Hybrid *Hibiscus* Seed Oil Compositions

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ABSTRACT: The seeds of cultivated *Hibiscus* spp. were extracted with supercritical carbon dioxide, and the resulting extracts were analyzed to identify the major TG components as the corresponding FAME. The seed oils were composed predominantly of oleic and linoleic FA (69.6–83.4%) with lesser amounts of palmitic acid (14.8–27.0%). Minor amounts of C14, C18, and C20 saturated FA were also detected. The oil content of the seeds was determined to be between 11.8 and 22.1 wt% for hybrid varieties and between 8.9 and 29.5 wt% for the native species from which the hybrid varieties were developed. The protein content of the defatted seed meal averaged 20% for the hybrid varieties. The composition of the extracted hibiscus seed oils suggests potential edible applications.

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KEY WORDS: Defatted meal, fatty acid composition, *Hibiscus*, oilseeds, triglycerides.

Members of the genus *Hibiscus* grow in diverse environments around the world and produce numerous natural products with both edible and industrial applications. The canes provide a source of fiber and crude protein. The flowers and green tissues of many species are edible. The roots and bark have been used in the practice of traditional medicine. Kenaf (*H. cannabinus*), for example, is cultivated and harvested in the United States as a fiber source for paper products and building materials (1–3). It has also been investigated as a forage crop and silage material for livestock feed (4,5). The large and colorful flowers produced by many species are prized by gardeners and cultivated as ornamental plants, with new varieties introduced regularly. However, the seeds are a potential source of TG, phytosterols, phospholipids, proteins, and other natural products that remain largely underutilized (6–9). In this work the seed oils were extracted from 16 cultivated hybrid and 35 native *Hibiscus* species and subsequently analyzed to determine the oil content, FA composition, and elemental composition of the defatted meal.

EXPERIMENTAL PROCEDURES

Seeds of *Abutilon hybridum*, *H. calyphyllus*, *H. dasycalyx*, *H. grandiflora, H. hamabo*, *H. laevis*, *H. martianus*, *H. moscheutos*, *H. mutabilis*, *H. striatus lambertianus*, *Malvaviscus drummondii*, *Pavonia hastata, P. lasiopetala*, *Sida spinoza*, and the related hybrids were obtained from The Village Botanica, Inc. (Waller, TX). The plants were grown on irrigated plots located in Waller, Texas. Seed pods were harvested in the fall of 2001, and the seeds were separated from the pods by hand. Seeds were allowed to air-dry for several weeks. Moisture determinations were made by heating seed samples in a convection oven for 2 h at 105°C and measuring the resulting weight loss. Whole seeds were mechanically ground to a nominal particle diameter of 0.1 mm and stored at −20°C prior to extraction.

Lipid components were extracted from ground seeds using supercritical carbon dioxide (SC-CO_2). Extraction vessels were filled with 5-g samples and extractions were performed at 80°C and 53.7 MPa. A 1-min static hold was followed by a 50-min dynamic extraction with carbon dioxide at a flow rate of 2.0 mL/min. Collection vials were maintained at 20°C throughout the extraction, and the collected fractions were stored under nitrogen at −20°C prior to analyses. Extractions were performed in duplicate.

A 100-mg aliquot of each collected lipid extract was converted to the corresponding FAME by reaction at 25°C with 1 mL 0.5 N sodium methoxide (Sigma-Aldrich, St. Louis, MO). The esters were recovered in hexane, diluted, and analyzed by GC–FID. Injection volumes of 1 μL were used with a 100:1 split ratio. Inlet and detector temperatures were maintained at 250°C. Separations were performed with an SP2380 capillary column (30 m × 0.25 mm i.d.; Supelco, Bellefonte, PA). Helium carrier gas was set at a flow rate of 1 mL/min, and the oven temperature was programmed to begin at 100°C, hold for 5 min, ramp to 190°C at 3°C/min, and then ramp to 250°C at 5°C/min. Data were collected and integrated with Chemstation Software (Agilent Technologies, Palo Alto, CA). Identification of compounds was made by comparison of retention times to authentic standards (Sigma-Aldrich). Analyses were replicated, and variation between replicates was less than 2% relative SD.

Elemental analysis of the defatted meal for carbon, hydrogen, and nitrogen was performed with a LECO Model CHN-2000. The instrument was calibrated prior to use, and samples were analyzed in duplicate. Replicate analyses agreed to within 10% RSD. Protein content was estimated from the reported percent nitrogen by using the factor 6.25.

RESULTS AND DISCUSSION

SC-CO_2 has proven quite effective for extracting lipid components from oilseeds (10,11). It is a nontoxic alternative to

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TABLE 1
Elemental Analysis of Defatted Hybrid *Hibiscus* Seed Meal and FAME Composition of the Extracted Seed Oil (% dry weight basis)

Hybrid	C	H	N	Protein	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20
Georgia Rose 1	42.9	5.5	2.7	16.8	0.0	16.5	0.0	3.0	25.4	53.2	0.6	0.0
Georgia Rose 2	45.6	6.0	4.0	24.9	0.0	17.7	0.0	3.4	27.0	52.0	0.0	0.0
Gov. Anne	44.5	6.0	3.1	19.5	0.0	17.9	0.0	2.9	17.8	61.4	0.0	0.0
Grace Coolidge	43.8	6.3	2.9	18.0	0.0	19.6	0.0	3.2	26.9	50.4	0.0	0.0
Lou Hoover	46.9	6.2	3.0	18.7	0.0	17.7	0.0	0.0	30.1	50.4	0.7	0.5
Martha Washington	45.9	6.1	3.1	19.1	0.0	20.9	0.0	3.2	28.6	45.5	0.5	0.5
Mary Todd	44.6	6.1	2.8	17.5	0.0	17.9	0.0	3.1	47.3	22.3	0.6	0.0
Nathan's Star	46.2	6.1	3.7	23.4	0.0	17.4	0.0	3.3	27.1	52.2	0.0	0.0
Nathan's Triumph	45.4	6.1	3.0	18.5	0.0	14.8	0.0	1.9	28.5	52.6	0.7	0.0
New Pink	45.4	6.3	3.2	20.2	0.0	16.6	0.0	0.0	29.0	54.4	0.0	0.0
Pink Disco Belle	43.6	6.2	3.5	21.5	0.0	22.6	0.0	3.2	27.2	47.0	0.0	0.0
Quatro Roja	45.7	6.3	3.0	18.6	0.0	18.4	0.0	3.4	23.2	55.1	0.0	0.0
Razberri Ruffles 2	44.5	6.1	4.0	25.2	0.4	21.1	0.4	0.0	29.6	45.5	0.6	0.4
Rosalynn 1	45.5	6.2	3.2	20.2	0.0	27.0	0.0	2.8	27.7	42.6	0.0	0.0
Rosalynn 2	45.7	6.1	2.8	17.7	0.0	22.8	0.0	1.0	29.5	46.9	0.0	0.0
The Blues	44.5	6.3	5.0	31.7	0.0	24.1	0.0	2.2	32.9	40.8	0.0	0.0

the organic lipid solvents such as petroleum ether or hexane typically used for laboratory extractions. Additionally, the speed of an SC-CO_2 extraction and the relatively mild temperatures required present distinct advantages for the recovery of thermally sensitive natural products compared to conventional Soxhlet extraction. The separation and recovery of the extracted components from an SC-CO_2 extraction are achieved simply by reducing the pressure, which vaporizes

TABLE 2
Elemental Analysis of Defatted *Hibiscus* and Related Malvacaea Seed Meal and FAME Composition of the Extracted Seed Oil (% dry weight basis)

Species (morphology)	C	H	N	Protein	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20
Abutilon hybridum	44.1	6.2	3.4	22.4	0.0	21.1	0.0	2.7	22.2	54.0	0.0	0.0
H. calyphyllus Madagascar	44.7	6.0	3.1	19.5	0.0	21.0	0.0	2.7	27.1	49.2	0.0	0.0
H. dasycalyx (big pod)	44.9	6.1	3.1	18.9	0.0	20.6	0.0	0.4	27.8	51.3	0.0	0.0
H. dasycalyx (tight pod)	44.6	6.2	3.0	18.6	0.0	19.5	0.0	4.6	29.6	46.5	0.0	0.0
H. dasycalyx (red calyx)	45.1	6.2	2.0	12.5	0.0	17.4	0.0	4.1	29.3	49.3	0.0	0.0
H. grandiflora	46.0	6.1	3.4	21.3	0.3	17.2	0.0	0.7	32.9	47.3	0.5	0.8
H. grandiflora, Gulf Coast	45.2	6.0	2.1	13.0	0.0	19.7	0.0	2.9	27.5	49.9	0.0	0.0
H. hamabo	47.0	6.6	2.5	15.8	0.0	19.4	0.0	3.3	27.8	49.5	0.0	0.0
H. hamabo Hamabo	45.1	6.2	3.8	23.3	0.0	20.0	0.0	3.9	29.1	47.0	0.0	0.0
H. laevis (balloon pod III)	44.8	6.2	1.7	10.8	0.0	23.7	0.0	3.2	25.0	48.1	0.0	0.0
H. laevis (fuzzy seed)	45.4	6.1	1.7	10.8	0.0	20.6	0.0	3.1	27.1	49.2	0.0	0.0
H. laevis	44.6	6.2	2.6	15.7	0.0	17.9	0.1	2.0	28.5	50.1	0.2	0.3
H. laevis (superior calyx)	45.4	6.4	2.4	14.9	0.4	23.2	0.0	2.9	27.6	45.8	0.0	0.0
H. laevis (balloon pod I)	44.9	6.1	2.2	13.8	0.3	19.9	0.4	3.3	25.9	49.2	0.4	0.6
H. laevis (normal calyx)	40.6	5.4	2.5	15.7	0.3	17.5	0.3	0.0	31.3	48.6	0.6	0.0
H. laevis (balloon pod II)	46.0	6.5	2.3	14.1	0.0	16.0	0.0	2.6	28.2	51.1	0.6	0.5
H. laevis (giant)	46.1	6.3	2.7	16.6	0.0	21.0	0.0	2.8	27.6	48.7	0.0	0.0
H. martianus	45.4	5.9	3.2	20.0	0.0	18.0	0.0	4.1	14.1	63.9	0.0	0.0
H. moscheutos 'Arkansas'	44.3	6.3	3.2	19.4	0.0	17.3	0.0	2.9	24.4	55.3	0.0	0.0
H. mos. 'Caroline's Pink'	45.5	6.1	4.1	25.5	0.0	19.0	0.0	3.6	29.0	48.5	0.0	0.0
H. mos. (hairy)	45.1	6.2	3.6	23.0	0.0	20.4	0.0	1.7	22.6	55.3	0.0	0.0
H. mos. (hirsute pod)	44.8	6.2	3.5	21.4	0.0	19.7	0.0	3.7	27.2	49.5	0.0	0.0
H. mos. 'Lowrey's Pink'	45.9	6.2	4.1	25.4	0.0	17.7	0.0	2.8	30.2	49.4	0.0	0.0
H. mos. Moscheutos	46.0	6.4	2.4	14.9	0.0	18.3	0.0	2.1	15.6	64.0	0.0	0.0
H. mos. (short pod)	45.9	6.3	2.4	15.0	0.0	16.8	0.0	0.0	29.1	53.1	0.0	0.0
H. mos. (long pod)	43.6	6.2	3.0	18.5	0.3	16.2	0.3	0.0	31.3	50.9	0.7	0.4
H. mos. (smooth pod)	45.9	6.1	2.7	17.1	0.0	18.1	0.0	4.1	29.5	48.4	0.0	0.0
H. mos. (yellow)	44.4	6.1	3.2	20.0	0.0	25.7	0.0	3.7	19.9	50.6	0.0	0.0
H. mutabilis (fuzzy seeds)	46.0	6.4	2.8	17.2	0.0	23.5	0.4	0.0	18.4	55.3	0.7	0.4
H. mutabilis (short season)	45.5	6.1	2.1	13.2	0.0	20.4	0.0	3.5	25.4	50.7	0.0	0.0
H. striatus lambertianus	46.7	6.2	2.5	15.5	0.0	20.9	0.0	3.8	27.6	47.6	0.0	0.0
Malvaviscus drummondii	44.3	6.2	3.2	20.1	0.0	26.1	0.0	2.7	23.0	48.3	0.0	0.0
Pavonia hastata	48.4	6.3	3.0	18.5	0.3	25.3	0.7	3.7	14.0	49.2	0.0	0.0
P. lasiopetala	47.1	6.0	2.0	12.3	0.0	27.0	0.0	2.8	22.0	47.2	0.0	0.0
Sida spinoza	45.2	6.4	4.7	29.1	0.0	23.2	0.0	2.7	25.9	48.3	0.0	0.0

the CO_2. The extract remains in the collection vessel. The instrumentation developed to perform these extractions has become highly automated and is advantageous for investigations in which numerous small-volume samples are involved.

Results obtained from the analyses of the SC-CO_2-extracted seed oils and the corresponding defatted seed meals are presented in Table 1 for the hybrid varieties and Table 2 for the native species. These data represent the average values obtained from duplicate extractions and replicated analyses. The protein content of the defatted seed meal was estimated from the elemental nitrogen analysis and ranged from 16.8 to 31.7% for the hybrid varieties and from 8.4 to 29.1% for the native species. For comparison, two hibiscus species that have been cultivated for millennia as a food source, okra (*H. esculentus*) and roselle (*H. sabdariffa*), have reported seed protein values of 20.6 and 25.2%, respectively (12,13). Additional studies on these two species have shown the amino acid profile to compare favorably to a World Health Organization standard protein (14,15). Similar results are expected for the hybrid hibiscus seed meals although these analyses remain to be performed. In contrast, the reported protein content of kenaf (*H. cannabinus*) stalk is 2–12%, which supports its use as a forage crop (4,16).

FAME analysis shows that all of the extracted seed oils are highly unsaturated and composed primarily of oleic and linoleic FA. The highest levels of these FA, 83.4%, were found in the hybrid New Pink. The lowest levels, 69.6%, were found in the hybrid Mary Todd. The corresponding values for the unsaturated FA contained in seed oils from the native species was 82.2% for two *H. moscheutos* and 61.2% for *H. laevis* varieties (Table 2). Palmitic acid was the most prominent saturated FA. It was detected in all seed oils analyzed, with 27.0% in Rosalynn 1 and 14.8% in Nathan's Triumph. Corresponding values of 27.8 and 16.0% were measured for the native species, *M. drummondii* and *H. laevis*, respectively. Minor amounts of the saturated FA C14, C16, and C20 were detected. These high levels of unsaturated FA in hibiscus seed oil suggest possible edible applications. However, the average oil yield was 11.4%, expressed as the weight percentage of the total extracted lipids determined gravimetrically from the collected fractions and calculated on a dry basis of whole ground seed. The yields ranged from a low of 8.5% for a variety indigenous to Madagascar (*H. calyphyllus*) to nearly 20% for a hybrid species (Georgia Rose). With these relatively low oil yields and the small acreage in production, it is unlikely that hibiscus would be cultivated as an oilseed. However, these results are significant to the development of hibiscus extracts for use in nutraceutical and cosmetic preparations, where such attributes would be of benefit.

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