Processing of Crambe for Oil and Isolation of Erucic Acid

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ABSTRACT: Crambe seed *(Crambe abyssinica)* is an excellent, recently established source of high-erucic acid oil. Erucic acid has a number of important and potential applications. To develop this potential, a rapid bench-scale method was desired whereby purified erucic acid in up to several 100-g quantities could be produced from crambe seed. Using the method developed, oil was expressed from dried, intact seed; clarified, degummed, and bleached; and saponified and acidified to obtain the free fatty acids. Analysis by inductively coupled plasma of the free fatty acids showed negligible levels of phosphorus and most minerals. Erucic acid was twice crystallized from 95% ethanol at –14°C, resulting in a purity of 87.1%. This process yielded 365 g erucic acid crystals per kg bleached oil. Nuclear magnetic resonance analysis showed that the prepared erucic acid had an excellent pattern of correlation with a commercial standard. The time needed to convert 1 kg of crambe seed to erucic acid is about 48 h. Crystal filtration and drying stages under the current process conditions require 30% of the overall time. The method is suitable for producing adequate quantities of erucic acid for use in studies of its bench-scale conversion. There is obviously, still, a fruitful field of work to be explored in the formalization of refining procedures for crambe oil. It seems that crambe is destined to continue expansion into the high-erucic acid oil markets.

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Erucic acid (C22:1, Δ13) is an important fatty acid in the oleochemical industry. It is a naturally occurring fatty acid in seed storage triglycerides of the Cruciferae family. The current, major industrial source of erucic acid is high-erucic acid rapeseed oil (1). Rapeseed oil contains more than 40% erucic acid. However, another member of the Cruciferae family appears to be even more interesting as an industrial crop. Crambe (*Crambe abyssinica* Hochst. ex. R.E. Fries), having a significant amount of oil (32–36%) with a high-erucic acid content (52–59%) along with its wide climatic and agronomic adaptation, is a promising alternative crop (2–4).

In addition to oil, Massoura *et al.* (5) have reported that other valuable by-products can be obtained from crambe, such as protein meal and possibly fiber. Acceptance of crambe meal by the feeds industry is based on its attractive price and satisfactory performance as a feed for ruminant animals (6). Commercial crambe production began in the northern Great Plains in 1990, and North Dakota's production had reached 24,000 ha within the next 4 yr (6). Crambe has been grown commercially since 1990, and has replaced rapeseed as the dominant high-erucic-acid crop in the United States (6). Drought tolerance is another attractive characteristic of crambe. Tolerance of crambe to drought is equal to or slightly less than that of cereal grains. It is more drought-tolerant than corn, canola, or soybeans at all stages of growth (7).

At present, world consumption of high-erucic acid oils for industrial applications is estimated to be 57,000 MT per year, with the United States accounting for 16,000 MT. This is up from a 1991 industry average of 12,500 MT for the U.S. share. Other major industrial users are Europe and Japan (8). In 1996, it was estimated that the U.S. supply of industrial rapeseed oil was 2,300 MT of domestic production and 14,000 MT imported from Canada and Europe (8,9).

The primary market for high-erucic acid oils is erucamide, a slip agent critical to manufacture and use of polyolefin films (6). Films such as polyethylene are produced commercially for familiar products such as bread wrappers, shopping and garbage bags, shrink wraps, and plastic sheeting (2,6). One of the selling points of the erucic-acid-oil products is their enhanced biodegradability compared to their petroleum-based counterparts (9). Because crambe and rapeseed oils provide a high degree of lubrication, they are also used either directly as lubricants or in formulations. They are used as spinning lubricants in the textile, steel, and shipping industries; as cutting, metal-forming, rolling, fabricating, and drilling oils; and as marine lubes. Erucic acid can also be oxidatively cleaved to brassylic acid for use in the production of polyesters (3). The oxidative cleavage of erucic acid can be performed *via* ozonolysis (3) or by reaction with hydrogen peroxide in the presence of an inorganic oxide catalyst (10).

Current methods of erucic acid extraction are costly and the finished product contains by-products which impart undesirable properties (1). One study was published on the purification of erucic acid from crambe oil (11); several related studies based on rapeseed oil have also been reported (12,13).

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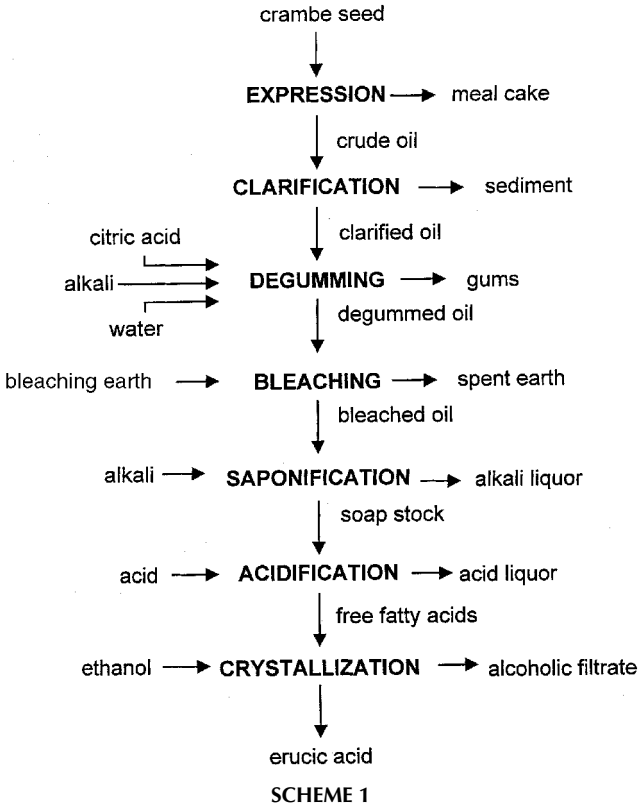
The objective of this study was the development of a bench-scale process for isolating erucic acid with adequate recovery and purity, using crambe seed as the starting material. The resulting erucic acid will be used for subsequent studies on its conversion to brassylic acid and related products.

EXPERIMENTAL PROCEDURES

Source of materials. Crambe seeds (*C. abyssinica* cv. BelEnzian) with a moisture content of 10.8% wet basis (w.b.) were obtained from an experimental station of North Dakota State University (Casselton, ND) and stored at 6°C until used. The seeds were placed in burlap bags and dried in a cabinet dryer (Standard Industries, Fargo, ND) with forced air at 59°C for 24 h to a final moisture content of 5.9% (w.b.). Finally, the dried seeds were placed in polyethylene bags, refrigerated until used, and brought to room temperature (23°C) before being pressed. Argon gas plasma was Praxair 99.999% pure. Mineral calibration standards were prepared from analytical grade reagents obtained from VWR Scientific Products (Minneapolis, MN). Erucic acid (90% purity) was from Sigma Chemical Co. (St. Louis, MO). The complete process for producing erucic acid from crambe seed is outlined in Scheme 1.

Oil expression and clarification. Oil expression was performed with a Komet screw oil expeller (Model S 87G, IBG Monforts GmbH & Co., Monchengladbach, Germany) equipped with an electrical-resistance heating ring attached around the outer surface of the press head. A digital thermometer (Digi-Sense Model 08528-20 Cole Parmer, Vernon Hills, IL) with a type-T thermocouple measured the temperature on the surface between the heating ring and the press head. Temperature was manually controlled at 120 ± 3°C by the on/off switch. Six replicates of 5 kg each were pressed with compression screw R8 at a screw speed of 20 rpm and with an 8-mm diameter restriction die. Samples of crambe seeds were fed by gravity into the hopper. The process was allowed 30 min to reach steady-state conditions before determining the suspended solids content of the oil. Steady state was judged to be attained based on constant thickness and consistency of the cake and by a constant flow rate of oil and cake.



SCHEME 1

The crude oil obtained from the expression process was allowed to stand for 1 d at room temperature then decanted into centrifuge tubes and centrifuged at 3250 × *g* for 20 min. The sediments from these two steps were combined and vacuum filtered over a Buchner funnel using Whatman No. 4 filter paper (Clifton, NJ) to recover additional clarified oil. The filtered solids were defatted in duplicate for determination of crude fat content using the American Association of Cereal Chemists (AACC) Method 30-25 (14). The defatted solids were weighed for calculation of suspended solids content of the crude oil. All the clarified oil from the six extractions was combined into one sample for subsequent processing.

Degumming and bleaching. Degumming of clarified oil was based on the Alfa-Laval combined degumming/neutralization procedure reported by Nilsson-Johansson *et al.* (15) but with citric acid as the complexing agent instead of phosphoric acid. The clarified oil was divided into four replicate batches ranging from 620–1430 g each, heated to 60–70°C, and intensely mixed with 0.3% (w/w) of 50% (w/w) citric acid using an Omni homogenizer (Model 2000, Omni Int'l., Waterbury, CT) with a 10 × 95 mm sawtooth rotor at 5000 rpm for less than 30 s. Immediately afterward, 2 M NaOH was added to neutralize the acid [5.5 mL/g 50% (w/w) citric acid] and the mixture homogenized again at the same temperature and time. Subsequently, the mixture was hydrated with the addition of 2% distilled water and slowly stirred at 50°C for 15 min to allow separation of flocculated gums. After separating the gums from the mixture by centrifugation at 3250 × *g* for 20 min, the upper oil layer was decanted off and saved. The wet gums were weighed.

Degummed oil was bleached according to the American Oil Chemists' Society (AOCS) Method Cc 8b-52 (16), with slight modifications: 5% (w/w) of grade F-105 bentonite acid-leaching powder (Filtrol grade 105, Engelhard Co., Chemical Catalysts Group, Jackson, MS) was added to the oil instead of the 4% (w/w) official activated bleaching earth recommended for green oils with high chlorophyll content; then, a short-step filtration using a Buchner funnel with Whatman No. 4 filter paper under vacuum conditions was conducted instead of using the refining apparatus and Whatman No. 2 filter paper in the official method. Bleaching was performed in seven replicates of 590–960 g each, and the loss of oil that adhered to the used bleaching earth was calculated by weight loss.

Saponification. Two 600-g batches of bleached oil were each divided into three 200-g samples and saponified under reflux. The procedure was a modification of AOCS method Cd 3-25 (16) using water in place of ethanol and NaOH in place of KOH. On the basis of the saponification value, 350 mL of aqueous 2 M NaOH solution, providing a 13% excess, was added with stirring to 200 g warm (50°C) bleached oil in a 4-L flask. The mixture was boiled at 108–112°C with reflux for 6 h until the sample became a homogeneous, milky liquid giving a heavy, white, soapy upper layer upon cooling. The contents were cooled for 1 h at room temperature (23°C) and 4 h at 6°C. The alkali liquor was drained from the cake of soap stock.

Acidification. The soap stock was slurried with one-half volume of 30% (w/w) HCl, then additional acid was added and the mixture was brought to a boil. The acid solution was added slowly and with constant agitation until the soaps were dissolved and pH 1 was achieved. This yielded a brownish-yellow mixture of free fatty acid (FFA) and acid liquor which was cooled for 1 h at 23°C and 4 h at 6°C to produce two well-separated phases without emulsion. The upper FFA layer was removed and gently washed with 300 mL distilled water.

Isolation of erucic acid. The FFA was dissolved in 95% ethanol (6 mL/g of FFA) at 38–42°C on a steam bath, and cooled to the crystallization temperature (−14°C) in a walk-in freezer. The mixture was allowed to crystallize overnight. The crystals were recovered from the mixture on Whatman No. 1 filter paper, washed twice with 100 mL of cold 95% ethanol, then held over vacuum on an unchilled Buchner funnel until the last traces of alcohol were removed. The alcoholic filtrate was held at −14°C for an additional 1 d, producing a second crop of crystals which were recovered, rinsed, and dried as described above. The first and second crops were combined to form the first crystallization product.

A second crystallization was carried out by dissolving the first crystallization product in 95% ethanol (6 mL/1 g of FFA), cooling the solution to −14°C, and collecting the crystals as described for the first crystallization, except that a second crop of crystals was not collected.

Moisture and fat analysis. Moisture content was determined in triplicate by AACC Method 44-19 (14). The crude fat was determined in duplicate by AACC Method 30-25 with three modifications: (i) the samples were ground on a Brinkmann mill Model ZM1 (Brinkmann Retsch GmbH & Co. KG, West Germany) to pass through a 1-mm screen prior to the crude fat extraction, (ii) hexane was used instead of petroleum ether, and (iii) the hexane from the crude fat was removed with a Brinkmann Model R111 Rotavapor (Brinkmann Instruments, Inc., Westbury, NY).

Fatty acid analysis. The fatty acid content of crambe oil and crystal fractions was determined by gas chromatography. An aliquot of the fraction was esterified with diazomethane in diethyl ether solution according to the procedure of Metcalfe and Wang (17). The sample was then injected into a Hewlett-Packard 5890 gas chromatograph containing a DB-23 capillary column (25 m × 0.25 mm, J&W Scientific Incorporated, Folsom, CA), which was held at 185°C for 15 min, increased to 220°C at 10°C per min, held at 220°C for 1 min, increased to 240°C at 20°C per min, and finally held at 240°C for 0.5 min, for a total run time of 11 min. Individual fatty acids were identified by retention times, and quantified against a standard fatty acid mixture. Two independent samples were analyzed and the fatty acid composition was calculated as the area percentage of methyl ester peaks.

Saponification value. The amount of alkali required to saponify crambe oil was determined in duplicate by AOCS Method Cd 3-25 (16).

Nuclear magnetic resonance (NMR) analysis. NMR data were obtained at 295 Kelvin (K) in $CDCl_3$ solution using a JEOL Model GSX-270 instrument (Japan Electronics Optic Ltd., Tokyo, Japan) operating at 270 MHz (^1H). Chemical shifts are reported in ppm relative to $Si(CH_3)_4$.

Color. The color of the whole seeds; crude, degummed and bleached oil; FFA; erucic acid crystals; and the oil from the alcoholic filtrate recovered after each crystallization was measured using a Gardner Colorimeter (Model XL-23, Gardner Instruments, Bethesda, MD) with a reference standard white tile ($L = 91.94$, $a = -1.03$, $b = +1.14$). Readings of L (measure of light reflectance), a (represents red when positive and green when negative), and b (yellow when positive and blue when negative) were expressed according to Hunter values. Color of oil samples was measured within 1 wk. Two samples were analyzed from each process step and duplicate readings from each sample were recorded.

Mineral analysis. The mineral content of crambe products was determined using a nitric acid digestion procedure described by Havlin and Soltanpour (18). Samples weighing 500 mg (solid materials) or 1 mL of oils and liquid fatty acid products were transferred to 50 mL Folin-Wu tubes with graduations at 12.5, 25, 35, and 50 mL. Concentrated nitric acid (10 mL) was added to each sample and allowed to predigest at room temperature overnight. Samples were then heated in an aluminum block digester at a temperature of 125°C until the volume was reduced to 2–3 mL and clear (although not necessarily colorless). The samples were then removed from the digester block, allowed to cool, and subsequently brought to 12.5 mL with concentrated nitric acid. Deionized water was then added to dilute the samples to a final volume of 50 mL giving a matrix of 25% nitric acid. Elemental determination was immediately done by inductively-coupled plasma emission spectroscopy (ICP-ES) (Model PS 1000; Leeman Labs., Inc., Hudson, NH) using an ionized argon gas plasma. Calibration standards were prepared using 1000 mg/L stock solutions and diluted with a 25% nitric acid matrix for Ca, Mg, P, S, Cu, Fe, K, and Na. All standards were prepared using a weight by volume dilution in concentration ranges of 4 to 40 mg/L based on a preliminary concentration scan with the ICP-ES instrument. Samples with concentration values outside the calibration range were diluted 10-fold to bring them within the range of instrument calibration.

Statistical analysis. Duncan's Multiple Range Test was performed to determine the difference in mean values of replicates. Significance was determined at $P < 0.05$ (19).

RESULTS AND DISCUSSION

Oil expression and clarification. In industry, oilseeds undergo extensive preparation prior to expeller pressing. For example, oilseeds may be dehulled to eliminate nonoil-bearing matter, conditioned and flaked to rupture the oil cell walls, and then cooked to denature protein and decrease oil viscosity. Each type of oilseed has unique requirements with respect to expeller configuration. Expeller design is especially important if the press cake will not undergo subsequent solvent extraction. Where solvent extraction is not employed, the oilseed may be pressed to a residual oil content in the cake of less than 7%.

This low residual oil content, which is essential in industry, cannot be easily duplicated on the bench scale and was not an objective of this study. We opted to eliminate seed preparation other than drying, in order to have a simple, quick method for producing 1–2 kg crambe seed oil. A preliminary set of tests was performed using a Komet S87 screw oil expeller to determine a suitable combination of seed moisture content (4.1, 5.9, and 10.8%), expeller temperature (100 and 120°C), screw speed (20 and 25 rev/min) and restriction die size (6, 8, and 10 mm). Residual oil content decreased with decreasing seed moisture content, increasing expeller temperature, and decreasing screw speed (Table 1). Use of the low moisture content (5.9%) was particularly important, but further reduction in moisture content (4.1%) was not beneficial and resulted in more sediment in the oil. Residual oil content decreased with decreasing restriction die size to 8 mm, but use of the 6-mm die plugged the expeller. Oil extraction rate increased with increasing screw speed, but this advantage was offset by higher residual oil in the cake.

By using the conditions selected from preliminary tests (5.9% moisture, 120°C, 20 rev/min, and 8-mm die), the rate of oil production in the full extraction tests averaged 0.64 ± 0.03 kg/h with 10.6 ± 0.1% residual oil in the cake. The oil samples evaluated for sediment contained 2.52 ± 0.06% solids. The seed rate to the expeller averaged 4 kg/h and initial oil content was 26.6 ± 0.2%, thus 60% of the seed oil was recovered. All of these weights and contents are shown on a dry basis. Cake temperature varied from 101 to 103°C and oil temperature was 90 to 93°C. The cake emerged as a cohesive, rope-like mass, leathery in texture at first but becoming hard upon cooling.

It was concluded that this simple, quick method for producing crambe seed oil was suitable for producing oil for subsequent process studies. Lower residual oil content and higher oil production rate might be attained with further seed preparation, such as dehulling, flaking, and cooking, but this is not ensured given the small scale of the expeller used in this study. Also, because the entrance from the hopper into the expeller intake is 5 cm in width, flaked seeds would not flow uniformly or unaided into the expeller, whereas intact crambe has excellent flowability.

Degumming and bleaching. Degumming of crude vegetable oils reduces the content of phosphatides, metallic prooxidants and other minor components (20,21). Water-degumming typically reduces the phosphorus content of crude soybean oil from about 800 to 150–250 mg/kg (21). In conventional processes, vegetable oil is first water-degummed and subsequently refined with caustic or with phosphoric acid/caustic. The effluent problems of edible oil refining are caused mainly by these caustic treatments (22). However, the combined acidification with citric acid and neutralization procedure used in this study should not cause significant effluent problems. Citric acid is a powerful complexing agent and allows easy separation of gums. The complexes with phosphatides from crambe oil should be hydratable and thus pass into water added after the acid treatment.

In this study, the phosphorus content of the crude crambe oil was reduced by degumming from 201 to 129 mg/kg (Table 2). The percentage oil recovery of crambe oil from combined degumming/neutralization was 95.7 ± 0.4%, based on the following definition:

$$\text{oil recovery } (\%) = \frac{\text{weight of oil recovered at end of step}}{\text{weight of oil used at start of step}} \times 100 \quad [1]$$

Yet, the degumming procedure used is reported to reduce the phosphorus content of soybean and rapeseed oil to 2 mg/kg (15). Alkali refining was not used between degumming

TABLE 1
Preliminary Evaluation of Selected Pressing Conditions for Crambe Seed[a]

Seed moisture content (% w.b.)	Expeller temperature (°C)	Screw speed (rev/min)	Restriction die size (mm)	Residual oil in cake (% db)	Oil extraction rate (kg/h)	Sediment in oil (%)
4.1	120	20	8	11.1 ± 0.3^{c,d}	0.61 ± 0.00^{b}	4.4 ± 0.3^{a}
5.9	120	20	8	10.7 ± 0.2^{d}	0.60 ± 0.00^{b}	3.3 ± 0.3^{b,c}
10.8	120	20	8	13.7 ± 0.1^{a}	0.42 ± 0.00^{d}	2.9 ± 0.3^{b,c}
5.9	120	25	8	11.2 ± 0.0^{c,d}	0.71 ± 0.00^{a}	3.4 ± 0.1^{b}
5.9	110	20	8	11.9 ± 0.8^{b,c}	0.56 ± 0.01^{c}	2.9 ± 0.3^{b,c}
5.9	120	20	6	N.A.	N.A.	N.A.
5.9	120	20	10	12.7 ± 0.04^{a,b}	0.55 ± 0.02^{c}	2.3 ± 0.5^{c}

[a]Mean values ± standard deviation of duplicate tests performed by pressing 1 kg seed per test using a Komet screw oil expeller (Model S 87G, IBG Monforts GmbH & Co., Monchengladbach, Germany). Values within a column followed by the same roman superscript are not significantly different at $P < 0.05$, based on Duncan's Multiple Range Test. N.A., not applicable, expeller was plugged; w.b., weight basis.

TABLE 2
Mineral Analysis (mg/kg) in the Crambe Seed, Oil, and Free Fatty Acid[a]

	Ca	Mg	K	Na	P	S	Cu	Fe
Whole seed	9093 ± 11[a]	2999 ± 88[a]	11479 ± 556[a]	93 ± 2[a]	6563 ± 94[a]	8355 ± 41[a]	9 ± 2[a]	55 ± 26[a]
Oil								
Crude	129 ± 7[b]	70 ± 4[b]	64 ± 13[b]	3 ± 6[c]	201 ± 2[b]	99 ± 6[b]	3 ± 1[b]	6 ± 2[b]
Degummed	95 ± 6[b,c]	46 ± 3[c]	4 ± 7[c]	31 ± 5[a,b]	129 ± 10[c]	89 ± 8[b]	2 ± 2[b]	4 ± 3[b,c]
Bleached	74 ± 3[c]	29 ± 2[d]	9 ± 10[c]	18 ± 3[b,c]	57 ± 4[d]	55 ± 5[c]	2 ± 1[b]	4 ± 1[b,c]
Free fatty acids	2 ± 1[d]	1 ± 0[e]	6 ± 2[c]	187 ± 29[a]	0.0 ± 0[c]	44 ± 2[d]	1 ± 0[b]	3 ± 1[c]

[a]Values within a column followed by the same roman superscript are not significantly different at $P < 0.05$, based on Duncan's Multiple Range Test.

and bleaching, in order to retain free erucic acid prior to the saponification step. Instead, degumming was followed directly by bleaching, which removed much of the remaining phosphatides and oxidation products as well as coloring substances. The phosphorus content was further reduced, by bleaching, to 57 mg/kg with a 96.5 ± 0.3% recovery of crambe oil. Thus, phosphorus was still high even after degumming and bleaching. Other studies found that cooking the seed prior to pressing insolubilizes phosphatides which can then be removed by hydrating with steam or hot water (23). Consequently, the seed should have been cooked prior to pressing to achieve low phosphorus content in the degummed, bleached oil.

Saponification and acidification. Saponification of crambe oil with aqueous NaOH was performed to hydrolyze the neutral oil to free fatty soaps and glycerol. The required proportion of NaOH solution was based on the saponification value of 175 ± 0.9 mg KOH/g crambe oil with allowance of a 13% excess.

The oil and NaOH solution were initially mixed at room temperature to prevent lumping of the soap, known as "bunching" (24). Although reflux was not essential, this minimized loss of soap from the mixture and maintained a constant proportion of one of the key reactants, water. After boiling for 6 h, the soap lost its greasy appearance and the residual alkaline cooking liquor showed no turbidity on cooling. The product was a heavy, white soap stock with 51% moisture. The total dry weight of the soap stock averaged 598 ± 0.5 g per 600 g batch bleached oil. The alkaline cooking liquor remaining from this step contained the bulk of the glycerol and could contain some impurities such as excess alkali and sodium carbonate, as well as color and odor-imparting bodies (24).

The soap stock was mixed and heated with 30% (w/w) HCl to complete acidification to liberate the FFA. Because of their low specific gravity, the FFA came to the top as a brownish yellow layer. The acid liquor, which contained the residual glycerol, sodium and other undesirable impurities, formed the lower layer and was later discarded. Thus, the fatty acids were significantly purified by saponification and acidification, and therefore well suited for preparation of erucic acid. The recovered FFA averaged 581 ± 3 g per 600 g bleached oil. This 96.8% recovery compares well with the stoichiometric recovery of 96.1%. The stoichiometric value was calculated on the basis that glycerol and sodium were replaced by hydrogen, that impurities were negligible, and that no fatty acid was lost.

A common industrial procedure for hydrolysis of triglycerides uses high pressure and temperature with water and a zinc oxide catalyst (25). This produces less effluent; however, a suitable pressure vessel was not available in our lab.

Isolation of erucic acid. A simple and convenient bench-scale procedure for isolation of erucic acid (C 22:1, Δ13) from crambe oil was performed. Erucic acid was prepared from the FFA by two-step crystallization from 95% (v/v) ethanol at −14°C. The percentage recoveries of erucic acid were 64 and 99% in the first and second steps, respectively. The content of erucic acid was increased from 52.8 to 81.1% in the first crystallization alone (Table 3). The erucic acid recovery from the crude crambe oil and the concentrates of erucic acid was defined as:

$$\text{erucic acid recovery (\%)} = \frac{\text{product weight} \times \text{product erucic acid content}}{\text{starting weight} \times \text{starting erucic acid content}} \times 100 \qquad [2]$$

To obtain a satisfactory crystallization, it was necessary to acidify the soap stock prepared by saponification to pH 1. To completely and quickly obtain a clear solution of the FFA in ethanol, the mixture was heated to 38–42°C. The formation of crystals began 2 h after holding the solution at crystallization temperature (−14°C), although the solution was allowed to crystallize at least 18 h before the first crop of crystals were collected.

The filtrate was left one more day at −14°C and the small, second crop of crystals was recovered and dried. The two crops of crystals were combined for a total weight of 238 g of erucic acid concentrate obtained from the first crystallization. Samples of this erucic acid concentrate were tested by gas chromatography (GC).

A second crystallization raised erucic acid content to 87.1%. A third crystallization under the same conditions increased erucic acid content to 90.2%. A fourth crystallization would probably further increase the purity of erucic acid.

The weight of ethanol used per weight of FFA (ethanol/FFA ratio) is a critical variable that affects the rate of crystal formation, the amount of crystals formed, as well as the composition of the crystals. These three measures of the process outcome also vary with the temperature of crystallization (26). In a preliminary crystallization study, an

TABLE 3
Fatty Acid (%) Composition of Bleached Oil, Free Fatty Acids, and Crystallization Products Prepared from Crambe Seed[a,b]

Fatty acids	Bleached oil	FFA	First crystallization	Second crystallization	Alcoholic filtrate[c]
16:0	3.5 ± 0.2	2.3 ± 0.4	0.6 ± 0.3	0.2 ± 0.1	3.5
18:0	1.7 ± 0.7	0.9 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	1.1
18:1	14.1 ± 0.3	13.8 ± 0.3	1.1 ± 0.8	n.d.[d]	21.9
18:2	10.8 ± 0.3	10.6 ± 0.4	n.d.	n.d.	16.8
18:3	6.3 ± 0.2	6.0 ± 0.2	n.d.	n.d.	9.5
20:0	0.7 ± 0.0	1.0 ± 0.2	2.3 ± 0.6	2.1 ± 0.2	nil
20:1	6.6 ± 0.1	3.9 ± 0.1	n.d.	n.d.	6.2
20:2	0.4 ± 0.1	1.8 ± 0.1	1.1 ± 0.3	n.d.	2.9
22:0	0.6 ± 0.1	1.5 ± 0.3	5.7 ± 3.2	5.3 ± 1.0	nil
22:1	52.8 ± 1	51.5 ± 0.4	81.1 ± 0.05	87.1 ± 1.4	29.6
24:0	1.4 ± 0.6	0.6 ± 0.2	2.5 ± 1.5	2.3 ± 0.5	nil
Other	1.1	6.1	4.71	2.44	8.2

[a]Values are listed as mean ± standard deviation from two independent batches.
[b]95% (vol/vol) ethanol (6 mL/g of FFA) at –14°C.
[c]Calculated by material balance on the basis of the fatty acids contents in FFA and the second crystallization.
[d]n.d., not detected; FFA, free fatty acid.

ethanol/FFA ratio of less than 5 mL/g resulted in slow crystallization. Also, the crystals had an oily, wet appearance, which indicated high concentrations of the undesirable fatty acids. The ethanol/FFA ratio was subsequently increased gradually to 6 mL/g of FFA, at which point white crystals formed rapidly.

Low-temperature crystallization procedures employing aqueous and nonaqueous solvents in conjuction with other methods have been reported for the preparation of erucic acid from rapeseed oil (11–13). However, satisfactory recoveries of pure erucic acid were not obtained. The methyl ester of erucic acid was crystallized once from aqueous acetone at temperatures less than 20°C, giving no more than 20% recovery of 95% pure erucic (12). Direct crystallization of the mixed acid soaps from 90% methanol followed by three recrystallizations at 15°C produced crystals with better than 90% erucic acid. Recoveries of up to 48% were reported, depending on the original composition of the rapeseed oil acids (13).

The purification of erucic acid by low-temperature (–11°C) crystallization from aqueous ethanol was used by Hagemann *et al.* (11). The solvent system used for the purification of erucic acid was an ethanol/water ratio of 3:1 (vol/vol). The solvent per gram of FFA ranged from 4.0 to 5.8 mL. The third crystallization produced crystals containing 76% erucic acid with a recovery of 66%. The starting material contained 56% erucic acid and was derived from crambe oil. Our recoveries were similar; however, although their starting content of erucic acid was higher than our material, the erucic acid content of their crystals was much less. The crystallization of the methyl esters using aqueous acetone (12) resulted in substantially better purity than crystallization using methanol or ethanol, but recovery was poor.

More recently McNeill (1) reported a new approach to the isolation of erucic acid from vegetable oils by enzymatic hydrolysis using lipase. Fatty acids having less than 22 carbon atoms were selectively removed from triglycerides that also contained one or two erucic acid groups. This resulted in a glyceride fraction enriched in erucic acid and a FFA fraction. Hydrolysis of rapeseed oil by *C. rugosa* lipase for 48 h gave a glyceride fraction containing up to 95% erucic acid and up to 90% recovery. This approach deserves further consideration. However, the long process time required makes lipase, as yet, unsatisfactory for a commercially useful process.

Fatty acids composition. The contents of erucic and other fatty acids in samples of bleached crambe oil, FFA, and first and second products of crystallization were determined by gas chromatography (Table 3). The composition was similar to other reports of crambe fatty acid composition (27). Fatty acids in the crambe oil were predominantly erucic acid (52.8%) and C18 fatty acids. The fatty acid compositions of bleached oil and FFA were similar; the few inconsistencies were small and may be typical for GC analysis. Since levels of linoleic and linolenic acids remained the same, there was no oxidation of the sample during saponification and acidification.

The main fatty acids and their melting points reported in crambe oil (27) are: oleic (18:1), 16.3°C; linoleic (18:2), –6.5°C; linolenic (18:3), –12.8°C; arachidic (20:0), 75.4°C; gadoleic (20:1), 75.4°C; behenic (22:0), 80°C; erucic (22:1), 33.4°C; and lignoceric (24:0), 84.2°C. The major contaminants in the erucic acid concentrates were expected to be those fatty acids having a higher melting point. Although solubility is not generally dependent on melting point, both characteristics are influenced in a similar manner by carbon chain length and degree of unsaturation (28). This was borne out by the GC analysis. The most persistent impurities in the first and second crystallization products were behenic, lignoceric, and arachidic acids. The solvent (95% ethanol) and conditions in this study were particularly effective in the removal of C18 polyunsaturated acids from erucic acid.

NMR proved that the erucic acid concentrate prepared by our procedure (third crystallization; 90.2% erucic acid) had

an excellent pattern of correlation with the chemical structure from the sample of 90% pure erucic acid obtained from Sigma Chemical Co. The similarity in the behavior of the resonance signals for the experimental and commercial erucic acid samples was not surprising considering the higher purity demonstrated by GC analysis on the product from the second crystallization. Figure 2 shows the 270-MHz NMR spectra of the experimental and commercial erucic acid samples in the presence of $Si(CH_3)_4$. A discrete signal, with the expected peak area and first-order multiplicity, is observed for the magnetically nonequivalent protons in the molecule as far down the chain as H-5. With the exception of the singlet for the chain methylene protons (H-2) the multiplicity of other signals is present due to peak overlap, however, the general structure information can be obtained from these spectra. Thus, one can confirm spectrally the fact that the signals for the olefinic protons (H-4) are present in both spectra with positions magnetically distinguishable.

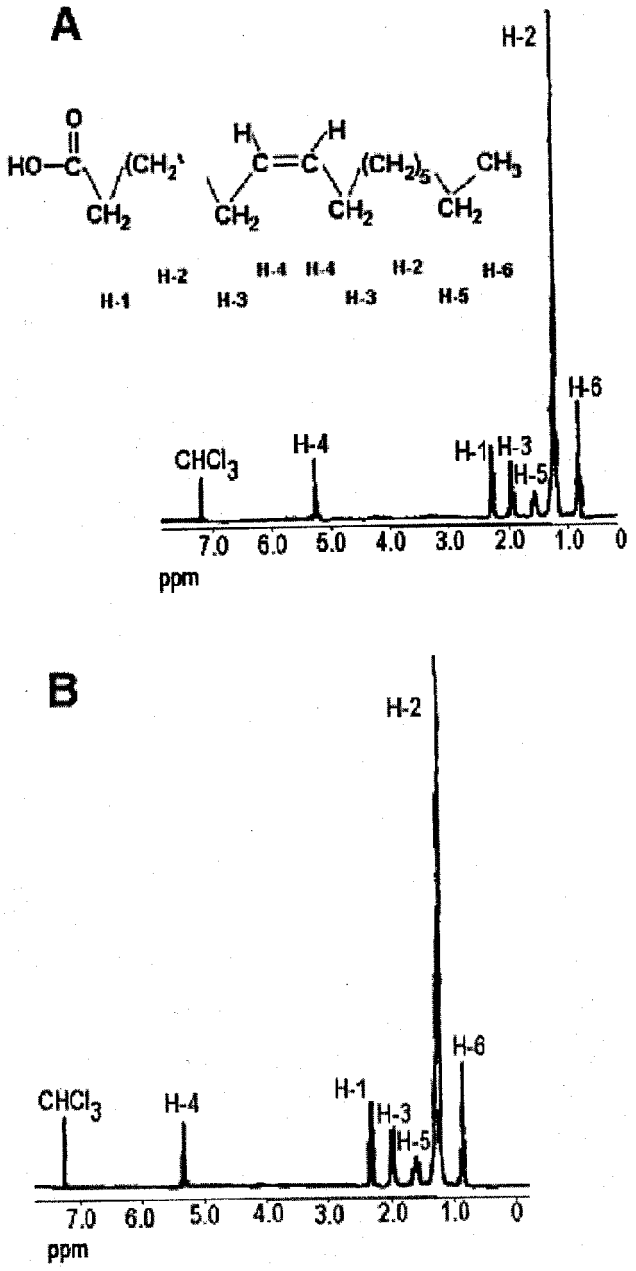


FIG. 2. ^{1}H 270-MHz nuclear magnetic resonance spectrum from structural-related information of the experimental (A) and commercial (B) erucic acid in $Si(CH_3)_4$.

The chemical structure of a simple molecule can often be elucidated by first-order interpretation of the NMR spectral data (29). The major features of the NMR spectra from 0 to 6 parts per million (ppm) for erucic acid are illustrated in Figure 2. The tracing is restricted to this region because this contains most of the signals for the protons of the sample, with the exception of the magnetically indeterminable signal of the carboxyl proton. The majority of the methylene protons have NMR signals with distinguishable peaks. We used the NMR study of erucic acid samples to show that this interpretative technique can help determine the purity of the erucic acid crystals. For example, the NMR spectrum revealed no phosphorus or other mineral contaminants with protons that cause NMR signals.

Color. Probably, the most widely used system of color measurement in oils is the Lovibond Tintometer (30,31). However, use of the Gardner Colorimeter avoids subjective human judgments on the color evaluation of crambe materials. The color of crambe seeds and different products are reported in Table 4. Some significant differences ($P > 0.05$) were found between degummed and bleached oils. The Hunter lightness (L) was lower in both materials, but the value of this color parameter increased from the degummed oil when the oil was bleached. These color differences ($P > 0.05$) can be explained on the basis of the carotenoid and chlorophyll components removed during the bleaching stage. In general, higher (L, a, b) values for erucic acid concentrates from the first and second crystallization were found as opposed to the FFA, as starting material for the preparation of these crystals. However, color differences between the crystals from the first and second crystallization were small ($L <$

TABLE 4
Color Scale Values of Crambe Seed, Oil, and Free Fatty Acids[a]

	Hunter values[b]		
Crambe materials	*L*	*a*	*b*
Whole seed	51.26[c]	+7.23[b]	+17.85[a]
Oil			
Crude (P)	6.45[f]	+5.75[c]	–0.38[f]
Degummed (P)	2.53[g]	+9.30[a]	–9.82[g]
Bleached (P)	7.85[e]	–0.29[f]	+0.22[e,f]
Free fatty acids (P)	12.25[d]	+0.54[e]	+4.02[d]
Erucic acid[c]			
1st (s)	91.90[b]	+0.51[e]	+8.75[b]
2nd (s)	96.18[a]	–0.2[f]	+5.37[c]
Oil recovered from alcoholic filtrate (P)	7.66[e]	+4.69[d]	+0.54[e]

[a] Standard values of the reference white tile: $L = 91.94$, $a = -1.03$ and $b = +1.14$. P, liquid; s, solid.
[b] Values within a column followed by the same roman superscript (a–g) are not significantly different at $P < 0.05$, based on Duncan's Multiple Range Test.
[c] From two crystallizations.

5, $a < 1$, $b < 4$). Interestingly, L was found to be higher than the value of the standard white tile utilized with purposes of comparison. The bright white color from the erucic acid concentrates presumably reveal that the treatment of FFA with alcohol at low temperature has an advantage of removing undesirable pigments that can interfere in the oxidative cleavage reaction of erucic acid.

Mineral analysis. Mineral composition of crambe seeds and different products are given in Table 2. In general, the mineral contents decreased in each progressive step of the process. Unexpectedly, the sodium content was much higher in the FFA. This might be due to incomplete removal of sodium which was added during saponification or contamination during the analysis.

The difference between the crude and bleached oils represents the components of the crude oil which are removed during refining. The range of materials found in crude oils with which the refiner has to cope includes: phosphatides, hydratables and nonhydratables; pigments, such as carotenoids, chlorophyll; and other compounds containing trace elements, e.g., iron, copper, calcium, magnesium, and sulfur. In this latter group the compounds presented may be copper and iron soaps, while the calcium and magnesium salts of phospholipids and phosphatidic acids are encountered as the greater part of the nonhydratable phosphatides (20). Sulfur compounds in crambe and rapeseed oil are the result of glucosinolates in the seed (32). The analysis on minerals carried out are crucial both to quality and economical refining procedures (30). After a deodorization process, the acceptable level of sulfur for commercial processing of rapeseed can be between 5 and 10 ppm for most purposes (30).

Citric acid chelates and thus deactivates trace element prooxidant catalysts, mainly copper and iron; the concentrations of nonchelated metals having some catalytic effect on oxidation is 0.005 mg/kg for copper and 0.03 mg/kg for iron (30).

Bleaching not only removes color pigments but also traces of metals and phosphatides conditioned by acid treatments after degumming with citric acid (20). In addition bleaching earths remove various polar compounds, and also hydroperoxides by catalytically decomposing them. This clean-up is essential for removal of catalyst poisons.

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REFERENCES

1. McNeill, G.P., Enzymatic Process for the Isolation of Erucic Acid from Vegetable Oils, U.S. Patent 5,633,151 (1997).
2. Lazzeri, L., O. Leoni, L.S. Conte, and S. Palmieri, Some Technological Characteristics and Potential Uses of *Crambe abyssinica* Products, *Ind. Crops Prod. 3*:103–112 (1994).
3. Gardner, J.C., Crambe: New Routes from Farm to Market, *INFORM 7*:986–989 (1996).
4. Mikolajczak, K.L., T.K. Miwa, F.R. Earle, and I.A. Wolff, Search for New Industrial Oils. V. Oils of Cruciferae, *J. Am. Oil Chem. Soc. 38*:678–681 (1961).
5. Massoura, E., J.M. Vereijken, P. Kolster, and J.T.P. Derksen, Isolation and Functional Properties of Proteins from *Crambe abyssinica* Oil Seeds, in *Progress in New Crops*, edited by J. Janick, ASHS Press, Alexandria, VA, 1996, pp. 322–327.
6. Carlson, K.D., J.C. Gardner, V.L. Anderson, and J.J. Hanzel, Crambe: New Crop Success, *Ibid.,* pp. 306–322.
7. Golz, T., Crambe, North Dakota State University, Fargo, ND, Alternative Agriculture Series, Number 4, January 1993, p. 2.
8. Chapman, P., HEAR Oil Supply Crimp Affecting Erucic Users, *Chemical Marketing Reporter,* Schnell Publishing Co., New York, NY, May 6, 1996, p. 10.
9. Dolack, P., HEAR Oil Prices Higher Due to Supply Tightness, *Ibid.*, March 4, 1996, p. 10.
10. Sibi, Mukund P., P. Boudjouk, and J. Ji, Method for Preparation of Carboxylic Acids, U.S. Patent 5,596,111 (1997).
11. Hagemann, J.W., K.L. Mikolajczak, and I.A. Wolff, Purification of Erucic Acid by Low-Temperature Crystallization, *J. Am. Oil Chem. Soc. 39*:196–197 (1962).
12. Taufel, K., K.O. Heims, and C. Franzke, Zur Darstellung von Erucasaure aus Rubol mit Hilfe der Tieftemperatur-Kristallisation, *Fette Seifen Anstrichm. 64*:1023–1026 (1962).
13. Chobanov, D., M. Agova, A. Popov, E. Chooparova, and C. Hadjikolev, Preparation of Pure Erucic Acid, *Chem. Ind.,* April 3, 1965, p. 606.
14. *Approved Methods of the American Association of Cereal Chemists*, American Association of Cereal Chemists, St. Paul, 1983, AACC Methods 30-25, 44–19.
15. Nilsson-Johansson, L., U. Brimberg, and G. Haraldsson, Experience of Prerefining of Vegetable Oils with Acids, *Fat Sci. Technol. 90*: 447–451 (1988).
16. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., American Oil Chemists' Society, Champaign, 1989, Methods Cc 8b-52, Cd 3-25.
17. Metcalfe, L.D., and C.N. Wang, Rapid Preparation of Fatty Acid Methyl Esters Using Organic Base-Catalyzed Transesterification, *J. Chromatogr. Sci. 19*:530–535 (1981).
18. Havlin, J.L., and P.N. Soltanpour, A Nitric Acid Plant Tissue Digest Method for Use with Inductively Coupled Plasma Spectrometry, *Commun. Soil Sci. Plant Anal. 11*:969–980 (1980).
19. Statistical Analysis System, *SAS/STAT User's Guide*, Version 6, 4th edn., Vol. 2, SAS Institute, Cary, 1990.
20. Hvolby, A., Removal of Nonhydratable Phospholipids from Soybean Oil, *J. Am. Oil Chem. Soc. 48*:503–509 (1971).
21. Segers, J.C., Superdegumming, a New Degumming Process and Its Effect on the Effluent Problems of Edible Oil Refining, *Fette Seifen Anstrichm. 84*:543–546 (1982).
22. Segers, J.C., Possibilities and Limitations for Decreasing the Environmental Load from the Refining of Edible Oils and Fats, *Ibid. 87*:541–543 (1985).
23. Thompson, J.C., and C.L. Peterson, An Automated Small Scale Oil Seed Processing Plant for Production of Fuel for Diesel En-

gines, *Vegetable Oil Fuels*, ASAE Publication No. 4-82, ASAE, St. Joseph, MI, 1982, pp. 260–268.
24. Kirschenbauer, H.G., *Fats and Oils: An Outline of Their Chemistry and Technology,* 2nd edn., Reinhold Publishing Co., New York, 1960, p. 115.
25. Othmer, K., Carboxylic Acids, in *Encyclopedia of Chemical Technology,* 4th edn., Vol. 5, edited by J.I. Kroschwitz, John Wiley & Sons, Inc., New York, 1993, p. 170.
26. Timms, R.E., Crystallization of Fats, in *Developments in Oils and Fats,* edited by R.J. Hamilton, Blackie Academic & Professional, New York, p. 205.
27. Sonntag, N.O.V., Composition and Characteristics of Individual Fats and Oils, in *Bailey's Industrial Oil and Fat Products,* 4th edn., Vol. 1, edited by D. Swern, John Wiley & Sons, New York, 1979, p. 423.
28. Formo, M.W., Physical Properties of Fats and Fatty Acids, *Ibid.,* p. 195.
29. Wineburg, J.C., and D. Swern, NMR Chemical Shift Reagents in Structural Determination of Lipid Derivatives: II. Methyl Petroselinate and Methyl Oleate, *J. Am. Oil Chem. Soc. 49*:267–273 (1972).
30. McGinley, L., Analysis and Quality Control for Processing and Processed Fats, in *Analysis of Oilseeds, Fats and Fatty Foods,* edited by J.B. Rossell and J.L.R. Pritchard, Elsevier Applied Science, New York, 1991, pp. 444–475.
31. Patterson, H.B.W., Important Tests Relating to Bleaching, in *Bleaching and Purifying Fats and Oils: Theory and Practice*, American Oil Chemists' Society Press, Champaign, 1992, pp. 217–218.
32. Daun, J.K., and D.I. McGregor, Glucosinolates in Seeds and Residues, *Ibid.*, p. 191.

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