Two-Stage Solvent Extraction of Seeds of *Hibiscus abelmoschus* Linn: Lipid and FA Compositions

D. Sahoo, K.S. Jena, P.K. Rout, and Y.R. Rao*

Regional Research Laboratory, Council of Scientific and Industrial Research, Bhubaneswar, Orissa 751013 India

ABSTRACT: The lipid and FA compositions of TAG seed oil of ambrette (*Hibiscus abelmoschus*), contrary to earlier reports, were found to contain only a small amount of epoxy FA. Higher HBr absorption values were shown to be due to the presence of fragrance components of the seed coat in the oil derived from intact whole seeds. Cyclopropene/cyclopropane acids in the FAME were determined to be about 1.5% by GLC, while epoxy acids were less than 1% by two different methods. Based on the physicochemical characteristics and lipid and FA compositions of the fresh and methanol-extracted seeds, ambrette TAG oil may be a candidate for edible use. The process of selective recovery of the expensive fragrant oily concentrate in higher yield in a first step and of an edible fatty oil in a second step makes it an attractive economic proposal.

Paper no. J10313 in *JAOCS 80,* 209–211 (March 2003).

KEY WORDS: Ambrette seed, *Hibiscus abelmoschus,* lipid and fatty acid composition, Malvaceae, selective extraction.

Hibiscus abelmoschus L. syn. *Abelmoschus moschatus,* Moerich of family Malvaceae, trivial name ambrette, is native to India (1). It is cultivated in the tropical regions of Asia, Africa, and South America for its seeds, which have a characteristic musky odor. The seeds contain about 16% of fatty oil. At present, the seeds are utilized only for the isolation of fragrance components, and the bulk of the TAG oil is wasted. Nee *et al.* (2) have shown that the essential (fragrant) oil is localized in the outer layers of the seed coat; it is practically absent in the embryo and the endosperm where the TAG oil is concentrated. These authors also studied the FA composition of the oil and did not report the presence of epoxy FA.

Hopkins and Chisholm (3) first investigated whole seed oil and reported the presence of about 4% epoxy oleic acids determined by the HBr titration method. Hashmi *et al.* (4) reported abnormally high values of epoxy and cyclopropene acids (6.4 and 14.4%, respectively). The seed oil has not received any further attention, as the seeds are utilized to a limited extent only for the extraction of the whole oil for fragrance use. With the widening gap between supply and demand of edible and industrial fatty oils, we have examined ambrette as a nontraditional edible oil seed. Lower economic returns on cultivation of traditional crops under rain-fed conditions was the other consideration in examining ambrette as an alternative crop.

Based on the results of Nee *et al.* (2), we decided to explore methods of obtaining the fragrance components and fatty oil separately. After making several unsuccessful attempts to separate the seed coat and embryo mechanically, we decided to try selective extraction of the whole seeds with polar solvents and found that the fragrance components were selectively extracted out. From the oleoresin obtained, a fragrant oily concentrate, free from fatty oils, was obtained in 0.3 to 0.35% yield. The physicochemical characteristics and FA and lipid compositions of the TAG oil obtained from the raffinate (selectively extracted) seeds, as well as from fresh seeds, are reported here. Contrary to the earlier reports, hexane-extracted ambrette fatty oil is found to contain less than 1% of epoxy FA and 1.5% of cyclopropane acids. Although the oil gives a positive Halphen test, methyl dihydrosterculate was the only unusual FAME detected in the GLC analysis of ambrette oil FAME.

EXPERIMENTAL PROCEDURES

The seeds required for the study were obtained from plants raised in the Aromatic and Medicinal Plants garden of the laboratory as well as those supplied by Global Herbs (New Delhi, India). Reagent-grade methanol and hexane were distilled before use. The whole seeds were first extracted by stirring with methanol in two stages, by which the fragrant oil present in the seed coat was removed.

Fresh as well as methanol-extracted seeds were flaked in a roller, and the flakes were extracted with hexane and chloroform/methanol (2:1) sequentially following published methods (5). The physicochemical characteristics of the hexane-soluble oil as well as total lipids content were determined by standard procedures (6,7) and are presented in Table 1. The oils gave positive Halphen tests. HBr titer values were determined at 27°C by an indirect HBr method according to Critchfield (8). Epoxy determination was carried out by a nonaqueous titration in pyridine (8) and gravimetrically through preparative TLC of the FAME. The hexane-soluble oil and the total lipids were separated into neutral, glyco-, and phospholipids by column chromatography on silica gel (9). Results are summarized in Table 2. The phospholipid was further separated on TLC, and the constituent lipids were identified following the literature procedure (5). The data given in

*To whom correspondence should be addressed at Forest and Marine Products Division, Regional Research Laboratory, Bhubaneswar, Orissa 751013, India. E-mail: yerramillirrao@yahoo.com

TABLE 1
Physicochemical Characteristics of the Hexane-Soluble Oil of Ambrette Seeds

Property	Raw seed oil	Methanol-extracted seed oil
Color and taste	Clear golden yellow liquid with a light odor of musk	Clear golden yellow liquid with a characteristic odor but without musk odor.
Density	0.907	0.904
Refractive index at 30°C	1.4750	1.4750
Acid value	2.7	1.3
FFA	1.3%	0.65%
Saponification value	193.7	193.4
Iodine value	92.5	92.3
Oxirane value (HBr method)	0.34	0.23
Oxirane value (pyridine hydrochloride method)	0.031	0.024
Epoxy fatty ester by preparative TLC of FAME	0.75	0.75
Unsaponifiable matter	1.8%	1.4%

TABLE 2
Lipid Composition[a] of Ambrette Seed Oils

Lipid/fat	Neutral lipid (%)	Glycolipid (%)	Phospholipid (%)
Total lipid (raw seed) (18.5%)	80.2	5.0	14.8
Total lipid (methanol-extracted seed) (16.7%)	81.8	4.1	14.1
Hexane extract (raw seed) (15.6%)	90.3	6.7	2.9
Hexane extract (methanol-extracted seed) (14%)	89.8	6.7	3.0
Total lipid (hexane-extracted seed flakes) (3.5%)	44.1	20.7	35.2

[a]Data represent average values (n = 3), with a variation of ±2%.

the tables are the average values with a variation of ±2% obtained from triplicate experiments. Due to the presence of epoxy FA in the oil, mineral acid-catalyzed transesterification was not used. These lipid fractions were saponified with alcoholic alkali, and after careful neutralization, the extracted FA were esterified with diazomethane. GC of FAME was carried out on a Shimadzu GC-17A gas chromatograph fitted with a 30 m × 0.25 mm WCOT capillary column coated with 0.25 µm film thickness of SUPELCOWAX 10 (Supelco, Bellefonte, PA), equivalent to CARBOWAX 20M; an FID detector; and a CR-6A data processor (Shimadzu). The GC analysis was carried out by the following temperature program: Initial temperature 160°C; increase at 2°C/min to 230°C; increase at 4°C to 250°C, hold for 5 min (total run time 45 min). The samples were then analyzed following the same program on a Shimadzu QP 5000 GC–MS instrument, and the peaks were identified by comparison of their mass spectra with spectra available in NIST library and by retention order. Quantification of peaks in the gas chromatogram was done by peak normalization method. The FA composition of the ambrette seed oil fractions is given in Table 3.

RESULTS AND DISCUSSION

We noted that the fatty oil extracted from raw seeds gave a higher HBr titer (0.34% oxirane value at 27°C) than oil extracted from methanol-extracted seeds (0.23% oxirane value). TLC of the oil as well as of its FAME on silica gel plates did not give spots corresponding to 12,13-epoxy oleic acid in TAG (R_f 0.43) or as the methyl ester (0.56), respectively (5). However, a spot with R_f 0.70 for oil and at R_f 0.67 for methyl esters gave a positive picric acid test characteristic of epoxy FA (10). Preparative TLC of the ambrette methyl esters gave only 0.75% of the epoxy compound. Determination of oxirane oxygen following nonaqueous titration in pyridine (8) showed only 0.03% of epoxy value equivalent to 0.56% vernolic acid, confirming the preparative TLC result. The GC analysis of FAME also did not show the presence of epoxy fatty esters, in agreement with the

TABLE 3
FA Composition (wt%) of Different Lipid Fractions of Ambrette Seeds[a]

	Total lipid (raw seed)			Hexane-soluble oil (raw seed)			Hexane-soluble oil (methanol-extracted seed)		
FA	NL	GL	PL	NL	GL	PL	NL	GL	PL
14:0	—	2.8	—	—	3.5	—	—	—	—
16:0	21.8	25.9	39.5	20.7	36.8	41.3	23.5	29.3	27.3
18:0	5.2	3.7	5.6	5.5	5.9	9.3	5.1	4.7	11.8
18:1	26.7	15.1	14.5	26.1	26.5	33.8	26.3	17.9	28.2
18:2	33.7	28.9	28.6	39.4	18.6	5.0	40.5	33.3	29.1
19:0 (CP)	2.7	2.3	1.7	1.5	1.4	3.0	1.6	1.4	1.4
20:0	0.4	0.6	0.6	0.3	0.3	—	0.3	0.3	—
22:1	2.0	2.4	0.1	0.2	2.4	1.2	0.5	1.7	0.2

[a]NL, neutral lipid; GL, glycolipid; PL, phospholipid; CP, cyclopropane acid; —, not detected. Data are average values (n = 3), with a variation of ±2%.

earlier report (2). GC–MS of the isolated epoxy ester showed it to be 9,10-epoxy stearic acid. Similarly, the mass spectrum of the peak corresponding to the cyclopropane acid in the GC–MS of the FAME was comparable to that of methyl dihydrosterculate $M^+ = 310$. The hexane-extracted oil contained about 90% neutral lipid. The phospholipid portion was analyzed by TLC and contained PC, PS, PI, and also digalactosyl diglyceride. The lipid and FA compositions and iodine value of the hexane-soluble oil make ambrette seed oil an excellent candidate for consideration as edible oil. The process of selective recovery in higher yield of the expensive fragrant oily concentrate and separate recovery of the fatty oil makes it an attractive economic proposal.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Vibhuti N. Misra, Director, Regional Research Laboratory, Bhubaneswar, India for permission to publish this work and to Technology Mission on Oil Seeds, Pulses, and Maize, Ministry of Agriculture, Government of India for a financial grant.

REFERENCES

1. Anon., *The Wealth of India: Raw Materials*, CSIR, New Delhi, 1959, pp. 75–77.
2. Nee, T.Y., S. Cartt, and M.R. Pollard, Seed Coat Components of *Hibiscus abelmoschus*, *Phytochemistry 25*:2157–2161 (1986).
3. Hopkins, C.Y., and M.J. Chisholm, Epoxy Acid in Seed Oils of Malvaceae and Preparation of (+) *threo*-12,13-Dihydroxyoleic Acid, *J. Am. Oil. Chem. Soc. 37*:682–684 (1960).
4. Hashmi, M., M. Khan, M.S. Ahmad Jr., S. Ahmad, and S.M. Osman, *Abelmoschus moschatus* Seed Oil: A Re-investigation, *J. Oil Technol. Assoc. India* (April–June):64–65 (1982).
5. Hammond, E.N., *Chromatography for the Analysis of Lipids*, CRC Press, Ann Arbor, 1993, pp. 21-63 and 65-11.
6. IS:548, *Methods of Sampling and Test for Oils and Fats (sampling physical and chemical tests)*, Bureau of Indian Standards, Bahadur Shah Zafar Marg, New Delhi, 1986, pp. 29–52.
7. *Official Methods of Analysis of the AOAC*, edited by W. Horwitz, P. Chichilo, and H. Reynolds, Association of Official Analytical Chemists, Washington, DC, 1970.
8. Critchfield, F.E., *Organic Functional Group Analysis*, Pergamon Press, 1963, pp. 131–136.
9. Hemavathy, J., and J. Prabhakar, Lipid Composition of *Calophyllum inophyllum Kernels*, *J. Am. Oil. Chem. Soc. 67*: 955–957 (1990).
10. Fioriti, J.A., and J.R. Sims, A Spray Reagent for the Identification of Epoxides on Thin Layer Plates, *J. Chromatogr. 32*: 761–763 (1968).

[Received April 29, 2002; accepted December 9, 2002]