A review of the volatile profiles showed that: (a) total ppm volatiles of in-process oils were primarily dependent on the concentration of residual hexanes; (b) crude soybean oil contained relatively few volatiles other than hexanes; (c) during processing of the oil, concentrations of later eluting volatiles increased; (note the development of decadienals that have been correlated to soybean oil off-flavors (8)); (d) deodorization removed nearly all of the hexanes and significantly reduced the concentration of later eluting volatiles; (e) using higher sampling temperatures (i.e., 220 C vs 170 C) resulted in higher estimated total volatile concentrations. However, for monitoring consistency and trends, the repeatability of the method, not the absolute numbers, was the critical factor.

Automating VPA and implementing systematic performance checks greatly simplified the routine operation of the GC system. Direct operator involvement was reduced by ca. 25 min per sample, compared with the manual valve switching and temperature control approach initially used. Sample tubes were prepared in advance by the more experienced operators for later use by less experienced operators. Therefore, minimal training was required to obtain satisfactory VPA on a routine basis. VPA provides a hard copy fingerprint of oil volatiles. Flavor panels would have a difficult time qualifying the flavors of the undeodorized products, whereas this GC technique easily qualitated and quantitated volatile flavor compounds in both in-process oils and finished products. Qualitative information is most important for monitoring production because each process oil or product has a characteristic volatile profile. Changes in the profile, in terms of volatiles being present or absent, are just as important as absolute concentrations when correlating VPA results to processing conditions and end-product oil quality.

Routine operation of the automated VPA technique has shown that the potential for this technique goes beyond verifying flavor scoring of finished products. VPA can also be used to: (a) monitor the effects of processing conditions; (b) evaluate the effects of storage conditions; (c) reduce the number of flavor panels; (d) follow oil quality changes during shipment; (e) monitor the changes in oil quality of finished products, e.g. margarine, mayonnaise, salad oil and salad dressing. All of these potential uses can lead to better product performance and even improved productivity by defining conditions for making a product right the first time.

In summary, the automated system for VPA developed in our laboratory not only met our objectives for reducing manpower requirements in an in-plant operation, but was found to be: (a) easy to operate; (b) consistent in performance; (c) adaptable to different analytical conditions; (d) useful in determining in-process and finished oil quality in a manner that has great commercial value.

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✲Changes in Lipid Class and Fatty Acid Compositions During Maturation of *Hibiscus esculentus* and *Hibiscus cannabinus* Seeds

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ABSTRACT

The major lipid classes and their constituent fatty acids were analyzed in maturing seeds of *Hibiscus esculentus* and *H. cannabinus*. The seeds matured in 40 and 45 days, respectively. The active accumulation period was from the 13th to 25th and 15th to 30th day after flowering, respectively. While a continuous increase in the content of triacylglycerols (TAG) was noticed in *H. esculentus*, TAG was at its peak in *H. cannabinus* on the 20th day after flowering. The contents of polar lipids were high in the immature seeds but decreased during maturation. The major fatty acids in both species were palmitic, oleic and linoleic. Cyclopropane fatty acids were present only in TAG of both species throughout maturation period. Cyclopropene and epoxy acids appeared in TAG in traces at the final stages of seed maturation. Oleic and linoleic acids were preferentially esterified at the secondary positions of TAG. The contents of palmitic and stearic acids at the secondary positions were sharply reduced as TAG accumulated.

INTRODUCTION

Changes in fatty acids, both common and unusual, during seed maturation, have received much attention (1,2). We have been carrying out such studies on oilseeds containing unusual acids, e.g., cyclopropene (3), conjugated trienoic (4) and petroselinic (5) acids. The changes in major lipid classes and the constituent fatty acids of 2 species of Malvaceae, *Hibiscus esculentus* Linn. (Okra) and *H. cannabinus* Linn. (mesta, Kenaf, 'Ambadi') form the subject of the present investigation. *H. esculentus* (HE) seeds appear to be a potential source of oil and protein (6,7). *H. cannabinus* (HC), though at present grown in India mainly for fiber (8), is becoming an important commercial source of oil (9). Both seed oils contain linoleic (18:2), oleic (18:1) and palmitic (16:0) acids as major components as in cottonseed oil (*Gossypium* species of Malvaceae) and small amounts of cyclopropenoid (CFA) and epoxy fatty acids (6-12).

MATERIALS AND METHODS

The seeds of HE (var. Pusa Savani) were obtained from the

National Seeds Corporation, Hyderabad, India, and seeds of HC from the Central Nucleus Jute Multiplication Farm, Budbud, W. Bengal, India. Seeds were sown (germination rate 95%) in prepared plots. Flowers were tagged and 100 or more seeds were collected from several plants at intervals.

Moisture contents were determined by heating the seeds in an airoven at 110 C until they reached a constant weight. Fresh seeds were weighed, crushed and soaked in chloroform/methanol (2:1, v/v) for 18 hr in the dark, then extracted 3 times at 2 hr intervals. The pooled extracts were concentrated at 40 C in a rotary vacuum evaporator, reextracted with petroleum ether (40-60 C), washed with 0.9 N sodium chloride solution to remove nonlipid impurities and dried over anhydrous sodium sulfate. An aliquot of the extract of the total lipids was used to determine lipid content. The Halphen test was carried out (13) to detect CFA and the picric acid test was used on Silica gel G plates (14) to detect epoxy fatty acids.

Preparative thin layer chromatography (TLC) was carried out on 1 mm Silica gel G layers to separate the total lipids into classes. Development with chloroform separated the total lipids into neutral and polar lipids. The neutral lipids were further separated on Silica gel G layers into triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG) and nonesterified fatty acids (NEFA) using hexane/diethyl ether/acetic acid (70:30:1, v/v/v). The polar lipids of HE were analyzed as such and those from HC were further separated into phospholipids (PL) and glycolipids (GL) by column chromatography on silicic acid. Acetone eluted GL and methanol was used to recover PL (15). Identity of the various chromatographic fractions was established using reference compounds.

Neutral lipid classes (TAG, DAG, MAG and NEFA) were estimated using methyl heptadecanoate (17:0) as an internal standard. From the gas chromatographic (GC) data of the component fatty acids of the various lipid fractions, mean molecular weights of the fatty acids were calculated and used to compute the factors to estimate the respective lipid classes. GL were determined from the hexose contents (16) and PL from phosphorus contents (17).

Fatty acid compositions of the various lipids were determined by GC. The lipids were converted to methyl esters by treatment with methanolic sodium methoxide at room temperature in the dark overnight. Samples that did not respond to the Halphen test were analyzed as such and those responding positively were treated with silver nitrate to convert CFA to stable ether and keto derivatives (18) before GC analysis. A Hewlett-Packard 5840 A unit equipped with flame ionization detector (FID) was used. A glass column (4 mm i.d. × 2 m) packed with 10% DEGS on Chromosorb W HP (80/100) and operated isothermally at 190 C at a carrier gas (nitrogen) flow of 30 mL/min was used, with on-column injection. The injection port and detector were maintained at 250 C and 300 C.

The presence of cyclopropane fatty acids was ascertained by GC analysis of the methyl esters of saturated acids isolated by silver ion TLC using hexane/diethyl ether (92:8, v/v). Similarly, heptadecenoic acid (17:1) was identified by GC analysis on a SE-30 column of the monounsaturated esters under the same conditions as with the DEGS column.

The isolated TAG were subjected to pancreatic lipase hydrolysis (19). The 2-MAG were isolated from the lipolysis products by TLC on Silica gel G using hexane/diethyl ether/acetic acid (60:40:1, v/v/v), converted to methyl esters and analyzed by GC.

RESULTS AND DISCUSSION

Changes in Lipid and Lipid-Class Contents

The seeds of HE matured in 40 days and of HC in 45 days (Table I). The active period of lipid accumulation was from the 13th to 25th day and from the 15th to 30th day after flowering, respectively. As maturation and lipid accumulation proceeded, the contents of PL and DAG, the precursors and the intermediates in TAG biosynthesis (20), decreased and TAG increased. MAG were found in HC but not in HE. The MAG may not be artefacts because NEFA, the other products of enzymic hydrolysis, were not found. The MAG pathway (21) may also be operating in HC to synthesize TAG. As TAG synthesis became active,

TABLE I

Changes in Contents (wt %)[a] of Lipids and Lipid Classes and the Constituent Fatty Acids of *H. esculentus* and *H. cannabinus* Seeds during Maturation

Days after flowering.		Content		16:0		18:0		18:1		18:2		18:3		Cyclic[b]		Others[c]	
HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC
								Total lipids									
9	9	2.2	5.9	25.7	24.3	1.4	0.2	8.6	18.7	54.8	49.4	7.2	3.9	0.5	<0.1	1.8	3.5
13	15	3.0	6.9	23.2	28.1	2.4	1.0	16.2	17.2	46.7	44.5	8.2	5.1	1.1	<0.1	2.2	3.9
16	20	8.6	15.3	29.6	22.6	4.4	1.6	16.9	24.1	43.8	49.4	1.6	1.4	3.4	0.4	0.4	0.4
19	25	12.3	22.8	33.2	22.1	4.5	2.0	15.1	26.4	43.2	48.4	1.2	0.5	2.4	0.4	0.4	0.2
25	30	20.2	23.0	31.6	22.2	3.8	2.9	15.2	29.6	45.6	44.5	0.3	0.5	1.5	0.4	2.2	<0.1
31	45	20.0	23.9	27.6	22.9	3.8	2.8	17.3	29.6	47.4	44.4	0.3	<0.1	1.6	0.3	1.4	0.1
35		17.9		38.7		2.6		16.3		41.0		<0.1		1.2		0.2	
40		18.3		39.0		2.6		17.4		39.8		<0.1		1.0		0.3	

TABLE I (Continued)

Days after flowering.		Content		16:0		18:0		18:1		18:2		18:3		Cyclic[b]		Others[c]	
HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC
								Triacylglycerols									
9	9	4.6	36.4	19.2	24.4	3.1	2.9	22.1	25.7	35.0	39.8	7.9	1.4	<0.1	0.6	12.7	5.2
13	15	22.1	42.5	24.1	26.9	2.2	2.7	28.3	23.4	40.2	40.8	4.5	1.4	0.6	0.5	<0.1	4.3
19	20	70.0	80.7	32.7	21.6	8.4	3.4	25.6	28.9	28.0	41.8	0.9	0.5	2.4	0.3	2.1	3.5
25	30	76.0	80.1	32.5	22.4	7.1	3.1	28.2	30.3	29.0	41.2	0.7	0.5	2.0	0.6	0.7	1.9
31	45	80.0	80.5	29.4	22.4	5.9	3.0	26.5	29.1	31.0	42.7	1.0	0.5	2.0	0.8	4.3	1.6
40		84.4		29.7		4.5		29.0		34.1		0.5		2.0		0.2	
								Diacylglycerols									
9	9	21.6	4.6	33.3	29.2	5.5	4.6	19.9	20.2	29.2	30.6	4.4	0.0	0.0	0.0	7.6	8.1
13	15	8.6	7.7	27.2	35.1	2.6	4.5	18.6	22.5	42.6	33.9	5.5	0.0	0.0	0.0	3.0	1.1
19	20	2.1	2.6	41.0	21.8	6.9	4.2	8.9	28.7	40.0	37.9	0.7	0.0	0.0	0.0	2.6	1.3
25	30	2.1	1.8	42.6	23.1	5.1	3.7	15.3	34.5	30.8	34.0	0.8	0.0	0.0	0.0	5.4	2.7
40	45	0.9	1.6	34.4	23.7	6.8	3.5	17.8	34.7	32.8	35.3	0.2	0.0	0.0	0.0	8.2	0.6
								Monoacylglycerols[d]									
	9		7.2		29.2		4.9		20.6		31.8		4.1		0.0		7.9
	15		3.9		41.4		5.5		18.4		22.9		5.0		0.0		6.9
	20		0.7		38.5		4.7		20.0		30.1		3.1		0.0		2.4
	30		0.6		35.6		4.5		18.2		29.8		3.0		0.0		6.8
	45		2.8		21.9		3.7		25.6		37.6		4.9		0.0		6.2
								Nonesterified fatty acids[d]									
9		4.0		41.6		1.9		4.5		44.8		6.4		0.0		0.8	
13		1.2		41.2		12.7		21.1		6.4		2.9		0.0		15.8[e]	
19		0.7		46.5		8.2		3.0		4.5		2.2		0.0		35.6[e]	
25		0.1		46.3		22.1		2.7		1.3		0.2		0.0		27.4[e]	
31		0.1		35.6		23.3		9.2		3.3		3.3		0.0		25.3[e]	
40		0.1		45.8		21.6		2.8		<0.1		0.0		0.0		29.8[e]	
								Glycolipids[f]									
9	9	50.5	9.1	41.6	34.6	1.9	17.8	4.5	14.6	44.8	1.6	6.4	1.2	0.0	0.0	0.8	30.1
13	15	65.5	14.8	35.9	31.4	2.2	17.9	12.6	12.5	38.3	9.7	10.0	4.9	0.0	0.0	1.0	23.4
19	20	9.8	2.3	34.3	31.9	5.1	12.5	6.7	14.4	46.8	27.0	4.1	4.9	0.0	0.0	3.1	9.5
25	30	5.7	0.8	39.8	33.3	4.8	10.1	15.5	15.6	31.9	17.5	1.7	3.5	0.0	0.0	6.3	20.0
31	45	6.0	2.2	40.0	34.3	4.5	9.8	20.8	23.2	28.9	17.9	2.3	3.2	0.0	0.0	3.6	11.4
40		1.8		37.5		4.8		27.9		24.2		1.6		0.0		4.1	
								Phospholipids									
	9		13.6		29.2		14.0		14.3		24.8		8.8		0.0		8.7
	15		7.5		32.7		10.0		16.3		31.4		6.5		0.0		3.0

TABLE I (Continued)

Days after flowering.		Content		16:0		18:0		18:1		18:2		18:3		Cyclic[b]		Others[c]	
HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC	HE	HC
								Phospholipids									
	20		1.8		30.9		11.0		17.2		31.6		4.4		0.0		4.7
	30		0.6		22.4		4.2		37;3		32.1		2.4		0.0		1.5
	45		0.4		38.0		5.8		21.3		28.2		2.3		0.0		3.7

a Total lipids in seeds on dry basis, lipid class in total lipids, and fatty acid in mixed fatty acids.

b Dihydromalvalic plus dihydrosterculic in *H.esculentus* (HE) and dihydrosterculic in *H.cannabinus* (HC).

c One or more of 12:0, 14:0, 16:1, 17:1, 20:0. Detailed data available on request.

d Monoacylglycerols were not detected in HE and nonesterified fatty acids in HC. Pigments and other minor lipid classes were not analyzed.

e Major components, 12:0 and 14:0.

f Mixture of glycolipids and phospholipids in HE.

TABLE II

Percentage Proportion[a] of Fatty Acids in the 2-Position of Triacylglycerols of *Hibiscus esculentus* and *Hibiscus cannabinus* Seeds During Maturation

Days after flowering		16:0		18:0		18:1		18:2	
HE	HC	HE	HC	HE	HC	HE	HC	HE	HC
13	–	36.4	–	47.2	–	80.5	–	22.5	–
19	20	9.7	1.8	5.2	10.4	57.8	39.0	46.5	46.0
25	–	8.6	–	6.1	–	49.5	–	49.0	–
31	30	12.2	2.3	7.9	4.6	42.6	38.5	44.7	47.0
40	45	4.9	5.2	5.1	11.9	44.1	35.7	57.8	46.5

[a] $\frac{\text{Percentage of particular fatty acid in fatty acids at the 2-position} \times 100}{\text{Percentage of the particular fatty acid in total fatty acids} \times 3}$.

GL contents were also reduced, perhaps because DAG was used more for TAG synthesis than for GL synthesis (22).

Changes in Fatty Acids

The major fatty acids of total lipids in both seeds were 18:2, 16:0 and 18:1 (Table I). No dramatic changes were observed in the contents of these acids, although a gradual increase was noticed in 18:1 of HC. Among the minor but significant components, the presence of cyclopropane fatty acids throughout the maturation period is noteworthy. Both dihydromalvalic and dihydrosterculic acids were present in HE, whereas only the latter was found in HC, also in lower concentrations. Appreciable portions of 18:3 were present in the initial stages. The observations on TAG were broadly similar to those on total lipids. CFA and epoxy fatty acids were found in the final stages in trace quantities. CFA and their dihydroderivatives were not found in DAG, MAG, NEFA, GL and PL. The CFA are postulated to be synthesized by the addition of a methylene group from methionine across the double bond of a monoenoic acid of phospholipids followed by desaturation (23,24). The absence of CFA and dihydro-CFA in lipid classes other than TAG of both the species suggests involvement of TAG. In NEFA of HE 16:0 and 18:0 were present in higher proportions and 18:2 in lower proportions than in other lipid classes. This could be expected because 16:0 is the primary product of de novo synthesis followed by 18:0 and very little desaturation occurs on the acyl moieties in the free form (25). GL and PL were analyzed together in HE and separately in HC. In general, GL and PL contained more 16:0 and 18:3 and less 18:2 compared with TAG.

Changes in Positional Distribution of Fatty Acids in TAG

The percentage proportions of individual fatty acids present

in the 2-position of TAG were determined by pancreatic lipase hydrolysis procedure (Table II). Normally, TAG of mature seed oils contain predominantly 16:0 and 18:0 in the 1,3-positions of TAG. But, considerable proportions of 16:0 and 18:0 were present in the 2-position of TAG in HE in the initial stages of maturation until the 13th day after flowering. As maturation proceeded and TAG synthesis became active, these proportions were drastically reduced and the distribution pattern became normal with 18:2 as the major acid in the 2-position. A gradual reduction occurred from a high value (80%) in the percentage proportion of 18:1 in the 2-position. This pattern was not observed in the TAG of HC because the samples were analyzed only after the TAG synthesis was complete. Once TAG synthesis was more or less complete, no change occurred in the positional distribution pattern in both species.

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Physical Refining of Edible Oil

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ABSTRACT

Physical refining of edible oils has received renewed interest since the early 1970s when the process was reintroduced on a large scale to refine palm oil in Malaysia. Subsequent laboratory and field tests have also shown that physical refining can be used as a substitute for caustic or chemical refining, not only for high free fatty acid (FFA) oils such as palm, but also on low FFA oils such as soybean oil. In either case, the physical refining system results in lower oil loss than chemical refining and also eliminates pollution problems associated with soapstock acidulation. In physical refining, however, the oil pretreatment and efficiency of the distillation are two very important factors that must be considered to guarantee continuous production of high quality products. This paper reviews the physical refining system as it is today and how it can be used on two different edible oils. An actual case study showing the effects of the pretreatment in a commercial operation is also presented.

INTRODUCTION

Physical refining of edible oils has been a principal topic of discussion in the fats and oils industry for the past few years. Until that time, edible oil refining was almost always thought of as a four-step process: degumming, deacidification, or neutralization, bleaching and deodorizing. Since the alkalai used in neutralization most often was caustic, the process was widely known as caustic or chemical refining. One problem of the chemical refining process, however, is the production of soapstock, a mixture of sodium salts of fatty acids, neutral oil, water, unused caustic and other compounds resulting from the reactions of the caustic with the various impurities in the oil. The disposal of this soapstock, or the waste streams from soapstock processing systems, has become very expensive as governmental regulations on plant waste water discharge quality tighten. Another problem associated with chemical treatment is that it also causes a loss in neutral oil, thereby reducing the overall yield of refined product. As long as the primary oils being refined, however, were those with naturally low free fatty acid contents (FFA), such as soybean, there were few attempts to introduce physical refining on a large scale. In the early 1970s, however, action by the Malaysian government forced the development of a large refining industry for their palm oil which had grown rapidly in the world market. A typical palm oil contained 3-5% FFA and, therefore, chemical treatment resulted in excessive losses in neutral oil and production of large quantities of soapstock. For this oil, physical refining offered an attractive alternative.

Block flow diagrams for both the chemical and physical refining processes are shown in Figure 1. The chemical method contains a water degumming step, which is used to remove impurities generally classified as gums; this is followed by caustic treatment for removal of additional impurities and most of the FFA; then bleaching to improve color, and finally deodorization or steam distillation to

[1] Presented at the 73rd AOCS annual meeting, Toronto, 1982.