Analysis of Oil and Meal from *Lesquerella fendleri* Seed

K.D. Carlson*, A. Chaudhry and M.O. Bagby
Northern Regional Research Center, ARS/USDA, Peoria, IL 61604

Attention is being focused on *Lesquerella* species as a source of hydroxy acids to replace imported castor oil. Genetic and agronomic improvement and utilization of the seed oil and meal are being studied. We have conducted laboratory experiments to extract oil from *L. fendleri* seed in preparation for extracting large quantities of seed. *L. fendleri* is a member of the Cruciferae family, and when seeds are crushed glucosinolates release isothiocyanates by the action of a thioglucosidase enzyme system. Therefore, our experiments included moist heat treatment of whole seeds to inactivate this enzyme. The seed was then flaked in a Wolf mill, and the flakes were exhaustively extracted with hexane. The oil was degummed and bleached, and then analyzed for hydroxyl (103), saponification (174), and iodine values (107), and for unsaponifiables (1.5%), FFA (1.13%) and P (10 ppm) contents. Hydroxy fatty acids, 55% lesquerolic (14-hydroxy-*cis*-11-eicosenoic) and 3% auricolic (14-hydroxy-*cis*-11,*cis*-17-eicosadienoic), and total fatty acid distribution were determined by gas chromatography of the methyl esters. The defatted meal was analyzed for residual oil (1%), protein (29.8%), non-protein nitrogen (0.7%), ash (6.45%), crude fiber (12.9%), and for distribution of amino acids. Defatted *L. fendleri* meal has an excellent distribution of amino acids, including favorable levels of lysine, methionine and threonine compared with soybean meal.

Castor oil, classified as a strategic material, is imported into the U.S. at an annual rate of some 80 million pounds valued at about 45 cents/lb. This quantity represents approximately one-seventh of the total world export of the oil (1). Uses of castor oil and its ricinoleic acid are many and include products for lubricants, plastics, coatings and pharmaceuticals (2,3).

As part of a research program to develop new uses for oils from prospective alternative crops, attention is being focused within USDA on *Lesquerella* species as a source of hydroxy acids to extend the useful range of the many product types derived from ricinoleic acid, and to supplement or replace imported castor oil. Over 70 species of *Lesquerella* are known within the family Cruciferae and are found primarily in western North America from Mexico to Canada with greatest concentration in the U.S. Southwest and Northern Mexico (1).

A.E. Thompson at the Agricultural Research Service's Water Conservation Laboratory in Phoenix, Arizona, has embarked on a program of germplasm evaluation and genetic and agronomic improvement of *Lesquerella* and has assembled a germplasm collection of 90 accessions from 23 species originally collected by USDA in the early 1960's (1). One species, *L. fendleri*, is considered to be a prime candidate for domestication, because it produces abundant seed both in the wild and under cultivation and appears to have abundant genetic variability for improvement through traditional plant breeding techniques. One desirable improvement would be oil yield, and another would be total hydroxy acid content.

Thompson has supplied us with several hundred pounds of *L. fendleri* seed for our studies. In this paper we describe how we processed the seed in the laboratory in preparation for oil extraction, some oil extraction experiments, and extensive characterization of the oil and meal products.

EXPERIMENTAL PROCEDURES

Analyses. Analyses following Official AOCS Methods (4) included: ash (Ba 5-49); crude fiber (Ba 6-61); free fatty acids (FFA) (Ca 5a-40); hydroxyl value (Cd 13-60); iodine value (Cd 1-25); moisture (Ac 2-41); nitrogen (Aa 5-38) and crude protein (N × 6.25); nitrogen free extract; non-protein nitrogen; oil (Ac 3-44); phosphorus (Ca 12-55); saponification value (Cd 3-25); and unsaponifiables (Ca 6b-53).

Amino acids were determined on defatted flakes with an amino acid analyzer after 6N HCl hydrolysis (5). The TesTape^R method of Van Etten *et al.* (6,7) (glucose-sensitive TesTape^R, Eli Lilly and Co., Indianapolis, Indiana) was used to check for thioglucosidase enzyme activity in seed and flakes by wetting a piece of TesTape^R with a slurry made from 40–60 mg of meal sample and 1 mL distilled water. The rapidity and intensity of the green color developed in the TesTape^R are indications of thioglucosidase enzyme activity and of glucosinolate content of the sample, respectively. When no color develops, the thioglucosidase has been inactivated. Then, if color develops when 10 mg of purified mustard myrosinase (8,9) is added to the meal slurry, the meal contains residual glucosinolate. Thin layer chromatography (TLC) was performed on 0.25 mm silica gel 60 F254 plates (E. Merck, Cincinnati, Ohio). Developing solvent was hexane/diethyl ether (60:40 or 50:50, v/v) with 1 part acetic acid. Visualization was by iodine vapor and/or H_2SO_4-charring. Methyl esters were analyzed with a Spectra Physics SP-7100 gas chromatograph (GC) equipped with a fused-silica capillary column using helium and temperature programming. Fatty acid methyl esters (FAME) were prepared from *Lesquerella* oils by transesterification with 0.28*M* sodium methoxide in methanol or by saponification in 0.5*N* NaOH/methanol followed by reaction with 10% BF_3/methanol. The former was used especially to prepare multigram quantities of FAME, and the latter method was preferred where FFA were known to be present in the oil. A third method was used on occasion to prepare FAME directly from full-fat seed meal or flakes by vigorously shaking (5–10 min) 100 mg of meal in a screw-cap vial with 1 mL of 0.28*M* sodium

*To whom correspondence should be addressed.

methoxide/methanol. The FAME were recovered in ether for GC analysis.

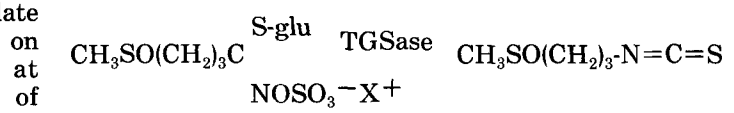
Conditioning and flaking L. fendleri *seed. L. fendleri* seed was obtained from A.E. Thompson. Seed moisture was adjusted upward from ambient (6.1%) by adding 15% by weight water to the hot seed (80–100°C). Seed was tempered 1 hr at ≥80°C. Such tempered seed gave a positive test for glucose with TesTape®, showing that the thioglucosidase enzyme was still active. These seed also flaked poorly due to the high surface moisture and stickiness. Thus, the seed was allowed to moisture equilibrate overnight and then was again stirred at ≥80°C for 90 min at which time the moisture content was 9.0%: the seed flaked easily, and a negative test for glucose with TesTape® (no thioglucosidase activity) was obtained. Addition of a few mg of mustard myrosinase enzyme to the slurry rapidly gave a strong glucose test showing that glucosinolate was still present. The tempered seed was flaked on a Wolf flaking mill (12-inch smooth rolls) set at 0.002–0.003 inch, which gave whole-seed flakes of uniform thickness when the moisture was 9.0%. For analytical samples, flakes of whole seed were ground in a Wiley mill through 20 or 30 mesh screens. Flakes were air-dried to ca. 5.0% moisture before oil extraction.

Oil extraction. Replicated batch extractions of oil were performed with 209 g of tempered and air-dried flakes by initial steeping in 350 mL of hot (50–60°C) hexane for 30 min followed by seven additional steeps, each with 150 mL of hot hexane for 30 min. The 8 extracts of each replicate were separately concentrated on a rotary evaporator, and their residual oil contents were determined. A sample of oil from each steep fraction was converted to methyl esters which were analyzed by GC for fatty acid distribution. Oil content of the seed and residual oil in the defatted flakes were determined by exhaustive butt flask extraction (AOCS).

Oil refining. In preliminary experiments, crude *L. fendleri* oil was degummed by vigorously mixing oil with either distilled water or saturated NaCl solution (5%, v/v). Hydrated gums were separated by centrifugation at 2,000 rpm for 15 min. The degummed oil was divided into four 1-mL portions, which were vigorously mixed (15 min, 40–60°C) with 50 mg of **one** of the following: Darco G60 charcoal or Harshaw/Filtrol bleaching earths—grade 13, 160 or Nevergreen. Nevergreen was most effective at reducing oil color. On a larger scale, 21-g replicates of crude oil were degummed by mixing the heated oil (15 min, 40–60°C) with saturated NaCl solution (5% v/v) followed by centrifugation (96% oil recovery). The degummed oils were bleached (15 min, 40–60°C) with Nevergreen bleaching earth (5% w/v) followed by centrifugation (90% oil recovery). On a still larger scale, 200-g samples of crude oil were degummed and then bleached with 2% Harshaw/Filtrol bleaching earths of grades 2c, 13, 22, 71, 113 and Super Filtrol, with and without 2% charcoal (w/v). Degummed and bleached oils were clear with a dark yellow-gold color (Gardner 10–12). The small amount of free fatty acids present in the oil was removed by shaking the oil with 10% Na_2CO_3 solution, centrifuging and decanting.

RESULTS AND DISCUSSION

One of our first concerns in this study was to properly prepare *L. fendleri* seed for oil extraction and thereby obtain quality oil and meal products for further characterization. As a crucifer, *Lesquerella* species produce seeds containing glucosinolate(s) and an associated enzyme system, thioglucosidase (thioglucoside glucohydrolase [EC 3.2.3.1]), abbreviated TGSase in this paper. When glucosinolate and TGSase are co-mingled upon cell rupture, aglucon products are released into the surrounding tissues. The major glucosinolate in *L. fendleri* seed, 3-methylsulfinyl propylglucosinolate, undergoes enzymatic hydrolysis to release glucose, HSO_4^- and 3-methylsulfinylpropyl isothiocyanate when seeds are crushed (10,11). These products generally end up in the seed meal thereby reducing



3-methylsulfinylpropyl-glucosinolate

3-methylsulfinylpropyl isothiocyanate (iberin)

palatability and feed value, but sulfur-containing products such as the isothiocyanate can also interfere with hydrogenation of the oil if they are carried into the oil during extraction.

Objectives in processing *Lesquerella* seed should include: inactivation of thioglucosidase at an early stage of processing; prevention of both enzymatic and thermal degradation of glucosinolate(s) during all processing stages; maximizing recovery of high quality oil; and production of a quality by-product meal.

Figure 1 shows the processing steps we used to extract oil. In addition to the oil extraction stage and meal desolventizing, proper control of time, temperature and moisture levels are important. For example, initial heat and moisture treatment of the seed for 1 hr did not fully inactivate the TGSase enzyme, although seed moisture (21.8%), temperature (>80°C) and time were all at appropriate levels. Probably, moisture had not penetrated sufficiently into the seed. The seeds also flaked poorly due to their sticky surfaces. When this seed was moisture-equilibrated overnight in a closed environment and then heated at >80°C for 90 min, the TGSase was fully inactivated. Excellent flakes (9.1% moisture) were obtained using a Wolf mill set at 0.003 inch clearance. From these preliminary results and our past experience with another crucifer (7), we expect that 10–15% moisture, 90–100°C and 15–60 min of tempering will be satisfactory for processing *L. fendleri* seed on a larger scale.

Oil was extracted from the tempered flakes by repeated steepings with fresh portions of hot hexane. This was a convenient method to use in the laboratory, and it allowed us to evaluate both the quantity and composition of oil removed by individual solvent fractions and with increasing solvent-to-flake ratios. The results of replicated solvent extractions, each with eight steeps, are shown in Figure 2. The solvent-to-flake ratio (w/w) after the first steep was 1.1, and after the

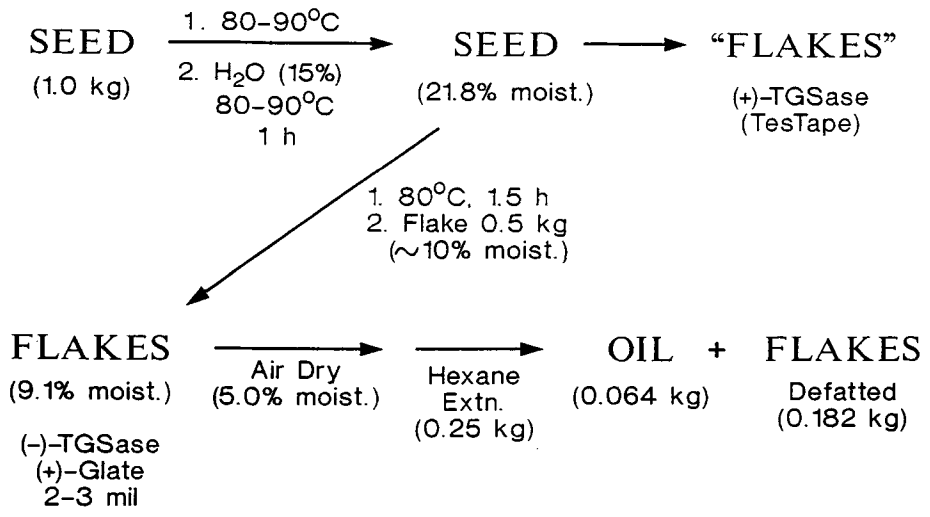


FIG. 1. Preparation of *L. fendleri* seed for oil extraction. (+)-TGSase and (−)-TGSase are positive and negative tests, respectively, for thioglucosidase enzyme activity. (+)-Glate is positive indication of the presence of glucosinolate after addition of myrosinase to thioglucosidase-negative wetted meal.

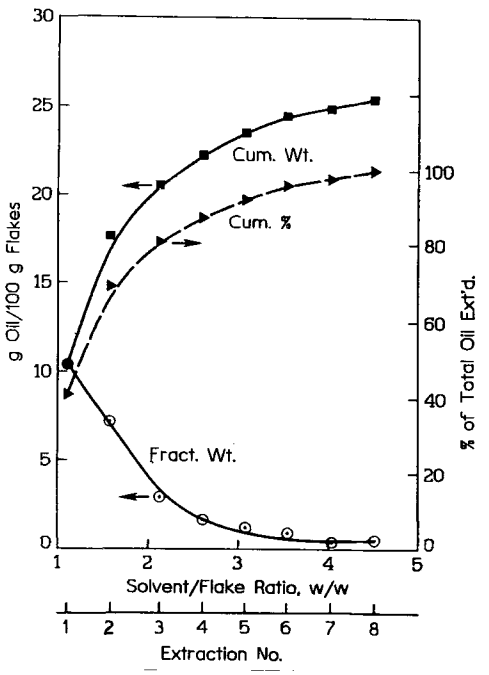


FIG. 2. Amount of oil extracted from *L. fendleri* flakes as a function of amount of hexane used.

eighth was 4.5. Forty-one percent of the oil was recovered in the first steep. By steep 3 (solvent-to-flake ratio = 2.1), 80% of the oil had been recovered, after which remaining oil was gradually recovered through the eighth steep. Total cumulative oil recovered was 25.5% by wt.

Crude oil was refined by degumming it with saturated NaCl solution (5%), followed by treatment with bleaching earths and/or alkali (10% Na_2CO_3 solution). Of the eight bleaching earths tried, the most effective were Super Filtrol, Nevergreen and grade 113. In combination with charcoal (2% w/w) all bleaching earths reduced the crude oil color from Gardner 14 to Gardner 10. As expected, the phosphorus level of crude oil (295 ppm) is greatly reduced by degumming and bleaching (10 ppm) (Table 1). Refining without alkali appeared to decrease slightly the FFA content and unsaponifiables. Otherwise little difference was noted in analyses between crude and degummed, bleached oils.

Compositions of the oils in the steep fractions, by GC analysis of the FAME, are compared in Table 2 with the mean composition of the oils exhaustively extracted from 4 multikilo lots of seed. Although the steep fractions showed greater compositional variability (>SD), there was no clear-cut advantage to segregating initial and later hexane miscellas in the hopes of obtaining an enriched hydroxy acid fraction. Lesquerolic (14-hydroxy-*cis*-11-eicosenoic) acid constituted 54.5% of the fatty acids, and auricolic (14-hydroxy-*cis*-11-*cis*-17-eicosadienoic) acid (2.9% of the fatty acids) represented about 5% of the total hydroxy acids.

Representative GC curves for FAME from *L. fendleri* oil and castor oil are shown in Figure 3. The castor oil contained 89% ricinoleic acid with less than 0.5% lesquerolic acid. Normal esters included 16:0 (0.9%), 18:0 (0.8%), 18:1 (4.2%), 18:2 (5.1%) and 18:3/20:0 (1.0%). Besides lesquerolic (54.5%) and auricolic acids (2.9%), the *L. fendleri* oil contained ca. 0.6% ricinoleic acid and largely unsaturated (93%) C-18 acids, 18:1 (16.9%), 18:2 (7.6%) and 18:3 (13.2%). When separated from the hydroxy esters, the normal ester fraction was an interesting mix of 18:1 (41%), 18:2

TABLE 1

Analyses of *L. fendleri* Oil

Oil	Hydroxyl value	Sapon. value	Iodine value	Unsaps. (%)	FFA (%)	P (ppm)
Crude	103	174	106	1.82	1.25	295
Refined[a]	103	174	107	1.52	1.13	10

[a]Degummed and bleached.

TABLE 2

Composition of *L. fendleri* Oils

	Fatty acid methyl ester							
Sample	14-OH-11,17 20:2	14-OH-11 20:1	18:3	18:2	18:1	18:0	16:1	16:0
Extraction fractions								
Mean (16)[a]	2.7	52.5	13.2	7.9	17.6	2.0	1.4	1.4
SD	0.2	2.2	0.4	0.3	0.9	0.1	0.9	0.3
Wt'd ave (2)[b]	2.8	53.2	13.4	8.0	17.3	1.9	0.8	1.2
Bulk samples								
Mean (4)[c]	2.9	54.5	13.2	7.6	16.9	1.9	0.5	1.1
SD	0.1	0.7	0.2	0.1	0.5	0.1	0.1	0.0

[a]Two reps. × 8 ext'ns. each.
[b](% × frn. of total oil ext'd)/total oil ext'd.
[c]Four multikilo lots.

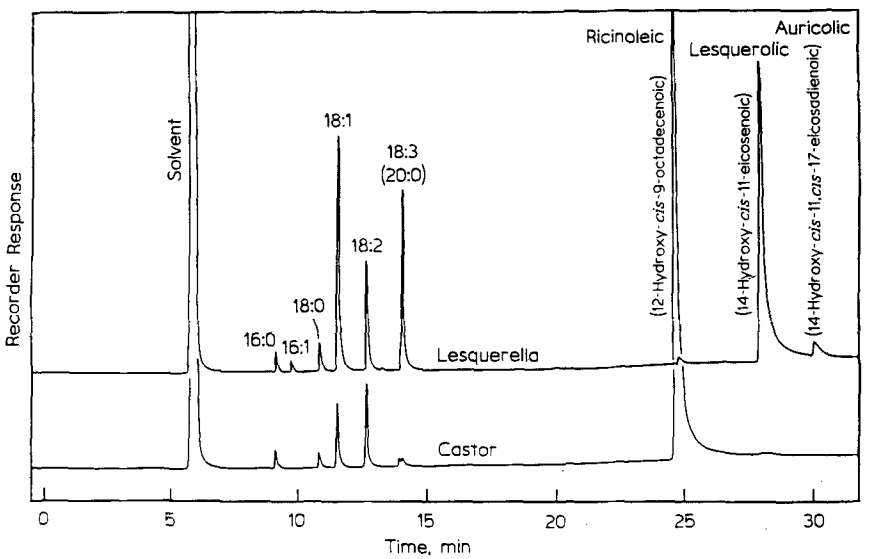


FIG. 3. GC analysis of *Lesquerella* and castor oil methyl esters (60M × 0.24 mm SP2340 cap. columns, 180–250°C at 3°C/min).

(18%) and 18:3 (32%), perhaps suitable for specific industrial uses.

The protein content of *L. fendleri* seed (22.1%) was raised by oil extraction to nearly 30% (29.8%) in the defatted flakes (Table 3). Non-protein nitrogen (NPN = 0.7%) content was partly associated with the glucosinolate content of the flakes, and nearly 50% of the flake dry weight was attributable to carbohydrates and other nitrogen-free components (NFE = 49.4%). Crude fiber (12.9%) and ash contents (6.5%) are of interest with regard to the potential feed value of the defatted flakes. However, neither mineral composition of the ash nor feed value of the meal has been determined.

Nearly 96% of the meal nitrogen was recovered as amino acids. Table 4 shows the distribution of amino acids in *L. fendleri* compared to soybean meal and another crucifer, *Crambe abyssinica*. The current val-

TABLE 3

Analysis of *L. fendleri* Seed and Meal

Sample	Moisture (%)	Oil (%)	TKN (%)	Protein (%)	NPN (%)	Ash (%)	Crude fiber (%)	NFE (%)
Seeds	7.42	26.2	3.53	22.1	ND*	ND	ND	ND
Flakes defatted	5.99	0.8	4.77	29.8	0.70	6.45	12.87	49.4

*ND = not determined.

TABLE 4

Amino Acid Analyses for Defatted *L. fendleri* Flakes

Amino acid	Amino acid, g/16 g nitrogen			
	L. fendleri	*L. fendleri*[a]	Soybean[b]	Crambe[c]
Alanine	3.72	4.50	4.29	3.8- 4.2
Arginine	8.73	7.86	7.27	5.7- 7.3
Aspartic acid	7.14	7.23	11.78	6.0- 7.6
Cystine	1.99	1.78	0.93	2.6- 2.8
Glutamic acid	13.20	13.70	18.63	14.2-17.0
Glycine	5.05	5.94	4.30	4.7- 5.3
Histidine (E)*	2.30	2.53	2.55	2.2- 2.7
Isoleucine (E)	3.37	3.55	4.58	3.7- 4.1
Leucine (E)	5.45	5.81	7.75	5.9- 6.8
Lysine (E)	5.67	6.64	6.43	4.9- 5.7
Methionine (E)	1.77	1.34	1.13	1.6- 1.9
Phenylalanine (E)	4.12	3.82	5.01	3.4- 4.0
Proline	6.07	6.67	—	5.5- 6.2
Serine	4.43	4.64	5.45	3.5- 4.1
Threonine (E)	4.08	4.45	3.93	3.1- 4.6
Tyrosine	3.10	2.98	3.75	2.5- 3.0
Valine (E)	4.52	4.78	4.58	4.5- 5.6

[a]Reference (12).
[b]Reference (5).
[c]Reference (13).
*E = essential amino acid.

ues are in general agreement with those reported in 1962 by Miller *et al.* (12). Note particularly the favorable lysine, methionine and threonine contents of *Lesquerella* meal. One can presume that *L. fendleri* meal would provide quality protein for animal feeds, but feeding studies are required to determine palatability of the meal, weight gain and feed efficiency of consuming animals and the effect of glucosinolate and aglucon products on the acceptability and/or toxicity of the meal.

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