Lipids of Guar Seed Meal (*Cyamopsis tetragonoloba* L. Taub)

Satya P. Singh* and Basant K. Misra

The lipids (7% by weight) of guar seed meal, a waste product of the guar gum industry, have been separated and identified by thin-layer chromatographic techniques. Physical and chemical constants of the oil were determined by conventional methods, and the fatty acid composition was determined by gas–liquid chromatography with 24% saturated acid, 29% oleic acid, and 47% linolic acid. The fatty acid composition is generally similar to that of common edible oils.

For a long time guar (*Cyamopsis tetragonoloba* L. Taub) has been grown in the Indian subcontinent for use mainly as fodder and to a small extent as a green vegetable. During the last two decades guar has drawn the attention of researchers and technologists as a source of gum which is a significant foreign-exchange earner. In spite of several attributes, the future of the gum industries depends to a greater extent on the utilization of guar seed meal which remains a waste because of the presence of several toxic and foul-smelling components (Couch et al., 1966; Harborne, 1967; Bajaj et al., 1978).

Guar seed meal contains 45–55% protein and 5–7% lipids (Subramanian and Parpia, 1975). Guar oil may have value in the pharmaceutical and food processing industries.

EXPERIMENTAL SECTION

Fresh guar seed meal was purchased through a stockist (Delhi area) of gum industries in two lots of 2 kg each. The foreign material like small pieces of endosperm (~0.3%) was removed by hand picking. Both the lots were made moisture (2–3.5%) free and mixed together. Seed meal was finely powdered (80 mesh) and stored in glass bottles at room temperature. It was divided into two groups for analysis.

Total Lipid Extract. Total lipids were extracted from powdered meal by a mixture of chloroform, methanol, and water (Weber, 1969) by using the cold percolation technique (Kartha and Sethi, 1957). The total extract was dried over anhydrous sodium sulfate and filtered. The extract was evaporated nearly to dryness under vacuum. After the lipids had been redissolved in a known volume of chloroform–methanol–water (86:14:1) and filtered, two aliquots of 1 mL each were dried to a constant weight for the determination of total lipid content.

Refining of Lipids. The crude lipid obtained by the cold percolation method was refined by washing with 1% potassium hydroxide and finally with distilled water several times. The refining removed free fatty acids as water-soluble potassium soaps. The refined portion was dried over anhydrous sodium sulfate, the solvent distilled off under vacuum, and the lipid weighed to constant weight.

The hydrolysis of refined lipid was achieved by refluxing with alcoholic potassium hydroxide solution for 1 h. Ethanol was removed, and dried soap taken up in water, acidified with 1:1 sulfuric acid, and warmed to melt. The fatty acids were extracted with solvent ether and subjected to alkali washings in order to separate unsaponifiable matter.

Mixed Fatty Acids. From the pooled alkali washings as obtained above, the mixed fatty acids were regenerated and extracted as described by Singh (1975).

Division of Biochemistry, Indian Agricultural Research Institute, New Delhi-110012, India.

Table I. Column Chromatography of Guar Meal Lipids

lipid class	wt, %[a]
(1) hydrocarbons sterol esters	1.1
(2) triacylglycerols	94.6
(3) free fatty acids	1.0
(4) sterols	0.9
(5) partial acylglycerols	1.8
(6) polar lipids	0.5

[a] Results are the mean of duplicate tests.

Thin-Layer Chromatography (TLC). The separation of lipid extract was carried out essentially according to the method of Malins and Mangold (1960). The lipid samples were spotted on a standard 20 × 20 cm plate which was developed by using a petroleum ether–diethyl ether–glacial acetic acid (80:20:1 v/v) solvent system. After the plate was dried in a stream of nitrogen, the spots were visualized by spraying the plate with 50% sulfuric acid, followed by charring at 200 °C for 20 min. The triacylglycerol, free fatty acid, and phospholipid components were identified by comparison of R_f values against those of authentic reference samples of tripalmitin, palmitic acid, and lecithin.

Column Chromatography Followed by Thin-Layer Chromatography. The total lipid extract was fractionated on silicic acid column (15 mm i.d.). The silicic acid (100 mesh) specially prepared for chromatographic analysis was washed with water and methanol to remove fines and impurities and then activated at 120 °C overnight and again for 1 h immediately before the column was prepared. For each column 20 g of silicic acid was required and 300 mg of lipids was applied. The solvent system used was the same as that described by Hirsch and Ahrens (1958) and Weber (1970).

Sixty milliliters of 4% diethyl ether in petroleum ether eluted the hydrocarbons and sterol esters. A larger quantity (600 mL) of the same solvent mixture extracted triacylglycerols and an additional 50 mL extracted free fatty acids. Sterols were eluted with 250 mL of chloroform and partial acylglycerides with an additional 350 mL of chloroform. The polar lipids were removed from the column with 300 mL of methanol. The lipid classes were checked and purified further, if necessary, by thin-layer chromatography (TLC). These plates were developed by using following system(s): (1) petroleum ether–diethyl ether–acetic acid (80:20:1 v/v) as described by Mangold (1961) and/or (2) double-development technique using diethyl ether–benzene–ethanol–acetic acid (40:50:2:0.2 v/v) and petroleum ether–diethyl ether–acetic acid (90:10:1 v/v) as described by Freeman and West (1966) and/or (3) benzene–methanol–acetic acid (98:2:1 v/v) as adopted by Yoshida and Kajimoto (1972).

The spots were located either by exposing the plate with iodine vapor or spraying them with 50% sulfuric acid followed by charring at 200 °C for 20 min and/or by spraying with 50% phosphomolybdic acid followed by heating.

0021-8561/81/1429-0907$01.25/0

Table II. Physical and Chemical Constants of Guar Meal Oil and Other Edible Oils[a]

source of oil	sp gravity at 30 °C	refractive index at 40 °C	saponification value	iodine value	unsaponifiable matter, %	acid value	Bellers' turbidity test	hydroxyl value
guar meal[a]	0.914	1.4680	205	108	2.0	3.0	36	20.3
groundnut[b] (Gr. I[c] edible)	0.913	1.4640	195	98	1.0	2.0	41	
cottonseed[b] (refined edible)	0.920	1.4660	198	112	1.5	0.5		

[a] Results are the mean of duplicate tests. [b] Upper values of the limit are shown here. [c] Gr. I = best quality.

Table III. Fatty Acid Composition of Lipid Extract and Free Fatty Acid Pool of Guar Seed Meal

fatty acid	composition, wt %: lipid extract (refined)	free fatty acid pool
14:0	Tr[a]	Tr
16:0	17.9	20.4
18:0	5.8	4.6
18:1	29.0	32.4
18:2	47.2	42.5
18:3	nil	Tr

[a] Tr = traces.

Physical Characteristics and Oil Constants. Since triacylglycerols are the preponderant lipids in the guar seed meal like other seed oils, the use of common term "oil" in place of lipid has been made in the text. Specific gravity and the turbidity point of the oil were examined by using conventional standard laboratory procedures (Kirschenbauer, 1960). The hydroxyl value was calculated from the acetyl value determined by the modified method (Mathur, 1966) of Kartha and Mathur (1963). Free fatty acids were estimated by titrating the lipid solution with 0.01 N alcoholic potassium hydroxide solution; saponification value, acid value, and iodine value were determined by conventional AOAC (1955) methods. The refractive index was measured with an Abbe refrectometer; the content of unsaponifiable matter was computed in the manner as described by Singh (1975).

Gas–Liquid Chromatography (GLC). The methyl esters of both free and mixed fatty acids of oil were prepared essentially according to the method of Morrison and Smith (1964). For the analysis of fatty acid methyl esters, an F & M 1609 Model gas chromatograph equipped with a flame ionization detector (FID) and with a column of stainless steel ($^1/_4$ in. o.d. × 8 ft) was used. It was packed with 5% ethylene glycol succinate on acid-washed 60/80 Chromosorb W. Nitrogen at a flow rate of 6000 mL/h was used as the carrier gas. The temperature of the column and injection port was maintained at 200 and 300 °C, respectively. The detector temperature was also 300 °C. The fatty acids were identified by comparing the retention times with those of reference standards. Gas chromatographic peak areas were determined by multiplying peak height by width at half-height.

Colorimetric Estimations. (1) Acylglycerol content was reexamined by estimating it colorimetrically (Boekenoogen, 1968). (2) The presence of phospholipid was also ascertained by 1-amino-2-naphthol-4-sulfonic acid–molybdate color reagent (Fiske and Subbarow, 1925).

Infrared Spectroscopy. The O–H stretching vibration at 3 μm was measured on a Perkin-Elmer Model 457 infrared spectrometer to confirm presence of free hydroxyl groups in the oil sample.

RESULTS AND DISCUSSION

Guar seed meal contains 7% lipids by chloroform–methanol–water extraction. Seven spots were visible on the plate. The dominant peak was identified as triacylglycerol. Other peaks correspond to partial acylglycerols, free fatty acids, and phospholipids. Higher polar lipids remain at the base line in the TLC separation.

Table I lists components separated by column chromatography. Triacylglycerol (94.6%) is the largest fraction. Other fractions include hydrocarbon and sterol esters (1.1%), free fatty acids (1.0%), sterols (0.9%), partial acylglycerols (1.8%), and polar lipids (0.5%).

Table II compares physical and chemical constants of extracted guar meal oil against edible grades of peanut and cotton seed oils. The data for peanut and cotton seed oils are taken from the Indian Agricultural Produce Act of 1937 (1961). As expected, in comparison of crude and refined oils, guar oil is darker in color and higher in acid value than the other oils; other analytical constants are quite similar for all three oils.

The colorimetric estimation of acylglycerols and phospholipids confirms results obtained by column chromatography. The infrared spectroscopic analysis revealed the presence of a free hydroxyl group in this oil.

The fatty acid composition of the refined guar meal oil and that of the fatty acids separated from crude oil are quite similar (Table III). Guar oil contains traces of myristic acid (14:0), ~20% of palmitic acid (16:0), and ~5% of stearic acid (18:0) for a total of ~25% of saturated acids. The unsaturated components include ~29% oleic acid (18:1), ~47% linoleic acid (18:2), and traces of linolenic acid (18:3).

Guar oil has potential as an edible oil. Its linoleic acid content of 47% is close to that of common edible oils such as cotton seed oil, soybean oil, and corn oil where linoleic acid contents are generally in the 50% range (Neelakantan, 1973). Nearly 0.6 million ton of guar meal is available annually in India alone. Extraction could yield ~80 million pounds of oil which could supplement nutritional requirements now being met by conventional oils, specially peanut, cottonseed, and rapeseed oils.

LITERATURE CITED

AOAC "Official Methods of Analysis", 8th ed.; Association of Official Agricultural Chemists: Washington, DC, 1955.
Bajaj, K. L.; Madhu Rani; Arora, J. K. *Plant Biochem. J.* **1978**, *5* (2), 140.
Boekengoon, H. A. "Analysis and Characterisation of Oils, Fats and Fat Products"; Interscience: New York, 1968; Vol. 2.
Couch, J. R.; Cregar, C. R.; Bakshi, Y. K. *Proc. Soc. Exp. Biol. Med.* **1966**, *123*, 263.
Fiske, C. H.; Subbarow, Y. *J. Biol. Chem.* **1925**, *66*, 375.
Freeman, C. P.; West, D. J. *J. Lipid Res.* **1966**, *7*, 324.
Harborne, J. B. "Comparative Biochemistry of Flavonoids"; Academic Press: London, 1967.
Hirsch, J.; Ahrens, E. H., Jr. *J. Biol. Chem.* **1958**, *233*, 311.
Indian Agricultural Produce (Grading and Marketing) Act of 1937, 4th ed.; Government of India Press: Delhi-110008, India, 1961; Schedules V and XII.
Kartha, A. R. S.; Mathur, J. M. S. *Indian J. Chem.* **1963**, *1*, 494.
Kartha, A. R. S.; Sethi, A. S. *Indian J. Agric. Sci.* **1957**, *27*, 211.
Kirschenbauer, H. G. "Oils and Fats", 2nd ed.; Chapman and Hall: London, 1960; Chapter V, p 55.

Malins, D. C.; Mangold, H. K. *J. Am. Oil Chem. Soc.* **1960**, *37*, 576.
Mangold, H. K. *J. Am. Oil Chem. Soc.* **1961**, *38*, 708.
Morrison, V. R.; Smith, L. M. *J. Lipid Res.* **1964**, *5*, 600.
Mathur, J. M. S. *Curr. Sci.* **1966**, *3*, 203.
Neelkantan, K. A. *Sci. Today* **1973**, *Nov*, 34.
Singh, S. P. Doctoral Thesis, Meerut University, Meerut, India, 1975.
Subramanian, N.; Parpia, H. A. *PAG Compendium* **1975**, *D*, 215.
Weber, E. J. *J. Am. Oil Chem. Soc.* **1969**, *46*, 485.
Weber, E. J. *J. Am. Oil Chem. Soc.* **1970**, *47*, 340.
Yoshida, H.; Kajimoto, G. *Nippon Nogei Kagaku Kaishi* **1972**, *46*, 625.

Received for review October 3, 1980. Revised manuscript received April 27, 1981. Accepted May 14, 1981.

Composition of the Essential Oil of Soiling Dent Corn

Takayasu Kami

The essential oil of soiling dent corn was isolated by steam distillation of the fresh grass with a yield of 0.0031%. The essential oil was analyzed by combined gas chromatography–mass spectrometry and gas chromatographic comparison with authentic specimens, and 90 compounds consisting of hydrocarbons, aldehydes, ketones, alcohols, esters, acids, phenols, and miscellaneous were identified. Quantitative analysis was further carried out on the essential oil, and soiling dent corn oil was relatively rich in hydrocarbons and aldehydes.

For clarification of the connections between the aromas of forage crops and their palatability for domestic animals, a series of studies on the aromatic constituents of forage crops have been carried out. In the present work, the essential oil of soiling dent corn was analyzed by means of combined gas chromatography–mass spectrometry and gas chromatography, following that of the previous papers (Kami, 1975, 1977, 1978) on the analyses of the essential oils of Hybridsorgo, Sudangrass, and red and Ladino white clovers.

Since the corns (*Zea mays* L.) occupy a very important role in the feedstuffs of domestic animals, they are cultivated in a great extent all over the world. In Japan, similarly, the corns are the important forage crops and they are cultivated over the whole country from old times to the present. According to the classification from the starch of grain, dent species is especially applied to soilage and silage of dairy cattle, owing to the good adaptability, the high nutrition, and the abundant yield.

EXPERIMENTAL SECTION

Materials. Soiling dent corn (Nagano No. 1, early variety) was cultivated on a farm of the Faculty of Applied Biological Science, Hiroshima University, and the aerial parts were harvested in Oct 1977 by mower. The harvest time corresponded to the milky stage of the seed of dent corn.

Isolation of the Essential Oil. The fresh aerial parts (220 kg), after chopping, were steam distilled in 28-kg lots for 1 h under 0.8 kg/cm^2 distillation kettle pressure, and yielded about 130 L of cloudy distillate in a water-cooled trap and 7.3 and 1.0 g of colorless aqueous condensates in ice–water-cooled and dry ice–methanol-cooled traps, respectively. After the cloudy distillate of the water-cooled trap was saturated with sodium chloride, 2-L lots were extracted twice with 300 mL of distilled diethyl ether in the same way as the previous paper (Kami, 1975) to yield a dark brown oil with a sweet silage-like odor (6.783 g). The essential oil was stored in a sealed glass tube at 3 °C, as were the aqueous condensates from the ice–water- and dry ice–methanol-cooled traps.

Fractionation of the Essential Oil. A portion (2360 mg) of the essential oil was sequentially extracted to separate acidic (517 mg), phenolic (382 mg), and basic (54 mg) fractions according to the procedure previously described (Kami, 1978). The remaining neutral oil layer (1400 mg) was extracted first in *n*-pentane and then in diethyl ether with silicic acid to separate it into nonpolar (185 mg) and polar (813 mg) fractions (Kami, 1977). Among them, the acidic fraction was further converted into the methyl esters with diazomethane (Vorbeck et al., 1961).

Analysis of the Essential Oil. In the beginning, the unfractionated essential oil was analyzed by combined gas chromatography–mass spectrometry (GC–MS). A Hitachi Model RMU-6MG mass spectrometer was used. The GC column was a 0.28 mm i.d. × 50 m glass capillary column coated with PEG 20M, and the column temperature was programmed from 50 to 180 °C at 2 °C/min. The carrier gas was helium. The mass spectrometer was worked at 70-eV ionizing voltage, 3200-V ion accelerating voltage, and 200 °C ion source temperature. In the next phase, the fractions of the essential oil, except for the basic fraction, and the unfractionated essential oil were analyzed by programmed temperature gas chromatography (GC). An FID-type Yanagimoto GCG-550T gas chromatograph was used. The GC column was a U-shaped 2.5 mm i.d. × 2.2 m glass column packed with 5% PEG 20M on 80–100-mesh Chromosorb W, and the column temperature was maintained at 48 °C for the first 5 min and then increased at a rate of 4 °C/min to 230 °C (helium flow rate, 13 mL/min; injection port temperature, 280 °C). The basic fraction was heated with a 2 N solution of sodium hydroxide, and the regenerated gases of amines were analyzed by isothermal GC with a triethanolamine column at 65 °C (Kami, 1975). For the analysis of low-boiling compounds, the headspace vapors in ice–water- and dry ice cooled traps were directly chromatographed by using the same procedure as previously reported (Kami et al., 1972).

Percentage Composition of the Essential Oil. The relative peak areas in GC of the unfractionated essential oil and the fractions of the essential oil were calculated

Faculty of Applied Biological Science, Hiroshima University, Fukuyama 720, Hiroshima, Japan (formerly Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Hiroshima, Japan).

0021-8561/81/1429-0909$01.25/0