[c]Total triacylglycerols.
[d]2-Monoacylglycerols.
[e]Relative proportion of the fatty acid esterified in the 2-position (e = 2-MAG/(TAG × 3) × 100).

TABLE 2

Total Saturated and Unsaturated Fatty Acids of The Triacylglycerols and Their Corresponding 2-Monoacylglycerols (Mole %)

	Cotton		Kenaf		Roselle		Okra[a]		Okra[b]		Ketmia (Hollyhock)	
Fatty acid	TAG[c]	2-MAG[d]	TAG	2-MAG	TAG	2-MAG	TAG	2-MAG	TAG	2-MAG	TAG	2-MAG
Saturated	28.3	10.9	30.2	12.0	27.5	11.5	23.3	10.9	31.7	12.7	28.9	12.7
Unsaturated	71.7	89.1	69.8	88.0	72.5	88.5	76.7	89.1	68.3	87.3	71.1	87.3

[a]*Hibiscus esculentus* var. *baladi.*
[b]*Hibiscus esculentus* var. *romi.*
[b]Triacylglycerols.
[d]2-Monoacylglycerols.

by Khattab *et al.* (25), on one wild and three cultivated *Gossypium* varieties. A genome-specific pattern was not found for the fatty acid composition or for other chemical and physical properties.

Table 1 shows the fatty acids, esterified at the 2-position in the TAGs, after lipolysis have been analyzed and calculated (22). The results showed that palmitic acid C16:0 is preferentially esterified at the 1- and 3-position, as its proportion in the 2-position ranged only between 10.9 and 12.7%. This is in agreement with the general distribution pattern of the saturated fatty acids reported for vegetable oils (1–4,22,23,26–28; and private communication with H.M. El-Khalafy, M.M.M. El-Mallah and H.M. Fadel), and also agrees with the data about the triglyceride structure of cottonseed oil (12–15).

The distribution of C18:1 and C18:2 in the 2-position, like that in the whole TAGs, showed no typical pattern in the six oils. The proportion of oleic and linoleic acids in the 2-position of cottonseed oil was ca. 25 and 65%, respectively, while in okra (*H. esculentus* var. *baladi*) it was ca. 38% for both acids (Table 1). This fact is in good agreement with Gunstone *et al.* (29). They showed that unsaturated acids in cottonseed oil and many other unsaturated oils do not compete equally for the secondary hydroxyl group. Besides, Mattson and Volpenhein showed that oleic and linoleic acids are preferentially attached at the 2-position, allowing the excess of unsaturated C18 acids or other unsaturated and saturated acids to attach to the 1- or 3-hydroxyl position of glycerol molecule. These findings were in accord with the positional distribution theory of Van der Wal (14). Another explanation is that the specific distribution of saturated acids (e.g., palmitic) in the 1- and 3-positions forces higher proportions of unsaturated acids (C18:1 + C18:2) into the 2-position of TAGs (30). The proportions of the different fatty acids in the 2-position relative to those in the whole TAGs have been calculated as given in Table 1, [c]. Total molar saturated and unsaturated fatty acids of TAGs and their corresponding 2-MAGs for the six samples were grouped in Table 2.

Triacylglycerols. The component TAGs of the six seed oils of the Malvaceae have been calculated from the fatty acids found in the 1,3-position and those determined in the 2-position for each oil (14,15,21–23). The results are given in Table 3. About thirty triglycerides have been found in each sample. Mostly, every molecular species of the triglycerides was present in a wide range of concentration through the six samples. Thus the minor or major TAGs were not similar for these oils and a general analogy for the identical TAGs was not easily achieved. The same can be said about the literature data reported for cotton (13,15) and okra (11,15). The molar concentrations of the triglycerides were grouped in two ways in Tables 4 and 5. The molar proportion of the symmetrical isomers (Table 4) showed that the values of each trisaturated (SSS) and 1,3-diunsaturated (USU) are close in the six samples. The values of each of triunsaturated (UUU) and 1,3-disaturated (SUS) showed relatively wide variations of their molar concentrations in the six oil samples. However, in each oil sample, the sum of the symmetrical isomers represents about one third of the glyceride. The four component categories of the whole triacylglyceros [namely, triunsaturated (S_3), disaturated-monounsaturated (S_2U), monosaturated-diunsaturated (SU_2) and triunsaturated (U_3)] were grouped for each oil sample in Table 5. The molar proportions of each of these categories showed close values in most of the oil samples.

As a general conclusion, the data obtained here assumes asymmetric distribution of the fatty acids between position 1 and 3 of the TGs (31,32). This pattern is better described by the 1-random-2-random-3-random hypothesis (26,28,33), which assumes a separate fatty acid pool

TABLE 3

Component Triacylglycerols (TAGs, Mole %)

Sample TAG	Cotton	Kenaf	Roselle	Okra[a]	Okra[b]	Ketmia (hollyhock)
PPP	1.2	1.7	1.1	0.8	2.0	1.6
PPS	0.3	0.2	0.3	0.2	0.1	0.1
SPS	T	T	T	T	T	T
PPO	1.3	3.2	2.2	1.8	2.2	2.0
PPL	3.3	2.2	2.4	2.2	3.7	3.6
SPO	0.2	0.2	0.3	0.2	0.1	0.1
SPL	0.4	0.1	0.3	0.3	0.1	0.2
OPO	0.3	1.5	1.1	1.0	0.6	0.7
LPL	2.3	0.7	1.3	1.7	1.8	2.1
OPL	1.7	2.1	2.4	2.6	2.0	2.3
POP	2.6	3.4	5.0	3.7	8.3	5.6
POS	0.6	0.3	1.3	1.0	0.6	0.5
SOS	T	T	0.1	T	T	T
POO	2.8	6.5	9.9	8.6	9.0	7.2
SOO	0.3	0.3	1.3	1.0	0.3	0.3
POL	7.3	4.5	10.6	11.0	15.6	12.8
SOL	0.8	0.2	1.4	1.4	0.6	0.5
LOL	5.0	1.5	5.7	8.2	7.3	7.2
OOL	3.8	4.3	10.5	13.0	8.4	8.2
OOO	0.7	3.1	4.9	5.2	2.5	2.3
PLP	7.1	9.0	3.7	2.5	5.5	5.4
PLS	1.7	0.9	1.0	0.6	0.4	0.4
SLS	0.1	T	0.1	T	T	T
PLL	19.5	11.9	8.0	7.4	10.2	12.0
SLL	2.3	0.6	1.0	0.9	0.4	0.5
PLO	7.5	17.2	7.3	6.0	5.9	6.8
SLO	1.0	0.8	1.0	0.7	0.2	0.3
OLO	2.0	8.2	3.6	3.5	1.6	2.2
OLL	10.3	11.3	7.8	8.8	5.5	8.0
LLL	13.5	3.9	4.2	5.5	4.8	7.0

[a]*Hibiscus esculentus* var. *baladi.*
[b]*Hibiscus esculentus* var. *romi.*
P, palmitic; S, stearic; O, oleic; L, linoleic; and T, trace.

TABLE 4

Molar Proportion of the Symmetrical Glyceride Isomers

Sample type	Cotton	Kenaf	Roselle	Okra[a]	Okra[b]	Ketmia (hollyhock)
SSS	1.2	1.7	1.1	0.8	2.0	1.6
SUS	9.9	12.4	8.9	6.2	13.8	11.0
USU	2.6	2.3	2.4	2.7	2.4	2.8
UUU	21.3	16.6	18.4	22.4	16.1	18.7

[a]*Hibiscus esculentus* var. *baladi.*
[b]*Hibiscus esculentus* var. *romi.*
S, saturated; and U, unsaturated.

for each position of the oil triacylglycerols. Consequently, the concentration of a fatty acid in one of these pools is probably governed by the overall concentration of that acid in the oil. Also, genetic factors or ecological conditions play another role in the fatty acid distribution in the triglyceride molecule. Finally, more studies on the theories behind the selective position distribution of the fatty acids in the triacylglycerol molecules, and generally in the glycerol lipid classes, are needed.

TABLE 5

The Four Component Categories of Total Triacylglycerols

Sample type	Cotton	Kenaf	Roselle	Okra[a]	Okra[b]	Ketmia (hollyhock)
S_3	1.5	1.9	1.4	1.0	2.1	1.7
S_2U	17.3	19.3	16.4	12.3	20.9	17.8
SU_2	45.8	46.3	45.3	42.3	46.6	45.5
U_3	35.3	32.3	36.7	44.2	30.1	34.9

[a]*Hibiscus esculentus* var. *baladi.*
[b]*Hibiscus esculentus* var. *romi.*
S, Saturated; U, unsaturated; SU_2, monosaturated-diunsaturated; and U_3, triunsaturated.

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