The Triglyceride Composition of *Moringa concanensis* Seed Fat

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ABSTRACT

Moringa concanensis seed fat and its randomized product have been subjected to pancreatic hydrolysis. Glyceride compositions have been calculated from the original fatty acid composition and those of the monoglycerides produced by hydrolysis. The per cent GS_3 content of the interesterified product has also been determined by the combined techniques of thin layer chromatography on silver nitrate impregnated silica gel and colorimetry.

INTRODUCTION

Moringacea is a small family composed of the species, *Moringa olifera* (synonym, *M. pterygosperma*), *M. concanensis* and *M. aptera*, all native to India. *M. concanensis* is a rapidly growing tree, even in poor soil, and its green seed pods and flowers are used for food in India. Moringa oil (yield, 30-49% of the kernels) seems to be a promising one for exploitation in pharmaceutical and other allied industries because of its reportedly unusual resistance to the development of rancidity (1,2).

Several investigators (3-9) determined the fatty acid composition of *M. olifera* seed fat. Most of these studies were based on lead salt separation of the mixed fatty acids. Recently, Sengupta and Gupta (10) determined the fatty acid composition of the seed fats of *M. olifera* and *M. concanensis* by combination of the techniques of urea adduct segregation, UV spectrophotometry and quantitative paper chromatography. The fatty acid composition of this oil, as reported by these investigators, is interesting as it is composed of 25-30% saturated acid and 75-70% oleic acid. The triglyceride composition of the seed fat of *M. olifera* has been examined earlier (7,11) by the oxidation method of Kartha (12). This paper describes the determination of the triglyceride composition of *M. concanensis* seed fat by combination of enzymatic hydrolysis, thin layer chromatography (TLC) and gas liquid chromatography (GLC) techniques. The investigation was also extended to randomized oil to study the changes in the triglyceride composition effected by interesterification.

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EXPERIMENTAL PROCEDURES

Materials and Methods

M. concanensis seed kernels were procured from the market of Varanasi in Northern India. Chromatographic standards of tripalmitin, dipalmitin, monopalmitin, fatty acids and the methyl esters were obtained from The Hormel Institute, Sigma Chemical Co. and Calbiochem Inc., U.S.A.

Lipolysis was carried out as suggested by Coleman (13) at pH 8.5 and 37.5 C using a purified pork pancreatic lipase preparation with the addition of Ca^{++} ions and bile salts. The partial glycerides were separated on a thin layer

TABLE I

Characteristics of the Seed Fat and the Mixed Fatty Acids (MFA) of *M. concanensis*

Characteristics	Oil	MFA
Per cent free fatty acids (as oleic)	3.4	
Saponification equivalent	301.2	282.4
Iodine value, Wij's 30 min	67.2	71.2
Unsaponifiable, %	1.7	
$E^{1\%}_{1\ cm}$ at 268 mμ isomerized at 170 C for 15 min	—	Negligible
$E^{1\%}_{1\ cm}$ at 234 mμ isomerized at 180 C for 60 min	—	16.2
$E^{1\%}_{1\ cm}$ at 234 mμ unisomerized	—	Negligible
Per cent linoleic acid	—	1.8
Per cent monoethenoid acid	—	75.5
Per cent saturated acid (by difference)	—	22.7

TABLE II

Fatty Acid Composition of *M. concanensis* Seed Fat, mole %

Fatty acids	Mole %
$C_{14:0}$	0.1
$C_{16:0}$	15.6
$C_{17:0}$	0.2
$C_{18:0}$	3.0
$C_{20:0}$	2.7
$C_{22:0}$	4.3
$C_{16:1}$	3.9
$C_{17:1}$	0.1
$C_{18:1}$	66.0
$C_{20:1}$	2.4
$C_{18:2}$	1.7

(0.3-0.4 mm) of silica by developing with a solvent system of *n*-hexane, diethyl ether and acetic acid (85:15:1). The monoglyceride fraction, detected with 2'7'-dichlorofluorescein, was extracted with hot alcohol. The monoglyceride and the original triglyceride samples were then saponified, the free acids liberated, extracted and converted to methyl ester by acid catalyzed esterification process. GLC was carried out with an F and M analytical gas chromatograph (Model 700-R12) equipped with flame ionization detector. The column (6 ft x 1/4 in.) packed with 10% polyester of diethyl glycol adipate on 60-80 mesh gas chrom-Z was operated at 180 C with a carrier gas flow of 40 ml/min. Peak areas were determined as the product of peak height and the width at half height; the weight percentages obtained were converted to mole percentages.

The interesterified *M. concanensis* seed fat was fractionated on a silver nitrate-impregnated silica gel layer using the technique proposed by Barrett et al. (14) and the amount of GS_3 thus separated was determined quantitatively by the method of Litchfield et al. (15) based on the estimation of glycerol using the chromotropic acid color reaction.

The seed fat of *M. concanensis* was randomized at 30 C by the process suggested by Chakrabarty and Bhattacharyya (16). A solution of 100 g refined and bleached oil in 150 ml

TABLE III

Fatty Acid Composition (Mole %) of the 2-Monoglycerides

Fatty acids	Moringa oil	Randomized Moringa oil[a]
$C_{16:0}$	4.9	34.2
$C_{18:0}$	0.5	6.9
$C_{16:1}$	1.8	—
$C_{18:1}$	92.8	58.9

[a]Slip point 19 C; randomized at 30 C for 60 min.

TABLE IV

Triglyceride Composition[a] (mole %) of *M. concanensis* Seed Fat and Its Interesterified Product

Glycerides	Seed fat	Randomized product
SSS		
PPP	0.2	0.2
PPSt	0.3	0.5
StPSt	0.1	0.5
PStP	—	—
StStP	0.1	0.1
StStSt	—	0.1
Total	0.7	1.4
SSU		
PPO	1.1	3.2
PPU	0.2	0.6
StPO	0.8	5.4
StPU	0.2	1.0
PStO	0.1	0.7
StStO	0.1	1.1
StStU	—	0.2
PStU	—	0.1
Total	2.5	12.3
SUS		
POP	4.3	0.3
POSt	5.9	0.9
StOSt	2.0	0.8
PUP	0.1	—
PUSt	0.1	—
StUSt	—	—
Total	12.4	2.0
USU		
OPO	1.4	16.5
OPU	0.6	5.8
UPU	0.1	0.5
OStO	0.1	3.3
OStU	0.1	1.2
UStU	—	0.1
Total	2.3	27.4
SUU		
POO	20.9	5.5
POU	4.4	1.0
StOO	14.4	9.4
StOU	3.1	1.7
PUO	0.4	—
PUU	0.1	—
StUO	0.3	—
StUU	0.1	—
Total	43.7	17.6
UUU		
OOO	25.6	28.4
OOU	10.9	10.0
UOU	1.2	0.9
OUO	0.5	—
OUU	0.2	—
UUU	—	—
Total	38.4	39.3

[a]The fatty acids have been grouped as, 'P-$C_{14:0}$, $C_{16:0}$ and $C_{17:0}$'; 'St-$C_{18:0}$, $C_{20:0}$ and $C_{22:0}$; 'O-$C_{18:1}$' and 'U-$C_{16:1}$, $C_{17:1}$, $C_{20:1}$ and $C_{18:2}$.

TABLE V

Fatty Acid Composition of the Seed Fats of Moringaceae Family

Species	Per cent weight									
	Saturated acids						Unsaturated acids			
	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	$C_{18:2}$	$C_{18:1}$	$C_{16:1}$	$C_{20:1}$
M. pterygosperma										
(5)	1.5	3.6	10.8	—	6.3	0.1	3.8	68.9	—	—
(9)	1.6	3.9	11.5	—	6.6	0.2	—	72.2	—	—
(6)	—	5.5	7.8	2.7	1.2	5.3	0.8	75.8	0.9	—
(7)	—	9.3	7.4	8.1	—	—	—	65.7	—	—
(10)	—	3.1	8.0	7.8	3.5	5.8	0.8	71.0	—	—
M. concanensis										
(9)	—	5.5	7.8	2.7	1.2	5.3	0.8	75.8	0.9	—
(10)	—	11.7	3.8	2.4	4.1	0.6	0.9	75.5	—	—
(Present work[a])	0.1	14.3	3.0	3.0	5.2	—	1.7	66.3	3.5	2.6

[a]It also indicates the presence of $C_{17:0}$, 0.2% and $C_{17:1}$, 0.1%.

n-hexane was taken in a three-necked flask and to it was added, drop by drop, the catalyst sodium methoxide (methanolic solution, 0.4% w/w of the oil solution). Rearrangement was continued to the equilibrium state. After 60 min the randomized sample was isolated by the usual procedure after destroying the catalyst with dilute hydrochloric acid.

RESULTS

On analysis by standard procedure, the seed fat of *M. concanensis* and the mixed fatty acids obtained from it showed the characteristics given in Table I. Methyl esters of the mixed fatty acids were next analyzed by GLC. The results are shown in Table II.

The fatty acid compositions of the 2-monoglycerides obtained from lipolysis of the Moringa oil and its interesterified product as determined by GLC are given in Table III.

The triglyceride compositions of the *M. concanensis* seed fat and its randomized product were next calculated from the fatty acid composition of the original triglyceride and the 2-monoglycerides formed, using the assumption of Vanderwal (17) and Coleman (18). While calculating, the fatty acids have been grouped (19) as '$P\text{-}C_{14:0}$, $C_{16:0}$ and $C_{17:0}$'; '$St\text{-}C_{18:0}$, $C_{20:0}$ and $C_{22:0}$'; '$O\text{-}C_{18:1}$' and '$U\text{-}C_{16:1}$, $C_{17:1}$, $C_{20:1}$ and $C_{18:2}$'. The results are given in Table IV.

The percentage GS_3 content of the randomized *M. concanensis* seed fat as determined by the method developed by Litchfield et al. (15) was found to be 1.5.

DISCUSSION

The results of the present investigation along with the findings of the earlier workers on the fatty acid compositions of the seed fats of the Moringaceae family are shown in Table V.

The fatty acid composition of *M. concanensis* seed fat as determined by GLC thus agrees well with that determined by Sengupta and Gupta (10) utilizing the combined techniques of urea adduction and quantitative paper chromatography. Though the triglyceride composition of *M. concanensis* has not been investigated earlier, Rao et al. (7) and Kartha and Upadhyay (11) have studied earlier the triglyc-

TABLE VI

Triglyceride Composition (mole %) of Moringacea Seed Fat

Glycerides	*M. pterygosperma* (7)	*M. pterygosperma* (11)		*M. concanensis* (present work)
		North India	South India	
GS_3	1.4	—	—	0.7
GS_2U	23.4	16.0	18.3	14.9
GSU_2	25.6	37.3	25.2	46.0
GU_3	49.6	46.7	56.5	38.4

eride composition of *M. pterygosperma* seed fat of similar fatty acid composition utilizing the oxidation method. These data, along with the findings of the present investigation, are shown in Table VI.

Table III shows the preponderance of C_{18} unsaturated acids in the 2-positions of the triglycerides of *M. concanensis* seed fat. According to the theory of Gunstone (20) the 2-position of the triglycerides is preferentially esterified with C_{18} unsaturated acids as far as the composition allows and thereafter the remaining acids are distributed randomly among the unoccupied positions. The present results agree fairly closely with this theory but not completely, since they indicate about 5% of the saturated acids in the 2-position. The present findings agree more with the observation of Coleman (21), who reported 4.4% saturated acids in the 2-positions of the triglyceride of Karanja oil, containing 26.6% of saturated acid. The proportions of the six possible glycerides as calculated by the proposed formulae of Coleman (21) agree well with the findings of the present investigation (Table IV), the two sets of data being 'SSS-0.4, 0.7'; 'SSU-1.2, 2.5'; 'SUS-13.7, 12.4'; 'USU-1.0, 2.3'; 'SUU-45.7, 43.7' and 'UUU-38.0, 38.4' per cent, respectively. Table III further shows that during the process of interesterification the proportions of saturated acids in the 2-position increased from 5.4% in the seed fat to 41.1% in the randomized product. The triglyceride compositions of these products (Table IV) as calculated on the basis of the 1,3-random, 2-random distribution hypothesis also gives an idea about the changes in the proportions of the six possible glycerides effected by the process of interesterification. The content of GS_3 and GU_3 changed from 0.7% to 1.4% and from 38.4% to 39.3%, respectively, by this process of randomization. The proportion of GS_3 (1.4%) content of the randomized product as calculated from the lipolysis data is also in close agreement with that (1.5%) determined by the method developed by Litchfield et al. (15).

The high content of oleic acid (66.3%) and the low content of linoleic acid (1.7%) make the fatty acid composition of *M. concanensis* seed fat interesting for utilization in the pharmaceutical industry as a formulation ingredient. The 25.7% of triolein also indicates a possibility of exploitation of this seed. Further studies are being continued to develop a process for the preparation of oleic acid and triolein from the seed fat of *M. concanensis*.

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