Analysis of *Calotropis gigantea*, *Acacia caesia* and *Abelmoschus ficulneus* seeds

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ABSTRACT

The percentage contents of oil and protein in the seeds of *Calotropis gigantea* Linn. (Asclepiadaceae), *Acacia caesia* Willd., syn. *A. intsia* (Leguminosae) and *Abelmoschus ficulneus* Wight & Arn., syn. *Hibiscus ficulneus* Linn. (Malvaceae) were 30.8, 8.8 and 14.4, and 19.0, 11.7 and 20.0, respectively. The major fatty acid was 18:1 in *C. gigantea* and 18:2 in the other two seeds oils. Malvalic, sterculic and dihydrosterculic acids were present in small quantities in *A. ficulneus* seed oil. The major essential amino acids in the seed proteins were phenylalanine, lysine and histidine in *C. gigantea*, threonine and arginine in *A. caesia* and lysine and phenylalanine in *A. ficulneus*.

INTRODUCTION

In our survey of newer oilseeds for augmenting oil and protein resources we have come across *Calotropis gigantea* Linn. (Asclepiadaceae), *Acacia caesia* Willd. syn. *A. intsia* (Leguminosae) and *Abelmoschus ficulneus* Wight and Arn., syn. *Hibiscus ficulneus* Linn. (Malvaceae). Various parts of *C. gigantea*, a shrub, are used in indigenous medicine (1). The bark of *A. caesia*, a climber, is used as shampoo in rural areas (2). *A. ficulneus*, a shrub, yields white fiber useful for twine and light cordage. The mucilaginous extract of green stem is an efficient clarifier of sugar cane juice (3). The characteristics of the seeds and the extracted oils of these three plants as well as fatty acid and amino acid compositions are reported here.

EXPERIMENTAL

Materials

C. gigantea seeds were collected from the Laboratory campus. *A. caesia* seeds were purchased from Pocha Seeds Pvt. Ltd., Pune, India. *A. ficulneus* seeds were collected from West Godavari District, Andhra Pradesh, India.

Methods

Official and Tentative Methods of the American Oil Chemists' Society were followed for determination of moisture, oil, protein and ash contents of the seeds and physicochemical characteristics of the oils (4). Oil was obtained by cold extraction with a chloroform/methanol (2:1, v/v) mixture, purified according to Folch procedure (5) and used for analysis. The oil was treated with diazomethane to esterify the free fatty acids and then transesterified with methanol containing 1.0% sodium methoxide according to the procedure described by Schneider et al. (6). The methyl esters were purified on 0.8-mm layers of Silica Gel G using a mixture of petroleum ether (40–60 C) and peroxide-free diethyl ether (90:10, v/v).

The oils were examined for ultraviolet (UV) absorption in CCl_4 on a Beckman 26 UV-visible spectrophotometer and for infrared (IR) absorption as a liquid film on a Perkin-Elmer 221 spectrometer.

The oils as well as the methyl esters were qualitatively examined for the presence of hydroxy, epoxy and cyclopropene fatty acid (CFA) components by the sulfuric acid turbidity test (7), Fioriti's picric acid test (8) and the Halphen test (4), respectively.

The methyl esters of *A. ficulneus* seed oil responded positively to the Halphen test, hence they were treated according to the method of Schneider et al. (6), with anhydrous methanol saturated with silver nitrate for 20 hr at ambient temperature to convert cyclopropene fatty acids into stable ether and keto derivatives for analysis by gas liquid chromatography (GLC).

A Toshniwal gas chromatograph equipped with a flame ionization detector and a stainless steel column (2.4 m × 3.2 mm) packed with 10% EGSS-X on Gas Chrom Q, 80–100 mesh was used for the methyl esters of *C. gigantea* and *A. caesia* seed oils. The column temperature was maintained at 200 C and the flow rate of the carrier gas at 40 mL/min. Peak areas were calculated by multiplying peak height with width at half height. The methyl esters of *A. ficulneus* seed oil containing ether and keto derivatives of cyclopropene fatty acids present in small concentrations were analyzed in a Hewlett-Packard 5840 A gas chromatograph fitted with a flame ionization detector and a data processor. A glass column (1.8 m × 6 mm) packed with 10% DEGS on chromosorb W, HP, was used at 190 C. The nitrogen flow rate was maintained at 30 mL/min. Since the methyl esters of linoleic and dihydrosterculic acids present in *A. ficulneus* oil were eluted together, the latter was estimated separately by fractionating the methanolic silver nitrate-treated esters on 0.8-mm layers of Silica Gel G containing 9% silver nitrate using a mixture of petroleum ether and peroxide-free diethyl ether (90:10, v/v) as developing solvent and analyzing by GLC the saturated ester fraction containing the dihydrosterculic acid.

The methyl esters of *Sterculia foetida* seed oil were treated with methanolic silver nitrate and the resultant mixed esters containing ether and keto derivatives of CFA (sterculic and malvalic) were used as reference standards in GLC. Sterculic acid was isolated from the mixed fatty acids of *S. foetida* seed oil by repeated urea adductions followed by crystallization at −50 C of the nonadducted acids in methanol as described by Nunn (9). Sterculic acid was esterified with methanol and hydrogenated over Pd/C for use as reference compound for identifiying dihydrosterculic acid ester in GLC.

Proteins were extracted from the defatted seeds with alkaline 10% brine and precipitated by acidification (10). The amino acid composition was determined according to Hirs (11). The proteins (5–10 mg) were treated with 0.2 mL of a mixture of hydrogen peroxide (30%) and formic acid (5:95, v/v) and kept for 1 hr at 4 C. Cold water (2 mL) was added and the mixture was immediately lyophilized. Hydrochloric acid, 6 N (0.2 mL) was added and the tube was sealed under vacuum. After hydrolysis for 24 hr at 105 C, hydrochloric acid was removed under vacuum in a desiccator. The residue was dissolved in 0.2 N citrate buffer (pH 2.2) and analyzed using a Beckman Model 119 C Amino Acid Analyzer.

RESULTS AND DISCUSSION

The characteristics of the seeds and oils are given in Table I.

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TABLE I

Physicochemical Characteristics of *C. gigantea*, *A. caesia* and *A. ficulneus* Seeds and Oils

	C. gigantea	*A. caesia*	*A. ficulneus*
Seed			
Wt of 100 seeds (g)	0.8	26.2	1.5
Volume of 100 seeds (mL)	0.4	20.0	1.5
Moisture (%)	7.5	5.1	6.5
Oil (%[a])	30.8	8.8	14.4
Protein (%[a])	19.0	11.7	20.0
Ash (%[a])	4.6	2.0	2.7
Oil			
Refractive index at 25 C	1.4639	1.4717	1.4587
Acid value	26.0	1.9	0.1
Saponification value	191.3	188.3	187.2
Iodine value	89.3	133.0	113.8
Unsaponifiable matter (%)	1.2	1.8	1.3

[a]Dry basis.

The oil content in *C. gigantea* seed was appreciably higher than in the other two seeds. The protein content is almost the same in *C. gigantea* and *A. ficulneus* seeds while it is lower in *A. caesia* than in the other two.

The turbidity and Fioriti tests as well as IR spectra showed the absence of hydroxy and epoxy fatty acids in the seed oils. The UV spectra showed no conjugated unsaturation and IR spectra showed no *trans* unsaturation. The Halphen test showed the presence of cyclopropene fatty acids in *A. ficulneus* seed oil. The argentation TLC of methyl esters showed only the saturates, *cis* monoenes and *cis, cis* dienes in *C. gigantea* and *A. caesia* and also traces of trienes in *A. ficulneus*.

Oleic and linoleic acids together accounted for 74, 83 and 65%, respectively, in seed oils of *C. gigantea, A. caesia* and *A. ficulneus* (Table II). *A caesia* seed oil was rich in linoleic acid (69.7%). The GLC analysis of *A. ficulneus* esters after methanolic silver nitrate treatment showed the presence, ca. 0.3% each, of malvalic and sterculic acids. The GLC chart showed no peaks for the minor keto derivatives and degradation products of CFA since the concentrations of CFA are low. A correction factor of 1.219 was applied to ether derivatives of CFA since these amounted to 82% of CFA according to Schneider et al. (6). No response factors were determined, hence area percentages were given. Although Schneider et al. recommended enrichment of CFA esters by passage through an alumina column for oils containing low concentrations (<5%) of CFA, this was not often done (12–15), perhaps due to the degradation of CFA. The GLC analysis of the saturated ester fraction obtained by argentation TLC showed the presence of 2.7% dihydrosterculic acid.

The data in Table III show that the major essential amino acids in the seed proteins were phenylalanine, lysine and histidine in *C. gigantea*, threonine and arginine in *A. caesia* and lysine and phenylalanine in *A. ficulneus*. The high contents of threonine (29.8%) in *A. caesia* and of glutamic acid (23.4% and 17.3%, respectively) in *C. gigantea* and *A. ficulneus* are noteworthy. The potential availability and utilization of oil and protein of these three seeds deserve to be examined.

TABLE II

Fatty Acid Composition (Area %) of *C. gigantea, A. caesia* and *A. ficulneus* Seed Oils

Fatty acid	*C. gigantea*	*A. caesia*	*A. ficulneus*
14:0	0.0	0.0	0.2
16:0	16.7	9.5	26.5
16:1	0.0	0.0	0.8
18:0	9.0	7.5	3.3
18:1	47.2	13.3	23.2
18:2	27.1	69.7	41.7
18:3	0.0	0.0	0.4
20:0	0.0	0.0	0.6
Malvalic	0.0	0.0	0.3
Sterculic	0.0	0.0	0.3
Dihydrosterculic	0.0	0.0	2.7

TABLE III

Amino Acid Composition (Mol %) of *C. gigantea, A. caesia* and *A. ficulneus* Seed Proteins

Amino acid	*C. gigantea*	*A. caesia*	*A. ficulneus*
Cystine	0.0	0.0	1.8
Aspartic acid	9.9	7.8	10.8
Methionine	1.5	0.7	1.5
Threonine	3.1	29.8	2.8
Serine	4.8	3.8	2.9
Glutamic acid	23.4	11.9	17.3
Proline	7.9	3.0	12.3
Glycine	6.4	3.5	6.0
Alanine	4.5	3.3	3.7
Valine	4.6	3.1	5.9
Isoleucine	4.4	3.0	3.4
Leucine	6.6	5.5	6.5
Tyrosine	0.0	0.0	1.0
Phenylalanine	5.0	3.4	6.8
Histidine	4.1	2.6	1.3
Lysine	4.2	3.6	7.1
Arginine	9.6	15.0	8.9

ACKNOWLEDGMENTS

CSIR awarded Junior Research Fellowship to KSR. G. Narasimha Rao, Botany Department, Osmania University, Hyderabad, identified *A. ficulneus* plant. N. Sitaram, Centre for Cellular and Molecular Biology, Hyderabad, assisted in amino acid analysis.

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[Received August 25, 1982]

⁂Phospholipid Composition of Some Plant Oils at Different Stages of Refining, Measured by the Iatroscan-Chromarod Method

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ABSTRACT

Although the phospholipid composition of crude plant oils has been well studied, not much is known about the effect of the different refining processes on the individual phospholipids. This information is useful to the manufacturer to optimize the refining process. In this study corn, sunflower seed and peanut oils, at different stages of refining, were analyzed with the Iatroscan-chromarod method. The total phosphorus content of the samples was also determined with a classical method. The Iatroscan gave results of acceptable accuracy for the analysis of crude oils with phospholipid-phosphorus values between 145 and 536 ppm. However, for oils at further stages of refining, with phospholipid-phosphorus values between 1 and 10 ppm, less accurate results were obtained. For these oils, the Iatroscan results had to be supported by conventional thin layer chromatography. Degummed oils contained phosphatidylcholine (1.1–22 ppm P), phosphatidylethanolamine (1–2 ppm P), phosphatidylinositol (trace–10 ppm P) and phosphatidic acid (trace–5 ppm P). Further refined oils contained no phospholipids with the exception of two samples. Bleached sunflower oil contained about 1 ppm phosphatidylinositol and bleached peanut oil contained ca. 1 ppm phosphatidylethanolamine and 1 ppm phosphatidylcholine. Fully refined edible oils contained no phospholipids.

INTRODUCTION

Phospholipids occur in crude plant oils at levels ranging between 0.1 and 1.8% (1). The composition and characteristics of phospholipids in crude oils have been thoroughly studied (1, 2); however, very little is known about the phospholipid composition of oils at different stages of refining. Some phospholipids act as antioxidant synergists with the tocopherols in plant oils (1, 3, 4), but the general practice is to remove the phospholipids from the oils prior to neutralization (alkali refining) or steam distillation (physical refining (5).

Phospholipids cause losses of neutral oil during neutralization (6, 7) and their presence leads to oil discoloration during deodorization and steam distillation (5).

The phospholipids are largely removed from the crude oils by water or acid degumming. This process reduces the phospholipid content of sunflower seed oil about eight times, and that of soy oil 70 times (3). The total phosphorus content of oils is monitored to give a general indication of phospholipid removal.

Very little is known about the effect of degumming and the successive refining steps on the individual phospholipids. The main reason for the lack of information is the unavailability of suitably sensitive methods for phospholipid analysis in oils.

High performance liquid chromatography offers a limited solution. Ultraviolet detection at 200–210 nm proved to be useful for qualitative evaluation in our laboratory (Y. Totani, personal communication), but not for quantitative analysis of phospholipids (8). In this investigation, the Iatroscan thin layer rod technique was used to study the phospholipid changes in oils that underwent alkali and physical refining.

EXPERIMENTAL PROCEDURES

Instruments and Materials

The Iatroscan TH 10 TLC analyzer Mk III (Newman-Howells Ass. Ltd., England) was used, coupled to a Hewlett Packard 3390A integrator. Hydrogen flow rate was 175 mL/min and air flow rate 1,850 mL/min. Chromarods SII were used for separations. Merck (Darmstadt) silica gel 60 TLC plates (without fluorescence indicator, Art. 5721); silica gel 60 for column chromatography (70-230 mesh, Art. 7734); AR grade chloroform, methanol, acetone and glacial acetic acid were used. Tertiary butylhydroquinone (TBHQ) was supplied by Eastman Products (Kingsport, TN).

The following phospholipid standards were obtained from Sigma Chemical Co. (St. Louis, MO): L-α-phosphatidic acid, sodium salt (PA) no. P9511, L-α-phosphatidyl-