TABLE I

Phosphatidylcholine Content Determined by HPLC of Soy Lecithins

Sample	Content (%)[a]
Crude lecithin	18.4 ± 1.3[b]
Acetone-insoluble fraction	27.3 ± 1.0
2-Propanol-soluble fraction	57.6 ± 1.2

[a]Content (%) = (phosphatidylcholine [mg] /lecithins [mg]) X 100.
[b]Mean ± SD based on 3 samples.

Therefore, we could separate PC but we could not separate another 2 major components of the lecithin, PE and PI. Furthermore, PC was found to be completely separated from Sph. Guerts van Kessel et al. (3) and Hax and Guerts van Kessel (4) noted the difficulty of separating PC from SpH with UV detector in the range of 203-214 nm and with *n*-hexane/2-propanol/water mixture as eluting solvent, whereas Jungalwala et al. (2) reported that PC and Sph could be separated with acetonitrile/methanol/water mixture as eluting solvent using gradient system.

In order to quantify the amount of PC, aliquots of standard solutions ranging from 0.5 to 4.0 mg PC were chromatographed and the plot of peak area against PC concentration of the samples was found to be linear as shown in Figure 1. Based on this calibration curve, PC contents of crude lecithin, acetone-insoluble fraction and 2-propanol-soluble fraction were obtained from HPLC as shown in Figure 2 and the results are shown in Table I. The results indicate that PC contents of crude lecithin, acetone-insoluble fraction and 2-propanol-soluble fraction were 18.4 ± 1.3, 27.3 ± 1.0, 57.6 ± 1.2%, respectively, and these values were very close to the data obtained by Erdahl et al. (6) and Sullivan and Szuhaj (11). The HPLC method described in this paper is a rapid and simple analytical tool for the determination of PC in soy lecithins.

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[Received July 6, 1981]

⁂Cyclopropenoid Fatty Acid Content and Iodine Value of Crude Oils from Indian Cottonseed

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ABSTRACT

Cyclopropenoid fatty acid (CPFA) and iodine value (IV) of oils extracted from 30 varieties of cottonseed belonging to 4 botanical species, *Gossypium arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense*, have been reported. The CPFA determined by HBr titration method ranged from 0.66 to 1.51%. The IV of the oils ranged from 93.3 to 111.2. CPFA contents were significantly different among the species and nonsignificant within the species. The mean values of CPFA for different species were 0.67 (*G. barbadense*), 0.83 (*G. hirsutum*), 1.14 (*G. herbaceum*) and 1.40% (*G. arboreum*). There was no correlation between IV and CPFA content.

INTRODUCTION

Cottonseed oil contains cyclopropenoid fatty acid (CPFA) as malvalic and sterculic acids in its triglycerides. Although the concnetration of CPFA in cottonseed oil is small (1-7), the incorporation of low levels of cottonseed oil containing CPFA that give a positive Halphen test into the diet of laying hens results in unusual biological effects (2,8-11). The dietary CPFA can cause pink-white, pasty yolks in the eggs, defects in the reproductive capacity of the bird, growth inhibition, altered fatty acid distribution and fat accumulation (12-14). Cottonseed oil is widely used in food for human consumption and the possibility exists that the CPFA may produce deleterious effects in man. Thus, data on CPFA content in oils of different varieties of cottonseed will provide useful information. Data on CPFA content in cottonseed oil of American varieties have been reported whereas little is known about those of Indian cottonseed. The object of this study was to provide basic information on CPFA content of oils of different varieties of Indian cottonseed.

In this investigation, 30 samples of crude cottonseed oil belonging to 4 cultivated botanical species were analyzed for CPFA content, expressed as percentages of malvalic acid using the HBr-dilution technique (15) and iodine value (IV). Significant variation in CPFA content in cottonseed oil among different species was observed.

EXPERIMENTAL

Materials

Thirty cottonseed samples included in this study belonged to 4 botanical species; 11 from *G. arboreum*, 5 from *G. herbaceum*, 12 from *G. hirsutum* and 2 from *G. barbadense*. The cottonseed samples were obtained from cotton

breeders.

About 200 g of seeds was dehulled, kernels separated, and then ground in micropulverizer. The oil was extracted in a Soxhlet with petroleum ether (40-50 C), freed from the solvent under reduced pressure using a rotary vacuum evaporator below 60 C and stored in a refrigerator until used. Methyl esters were prepared from the oil by methanolysis, adding one part by wt of oil to 3 parts of absolute methanol in which 0.003 parts of metallic sodium in excess of free fatty acid equivalent of the glyceride had been dissolved (4).

Method

The methyl esters were subjected to selective alumina treatment to remove interfering substances. One hundred g of activated alumina, (4 times the weight of the sample) was slowly poured into the chromatograph tube (22 mm id and 600 mm length) containing a plug of absorbent cotton and a sufficient quantity of petroleum ether (bp 40-60 C) to cover the entire charge of alumina. The 25-g sample dissolved in an equal vol of petroleum ether was poured into the column when the solvent had drained to within about 5 mm of the top of the packing. It was allowed to percolate by gravity and was then eluted by addition of 250 mL of solvent from an automatic siphoning separatory funnel. The combined percolate was filtered and the solvent removed on a rotary evaporator under reduced pressure at a temperature below 60 C. The stripping flask was then cooled and the vacuum broken with the nitrogen to provide an oxygen-free environment. The alumina-treated methyl esters were subjected to HBr titration at 3 and 55 C in duplicate (15). The methyl esters of the oils were diluted to 15 times and CPFA were calculated as a percentage of malvalic acid according to the following formula (16).

$$\% \text{ Malvalic acid} = 28.04 \frac{NV}{W} \times 1.175,$$

where N is the normality of HBr solution; v, is the number of mL of HBr solution of normality required; W, is g of sample.

IV of the oil was determined before alumina treatment by Wijs method (17).

TABLE I

Cyclopropenoid Fatty Acid (malvalic acid %) and Iodine in Crude Cottonseed Oil of *G. barbadense* Species

Sample	Variety	Year grown	Location	Malvalic acid (%)	Iodine value
1	Sea Island	1978	Amravati	0.66	101.8
2	Suvin	1978	Tamil Nadu	0.67	103.5
Mean				0.665	102.7

TABLE II

Cyclopropenoid Fatty Acid (malvalic acid %) and Iodine Value in Crude Cottonseed Oil of *G. birsutum* Species

Sample	Variety	Year grown	Location	Malvalic acid (%)	Iodine value
1	Deviraj	1978	Dharangdhara	0.69	108.0
2	Khandwa-2	1978	Khandwa	0.72	106.7
3	J-34	1978	Shriganganagar	0.75	101.6
4	Laxmi	1978	Simoga	0.78	109.3
5	A-51-9	1978	Khandwa	0.82	104.2
6	Hampi	1978	Raychoor	0.83	106.3
7	C-Indore-1	1978	Bhiliwada	0.83	110.7
8	Hybrid-4	1978	Ratlam	0.87	109.5
9	Hybrid-4	1978	Barwaha	0.88	106.5
10	Deviraj	1978	Gokak	0.88	102.9
11	Khandwa-2	1978	East Nimar	0.93	102.1
12	Buri-1007	1978	Amravati	1.02	103.5
Mean				0.83	105.8
Standard deviation				0.092	3.070

TABLE III

Cyclopropenoid Fatty Acid (malvalic acid %) and Iodine Value in Crude Cottonseed Oil of *G. berbaceum* Species

Sample	Variety	Year grown	Location	Malvalic acid (%)	Iodine value
1	Suyodhar	1977	Bagalkot	1.05	103.9
2	Digvijay	1978	Kurjan	1.12	107.0
3	Wagad	1978	Morvi	1.15	106.0
4	V-797	1978	Viramgam	1.17	106.8
5	W-1	1978	Bellary	1.19	109.2
Mean				1.14	106.6
Standard deviation				0.055	1.911

TABLE IV

Cyclopropenoid Fatty Acid (malvalic acid %) and Iodine Value in Crude Cottonseed Oil of *G. arboreum* Species

Sample	Variety	Year grown	Location	Malvalic acid (%)	Iodine value
1	Virnar	1978	Ratlam	1.17	104.2
2	A.K.277	1977	Khamgaon	1.29	97.3
3	C.J.73	1978	Botad	1.30	107.9
4	G-1	1977	Ganganagar	1.37	111.2
5	Maljari	1978	Khargoan	1.41	101.3
6	A.K.H-4	1977	Akola	1.41	105.3
7	G-6	1977	Bhainse	1.42	102.9
8	A.K.235	1977	Adilabad	1.43	98.7
9	V-1	1977	Jalgaon	1.47	102.0
10	G-1	1978	Ganganagar	1.51	106.3
11	G-22	1977	Bidar	1.51	98.2
Mean				1.40	103.2
Standard deviation				0.103	4.316

RESULTS AND DISCUSSION

The data on CPFA (malvalic acid) contents and IV of the oils extracted from 30 varieties of cottonseed belonging to 4 different cultivated botanical species are summarized in Tables I-IV. The varieties are arranged in order of increasing percentages of CPFA. Standard deviations are given in each table.

Data presented in Table I for CPFA content for *G. barbadense* varieties did not show marked variation. Data prsented in Tables II, III and IV for *G. hirsutum*, *G. herbaceum* and *G. arboreum* species, respectively, showed some variation among the varieties within the species, but these variations were not significant.

The percentage of CPFA in the different varieties from all 4 species varied from 0.66 for Sea Island (*G. barbadense*) to 1.51 for G-1 and G-22 (*G. arboreum*). The mean CPFA value was 1.01%. The mean value of CPFA for different species was 0.665% for *G. barbadense*, 0.83% for *G. hirsutum*, 1.14% for *G. herbaceum* and 1.40% for *G. arboreum*. From the analysis of variance of these results, it was observed that there is significant difference in CPFA content among species. The varieties belonging to *G. barbadense* species contain the lowest percentage of CPFA and the varieties from *G. arboreum* species contain the highest CPFA. The order of increasing CPFA content rated by species can be represented as: *G. barbadense*, *G. hirsutum*, *G. herbaceum* and *G. arboreum*. These results are in excellent agreement with the data reported by Bailey et al. (1). They also observed similar significant differences in malvalic acid content (uncorrected data) among the 3 species, viz., *G. barbadense* (0.56), *G. hirsutum* (0.71) and *G. arboreum* (1.17). These results establish species (rather than variety or environment) as the major factor in determining CPFA content. Furthermore, analysis of variance of these data did not show significant differences in CPFA content within the species.

Variation in CPFA content of the same variety grown at two different locations was noted in Deviraj (Table II). The sample from Gokak (0.88%) has higher CPFA than that from Dharandhara (0.69%). However, these differences are statistically nonsignificant. On the other hand, CPFA content was almost the same in hybrid 4 from 2 different locations, Ratlam (0.87) and Barwoha (0.88).

Data on IV from all 4 species varied from 97.3 for A.K. 277 to 111.2 for G.1, both from *G. arboreum* species. Mean IV for different species were almost same and the grand mean for all the species was 104.6.

The relationship between CPFA and IV was studied. There was no correlation between CPFA and IV. This is in agreement with the findings of Bailey et al. (1) and Lawhon et al. (6).

ACKNOWLEDGMENTS

The authors thank Shri D.V.Mhadgut for doing statistical analyses and V. Sundaram, Director, for permission to publish the paper.

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[Received July 30, 1979]