Hydroxy-Unsaturated Oils and Meal from *Dimorphotheca* and *Lesquerella* Seed

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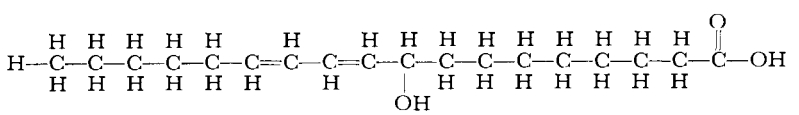
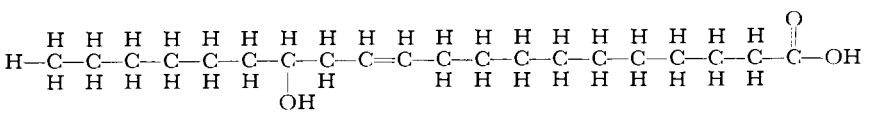
Methods were developed for recovery of oil and meal from two new oilseeds, *Dimorphotheca* and *Lesquerella*. Procedures are described for operating on a relatively small scale suitable for analytical work and on a relatively large scale suitable for preparation of quantities required for laboratory investigative and developmental research. Solvents do not remove oil from intact seed, so the seed coat must be broken; but the presence of active lipolytic enzymes in the crushed seed necessitates prompt extraction of oil after the seed is crushed to produce oils with low free fatty acid content. Use of readily available equipment is emphasized.

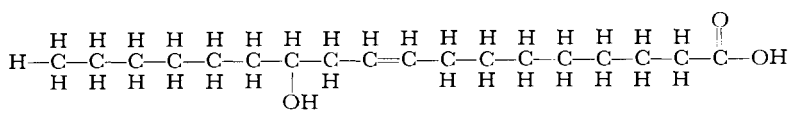
Castor is at present the only commercially available source of hydroxy-unsaturated oils, although a limited amount of isano oil has at times also been available. In the course of an extensive screening program in search of new and potentially useful industrial oils, two plants were revealed to have seeds relatively high in hydroxy-unsaturated fatty acids (*3*, *4*). The oil of *Dimorphotheca* contains about 60% of dimorphecolic (9-hydroxy-10, 12-*trans*, *trans* octadecadienoic) acid (I), and *Lesquerella*, about the same proportion of lesquerolic (14-hydroxy-11-*cis* eicosenoic) acid (II). The latter is a homolog of ricinoleic (12-hydroxy-9-*cis* octadecenoic) acid (III) of castor oil. Developmental research directed toward industrial utilization of these two oilseeds is currently under way here. Since these oils are not commercially available, they were prepared from the seeds. This paper describes procedures developed to produce the oils on a small and on a relatively large laboratory scale using equipment that was available.



Dimorphecolic Acid I



Lesquerolic Acid II



Ricinoleic Acid III

Materials

The seed was procured from the New Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture. The *Dimorphotheca* seed, chiefly *D. sinuata* [previously identified as *Dimorphotheca aurantiaca*, but according to Norlindh (*2*), *D. sinuata* is the proper designation], was obtained in about a dozen small batches ranging in size from about 100 to 6000 grams, totaling about 15 kg., and a single large batch of 42 kg. The batches contained different amounts of foreign material, but the cleaned seed appeared quite uniform in size, color, and maturity. The *Dimorphotheca* fruit, which somewhat resembles an elm seed, is a wafer-thin winged achene, tan to light brown in color, and heart-shaped. It averages 6 mm. across and weighs about 2 mg. The light, fragile wings (hull) comprise the major portion (about 60% by weight) of the achene, and when these are removed the light-tan colored, obovate, and relatively dense seed with a firm coat remains.

The *Lesquerella* seed, all *L. fendleri*, was received in a single 300-pound lot. It had been mechanically harvested in Texas in 1961 with a commercial combine used for harvesting grass seed. The seed contained only about 5% foreign material. *Lesquerella* seeds are bright yellow-brown disks, 1.0 to 1.5 mm. in diameter and weighing approximately 0.5 mg. each.

Decorticating and Cleaning. Although whole *Dimorphotheca* seed has been ground in a Mikro-Samplmill and extracted successfully, the authors found it expedient to remove the seed hulls to avoid processing nonproductive material. Preliminary attempts to decorticate the seed included abrasion between corrugated rubber mats and separation of the loosened hulls from the seed with a Bates Laboratory Aspirator. It was more efficient, however, to pass the whole seed through a Labconco mill with a jaw clearance of 0.034 inch. The hulls and the lightweight trash were then separated from the seed by gravitation and forced aeration in a McGill Rice Sample Sheller. Best results were obtained with the air intake set at 1/8 inch and a roller spacing of 0.040 inch. The seed fraction separated by this procedure was screened through a No. 8 Rice Sieve, having 0.086-inch round holes, to remove large heavy trash and unmilled seed. The amount of decorticated seed recovered was 40 to 45% of the untreated seed weight.

Since the *Lesquerella* seed was already threshed as received, it was necessary only to remove trash, foreign seeds, and a small amount of loose hulls. Procedures used for *Dimorphotheca* seed were

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only moderately successful when applied to *Lesquerella* seed. However, large lots of *Lesquerella* seed were easily and rapidly cleaned using a Hart Uni-Flow Tester of the Simon-Carter Co.

Crushing. Extraction of whole seed, before or after decortication, results in removal of only very minor amounts of oil even after prolonged treatment with hydrocarbon solvents. Accordingly the seed must first be crushed. Two methods of grinding the decorticated *Dimorphotheca* seed were developed, one suited primarily for analytical use, the other for preparative-scale work.

For analytical purposes, 50-gram samples of decorticated seed were ground in a Brabender Sample Grinder, which has a cutting action with relatively little crushing effect. Grinding for 2 minutes gave good reproducibility on extraction.

For kilogram-scale preparative purposes, the seed was fed through a 1/3-hp. electric meat chopper having 3/32-inch die perforations. This crushed and extruded the seed in spaghetti-like strands, well suited for extraction. The extruded material was hot (ca. 60° C.), but the oil did not appear to be damaged.

For preparation of *Lesquerella* seed, two methods were developed also.

For analytical-scale work, ca. 50 grams of cleaned seed were ground in a Brabender grinder in the same manner as *Dimorphotheca* seed, with satisfactory results.

For preparative-scale work, 0.5 to 5 kg. of cleaned seed were flaked or cracked in a 3- × 7-inch Farrel 2-roller rubber mill, using 0.006-inch spacing between the two smooth rolls.

Table I. Storage Effects on Acid Values of *Dimorphotheca* and *Lesquerella* Seed Oils

Material	Sample	Storage Temp., ° C.	Storage Time, Months or (Days)	Acid Value of Extracted Oil: Before storage	Acid Value of Extracted Oil: After storage
D. oil	1	2	36	6.0	6.4
	2	Room[a]	36	6.0	7.4
	3		36	6.0	6.4
L. oil	1	2	9	3.4	3.4
	2		17	3.7	7.0
	3	Room	18	3.5	3.9
	4		26	3.5	5.4
D. seed, whole	1	2	36	6.0	6.2
	2	Room	36	...	5.0
L. seed, whole	1		27	3.4	4.1
D. seed, crushed	1	−20	4	9.3	12.8
	2		32	6.0	63.2
	2	Room	(21)	7.0	18.5
L. seed, crushed	1	2	8	3.4	11.1
	1		11	3.4	14.6
	2		19	3.5	58.0
	3		23	3.5	79.0
	4	Room	(2)	3.7	11.8
	4		(7)	3.7	28.9
	4		22	3.7	115.3

[a] Normally ranges from 20° to 30° C.

Table II. Proximate Analysis of Meals from *Dimorphotheca* and *Lesquerella* Seed[a]

Seed	Meal, % of Whole Seed Wt.	% in Meal: Protein (% N × 6.25)	Ether-extractable	Crude fiber	Ash	N.F.E.
Dimorphotheca	25.9	58.7	2.9	4.6	8.5	25.3
Lesquerella	74.8	34.3	1.2	11.8	7.0	45.7

[a] All results reported on moisture-free basis.

Extraction of Oil

***Dimorphotheca* Seed.** In preliminary trials, ground, undecorticated seed was extracted with commercial pentane for 3 to 5 1/2 hours in Soxhlet extractors. Pentane was used to prevent heat damage to the oil. About 17 to 18% of the dry seed weight was obtained as solvent-free oil containing appreciable amounts of color and methanol-insoluble material.

Extraction of decorticated seed ground in a Brabender sample grinder gave a solvent-free oil yield of 38%. This oil was lighter in color than that obtained from the undecorticated seed. Examination of the ultraviolet spectrum of the oil indicated the presence of 62% conjugated diene (as dimorphecolic acid) and 3 to 4% conjugated triene (as eleostearic acid).

The seed prepared in larger batches by passing it through a meat chopper was extracted in kilogram lots in a large Soxhlet-type extractor. Commercial pentane was used, 3500 ml. for each batch, and the extraction was continued for 3 to 4 hours. Most of the solvent was removed from the oil on a steam bath. The final traces of solvent were removed in a rotary evaporator with reduced pressure at temperatures below 50° C. Yields ranged from 33 to 36% (13.5 to 16.5% on dry undecorticated seed). The oil typically had a density of 0.954 gram per ml. at 25° C., a viscosity of U-V (750 centipoises) on the Gardner scale, an acid value of 6, and a hydroxyl value of 116. It contained 61% dimorphecolate as dimorphecolic acid.

***Lesquerella* Seed.** Cleaned seed ground in a Brabender grinder was extracted with commercial hexane in batches of 40 to 60 grams in Soxhlet extractors for 20 hours. Typically, 25% of the dry seed weight was recovered as oil.

Seed prepared by cracking in a roller mill was extracted in 5-kg. batches with 20 liters of commercial hexane at 65° C. for 1 hour, with agitation. The extract was separated from the meal by centrifuging, and the meal was re-extracted with another 20 liters of solvent for 15 minutes. The combined extracts were concentrated in a steam-jacketed flash evaporator and finally in a rotary evaporator. Oil extraction of 23 to 24% on a moisture-free seed basis was achieved by this relatively fast method. The oil typically had a density of 0.930 gram per ml. at 26° C., a viscosity of I-J (240 centipoises) on the Gardner scale, an acid value of 3, and a hydroxyl value of 97. It contained 57% lesquerolate as lesquerolic acid.

Stability of the Oils

Acid values determined on oils extracted from *Dimorphotheca sinuata* and *Lesquerella fendleri* seed samples before and after storage are presented in Table I. Both *Dimorphotheca* and *Lesquerella* oils are apparently stable to hydrolysis under normal storage conditions. Oil in intact seed likewise is apparently stable for many months at room temperatures. However, oil in crushed seed does not exhibit like stability. The acid values recorded in Table I indicate a slow hydrolysis proceeding at −20° C. and +2° C. but a strikingly accelerated rate in the crushed seed samples held at room temperature. The *Dimorphotheca* and *Lesquerella* seeds referred to above were not dried before processing, but their moisture content was low enough (7 to 8%) to inhibit normal mold growth. No mold growth was observed. The stability of the oil in uncrushed seed, contrasted with rapid increase in free

fatty acid content of the oil in crushed seed held at room temperature, suggests that enzymatic hydrolysis of the oils in crushed *Dimorphotheca* and *Lesquerella fendleri* seeds occurs slowly at low temperatures and rapidly at room temperaperature. To prevent hydrolysis, seeds should be processed promptly after crushing.

Extracted Meal

The amino acid compositions of *Lesquerella* and *Dimorphotheca* meal proteins have been reported (*1*, *5*). The proximate composition (Table II) permits a partial evaluation of the meals for livestock feed use; bioassay and nutritional studies will be necessary for a full evaluation of their potential.

Acknowledgment

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PHOSPHATE EFFECTS ON MEAT

Effect of Inorganic Polyphosphates on the Solubility and Extractability of Myosin B

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The solubility of myosin B, as well as the extractability of proteins from myofibrils in the presence of pyrophosphate, tripolyphosphate, and hexametaphosphate with or without sodium chloride, magnesium chloride, and calcium chloride, has been studied under various conditions. Data on the solubility of myosin B as a function of pH and salt concentration correspond well to those of the extractability of proteins from myofibrils. The influence of inorganic polyphosphates on the solubility of myosin B may be classified into two types of binding with the protein, viz., direct binding of highly polymerized polyphosphate ion such as hexametaphosphate and subsequent di- or triphosphate binding following well known preferential cation binding with myosin B. The importance of the formation of univalent metal-myosinate and soluble divalent metal-polyphosphate complexes is also discussed.

POLYPHOSPHATES are used in the manufacture of sausage to improve water-holding capacity and binding properties although there have been many arguments about their effects on the quality of meat and meat products.

Fukazawa *et al.* (*9, 10*) reported that the factor necessary for the binding properties of sausage was myosin A in muscle structural proteins and indicated that the effect of phosphate was due to the dissociation of actomyosin into myosin A and actin. Recently, Sherman (*26*) mentioned the importance of ion binding with muscle structural proteins for the improvement of the water-holding capacity of comminuted meat. The understanding of the interaction of myosin with ions is important in the study of muscle function. A great amount of evidence has accumulated to illustrate that the interaction of ions with muscle proteins plays an important role in the contractile process and therefore deserves special study.

Myosin, one of the principal contractile proteins, has been shown (*5, 11, 19, 21, 22, 27*) to have a great affinity for ions. The present paper reports a study of the solubility of myosin B and of the extractability of structural proteins from myofibrils as influenced by polyphosphate ions.

The results obtained in the present study enable us to classify those phosphates into two groups on the basis of their effect on the solubility of myosin B and on the extractability of structural proteins from myofibrils. One group contains polyphosphates of comparatively low molecular weight, such as pyrophosphate (PP) or tripolyphosphate (TP), which react as a salt with salt-free myosin B. Their affinity to myosin B is greatly improved in the presence of high salt concentration and divalent cations. The other group is made up of highly polymerized polyphosphates, such as hexametaphosphate (HP), in which the ratio of Na_2O to P_2O_5 is very close to 1:1. They bind directly with salt-free myosin B, but their binding is rather inhibited at high salt concentration and in the presence of divalent cation.

Materials and Methods

The reagents were commercial products of analytical grade and were used without further purification.

Myosin B was prepared from rabbit back and leg muscle by the method de-