Chemical Survey and Erucic Acid Content of Commercial Varieties of Nasturtium, *Tropaeolum majus* L.

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Nasturtium (*Tropaeolum majus* L.) oil contains the highest levels of erucic acid of known seed oils (75–80%). A significant portion of the acid is attached to the 2-position of the glycerol, and trierucin is a major component (*ca.* 50%) of the oil. Seeds from eleven varieties of commercially available garden nasturtium (*T. majus*) were screened for oil content, erucic acid levels and fatty acid distribution. Oil contents ranged from *ca.* 6 to 11%, and erucic acid levels in the oils ranged from 62 to 80%. One sample of *T. speciosum* was also analyzed, and contained 28% oil, fatty acids from C_{16} to C_{28} and triglycerides up to C_{72}.

KEY WORDS: Erucic acid, GC/GC–MS, nasturtium, nervonic acid, oil, protein, *Tropaeolum majus*, *T. speciosum*.

The Cruciferae family is known to contain many species with seed oils rich in erucic acid; rapeseed and crambe being two members with erucic acid levels ranging from 45–60% in their oils. However, one of the highest levels of erucic acid (*ca.* 80%) occurs in the family Tropaeolaceae, in the seed of the common garden nasturtium, *Tropaeolum majus* L. (1–8). *Tropaeolum majus* is unique among high-erucic acid species because it deposits a significant portion of erucic acid in the 2-position of glycerol and, thus, trierucin is a major component of the oil (3,4). One drawback to *Tropaeolum majus* as a source of erucic acid is the low oil content of its seeds. However, a number of flower seed growers reported that they have mastered the growing of nasturtium seed in respectable commercial yields (Knapp, S., and W. Roath, personal communications). Our continuing interest in high-erucic acid oils (9) prompted us to chemically screen some of these commercially available varieties of *T. majus* for their oil and erucic acid contents. We also examined several *Tropaeolum* samples in the National Center for Agricultural Utilization Research (NCAUR; Peoria, IL) seed collection that we previously had not analyzed.

EXPERIMENTAL PROCEDURES

Eleven varieties of *T. majus* seed samples were generously supplied by Denholm Seeds (Lompoc, CA), Waller Flower Seed Co. (Gaudalupe, CA) and Bodger Seed Co. (El Monte, CA). Three additional samples of *T. majus* and one sample of *T. speciosum* were from the NCAUR's seed collection. Analysis of the seed meal followed Official American Oil Chemists' Society (AOCS) Methods (10): Ash (Ba 5-49); crude fiber (Ba 6-61); moisture (Ac 2-41); nitrogen (Aa 5-38); crude protein (N × 6.25); oil (Ac 3-52); and nitrogen-free extract (NFE) by difference. Nonprotein nitrogen (NPN) was determined by room temperature extraction of 1.0 g of defatted meal with 40 mL of 0.8 *N* trichloroacetic acid (30-min shaking). Following centrifugation (15 min at 2000 × *g*), a 25-mL aliquot was removed and analyzed by normal Kjeldahl procedure for nitrogen. Seed weights (g/1000) were determined by replicated weighings (25 seeds/weighing; 4 weighings).

Fatty acid methyl esters (FAME) were prepared as previously described (11). For each analysis, a 1-μL sample (10 mg/mL in hexane) of FAME was injected into a Spectra Physics SP-7100 gas chromatograph (GC) (Spectra Physics, San Jose, CA) equipped with a 30 m × 0.32 mm SP2340 capillary column (Supelco Inc., Belafonte, PA), He carrier gas and flame-ionization detector (FID). Oven temperature was programmed from 150 to 220°C at 3°C/min with an initial 2-min hold at 150°C and a final hold of 5 min at 220°C. A second set of conditions—temperature program from 180 to 250°C at 3°C/min with a 1-min hold at 180°C—did not resolve the linolenate (18:3) and eicosenoate (20:1) peaks, but hastened elution of the tetra- (24:1) hexa- (26:1) and octacosenoate (28:1) peaks. A reference sample of FAME (C_8–C_{24}; Nu-Chek-Prep, Inc., Elysian, MN) was used for routine identification of esters. Triglyceride oils were analyzed by injecting 1-μL samples (10 mg/mL in hexane) into a Hewlett-Packard 5890A GC (Hewlett-Packard Co., San Fernando, CA) equipped with a 2.5 m × 0.32 mm GB-1 capillary column (Foxboro/Analabs, North Haven, CT), He carrier and FID. Oven temperature was programmed from 170 to 350°C at 10°C/min with an initial 1-min hold at 170°C. Triglyceride references (trimyristin, tripalmitin, triolein and trierucin; Nu-Chek-Prep) aided in identifying triglycerides by carbon number (CN). FAME from *T. speciosum* oil were analyzed further by GC–mass spectrometry (GC–MS) with a Hewlett-Packard 5890 GC connected to a Hewlett-Packard 5970 Mass Selective Detector unit. The GC was equipped with a 12.5 m × 0.2 mm crosslinked dimethylsilicone-coated, fused-silica capillary column, and was operated with splitless injection. The GC oven was temperature-programmed from 120 to 200°C at 20°C/min and then from 200 to 280°C/min at 2°C/min.

RESULTS AND DISCUSSION

Tropaeolum seed samples analyzed in this study are listed in Table 1. NCAUR inventory number was assigned to each of the eleven varieties received from the three commercial seed companies (NCAUR 64469–64479). Four additional accessions were available from the NCAUR seed collection. Except for one sample of *T. speciosum* (NCAUR 64155), all seeds analyzed were of the species *T. majus*. Proximate analyses of these seeds are given in Table 1. There is significant variability in seed size (range 122–209 g/1000) and in crude protein (26.7%, range 21.7–30.8%) for *T. majus*. Mean values for other components are: 11% fiber, 5.8% ash, 0.6% NPN and 44.2% NFE (by difference). The oil content of *T. majus* seed averaged 7.6% (range 5.9–10.5%), in contrast to the one *T. speciosum* sample that had 28% oil. Linear regression analysis showed that there was a high negative correlation ($r = -0.8928$) between oil content and seed weight in *T. majus* (Table 2). This suggests that developing small-seeded varieties would be a desirable goal for plant breeders interested in

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TABLE 1

Source of *Tropaeolum* Seeds and Analyses of Their Defatted Meals

NCAUR number[a]	Seed source[b]	Variety[b]	Seed weight (g/1000)	Composition (%)							
				Moisture	TKN[c]	Crude protein	Crude fiber	Ash	NFE[d]	NPN[e]	Oil
64469	Denholm	Scarlet Jewel	134	10.24	3.55	22.2	11.9	5.44	49.8	0.5	8.2
64470	Denholm	Mahogany Jewel	135	11.29	3.47	21.7	11.5	5.39	49.6	0.5	7.4
64471	Denholm	Primrose Jewel	127	9.40	3.83	23.9	12.9	5.50	47.7	0.6	7.8
64472	Denholm	Jewel Mix	128	10.54	4.54	28.4	10.3	5.39	44.8	0.6	7.7
64473	Denholm	Golden Jewel	122	12.29	4.09	25.6	12.9	7.25	41.4	0.6	7.3
64474	Waller	Scarlet Gleam	149	12.44	4.93	30.8	10.5	6.44	39.3	0.6	6.9
64475	Waller	WhirleyBird 7-Blend	172	12.67	4.80	30.0	10.4	6.34	39.5	1.1	6.7
64476	Waller	WhirleyBird Gold	195	13.95	4.26	26.6	10.5	5.38	42.9	0.7	8.5
64477	Waller	WhirleyBird Mahogany	209	13.41	4.56	28.5	9.9	5.61	42.0	0.6	5.9
64478	Waller	Cherry Rose	178	12.07	4.62	28.9	11.5	5.86	40.9	0.8	6.3
64479	Bodger	Jewel Mix	148	11.86	4.20	26.3	11.2	5.68	44.1	0.7	7.4
47179	NCAUR	—	—	9.75	4.40	27.5	10.9	5.52	45.7	0.7	7.6
47180	NCAUR	—	—	11.28	4.28	26.3	9.4	5.17	47.3	0.6	8.2
49241	NCAUR	—	—	10.62	4.40	27.5	10.5	6.65	44.3	0.5	10.5
64155[f]	NCAUR	—	29	10.70	6.91	43.2	20.2	6.53	18.8	—	28.0

[a]Inventory number assigned at the National Center for Agricultural Utilization Research (NCAUR).
[b]Denholm Seed (Lompoc, CA); Waller Flower Seed Co. (Guadalupe, CA); Bodger Seed Co. (El Monte, CA).
[c]Total Kjeldahl nitrogen (crude protein = N × 6.25).
[d]Nitrogen-free extract (by difference).
[e]Nonprotein nitrogen.
[f]*Tropaeolum speciosum;* all other entries are *T. majus.*

TABLE 2

Linear Regression Analysis of Compositional Data from *Tropaeolum majus* L.[a]

Compositional pair[b]	Correlation coefficient
Oil content × seed weight	−0.8928
Crude fiber × seed weight	−0.6558
Crude protein × seed weight	0.4855
Crude protein × oil content	−0.5758
Crude protein × crude fiber	−0.5491
Erucic acid × seed weight	−0.6008
Erucic acid × oil content	0.2443
Erucic acid × oleic acid	−0.9540
CN 66 triglyceride × erucic acid	0.9529
CN 64 triglyceride × erucic acid	0.6571
CN 62 triglyceride × erucic acid	−0.6385

[a]Analyses on eleven commercial varieties (see Table 1; NCAUR 64469-64479).
[b]Y (first component) = a ± bX (second component). CN, carbon number.

developing higher oil content in the species. Assuming genetic compatibility, a cross between *T. majus* and *T. speciosum* might be a route to germplasm with respectable oil yield and high-erucic acid content. It is interesting to note that *T. speciosum* also has high crude protein (43.2%) and high crude fiber (20.2%) associated with its small seed size (29 g/1000). Crude protein and crude fiber are oppositely correlated with seed weight and negatively correlated with each other in *T. majus* (Table 2).

Table 3 shows fatty acid composition obtained on the fourteen *T. majus* and single *T. speciosum* seed oil samples. Both species provide oil with high monoene fatty acid contents (>96%), but with different fatty acid distributions (4,5). Entries are listed in order of increasing levels of erucic acid (22:1), which ranges from 17.3% in *T. speciosum* (NCAUR 64155) to nearly 80% in some *T. majus* varieties (NCAUR 64472). *Tropaeolum speciosum* is one of the richest known sources (5,12) of nervonic acid (43% 24:1), and possesses genetic machinery for producing a wide range of fatty acids distributed from 16:0 to 28:1 (Table 3). Other conspicuous features of this fatty acid distribution are a substantial pool of 18:1 (*ca.* 25%) and a small pool of eicosenoic acid (20:1, <1%), in contrast to the substantial and relatively fixed pool of 20:1 (*ca.* 15%) among *T. majus* varieties. There is no correlation between erucic and eicosenoic acid contents of *T. majus*, but there is a strong negative correlation between erucic and oleic acids (r = −0.9540; Table 2), the latter decreasing to <2% when erucic approaches 80% in some varieties (Table 3). Similar inverse relationships have been reported for these two acids in the Cruciferae (13–15). Downy and Craig (15) have demonstrated in rapeseed that both eicosenoic and erucic acid are formed by a genetically controlled chain-lengthening addition of acetate molecules to the carboxyl end of oleic acid. In other genera, including *T. majus*, long-chain acids, such as 20:1 and 22:1, also have been shown to be synthesized by oleic acid elongation mechanisms rather than *de novo* synthesis (7,8). As with oil content, erucic acid level is correlated with smaller-seeded varieties (r = −0.6008, Table 2), but as observed in rapeseed, oil and erucic acid are poorly correlated (r = 0.2443) with each other (15).

Within *T. majus* (Table 3), the varieties can be grouped into essentially three sets by oleic acid content (<2%, 4–8% and 17.5%) or by erucic acid content (<68%, 68–74 and >76%). Eicosenoic acid levels are least variable of the three major monoenes (mean = 14.9%, relative standard deviation = 7.51%). NCAUR 64477 has relatively high oleic acid (17.5%) content at the expense of erucic acid (62.3%), whereas NCAUR 47180 has the highest eicosenoic

TABLE 3

Gas Chromatographic (GC) Analysis of *Tropaeolum* Seed Oil Fatty Acid Methyl Esters[a]

NCAUR number[c]	Fatty acid methyl ester (% by GC)[b]													
	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	24:1	26:1	28:1
T. speciosum														
64155	0.9	0.1	—	24.6	1.7	0.5	—	0.6	0.2	17.3	0.4	42.5	9.8	1.5
T. majus														
64477	1.0	—	0.1	17.5	0.6	0.7	0.1	13.7	0.2	62.3	0.2	1.5	—	—
47180	1.1	—	0.1	6.5	0.4	0.6	0.1	21.1	0.4	68.1	—	1.2	—	—
64475	1.1	0.1	0.2	8.1	0.8	0.7	0.1	14.7	0.6	70.6	0.2	1.6	—	—
64474	1.0	0.1	0.2	4.1	0.7	0.8	0.1	15.7	0.5	71.6	0.2	1.3	—	—
47179	1.0	—	—	5.9	0.6	0.6	trace	16.1	0.5	72.0	0.2	2.9	0.3	—
49241	1.1	—	0.1	5.7	0.6	0.5	0.1	15.2	0.5	73.6	0.2	1.8	—	—
64470	0.8	—	0.1	1.9	0.3	0.4	0.1	16.4	0.6	76.7	0.2	1.5	—	—
64479	0.9	—	0.1	2.0	0.3	0.5	0.1	16.6	0.5	77.3	0.2	1.5	—	—
64469	0.8	—	—	1.7	0.3	0.5	0.1	15.5	0.6	78.6	0.2	1.6	—	—
64476	0.7	—	0.1	1.9	0.3	0.3	0.1	14.3	0.5	79.2	0.2	1.9	—	—
64473	0.7	—	0.1	1.3	0.3	0.4	0.1	13.5	0.6	79.5	0.2	1.7	—	—
64478	0.7	—	0.1	1.7	0.4	—	0.1	13.2	0.7	79.6	0.3	2.4	—	—
64471	0.7	—	0.1	1.5	0.3	0.3	0.4	14.5	0.6	79.7	0.2	1.7	—	—
64472	0.7	—	0.1	1.6	0.3	0.4	0.1	14.2	0.5	79.7	0.2	1.7	—	—

[a]Grouped first in order of increasing erucic acid content (22:1), and secondarily by change in 18:1 and 20:1 contents. — = Not detected.
[b]A 30 m × 0.32 mm SP2340 capillary column was used; oven temperature was programmed from 150 to 220°C at 3°C/min with an initial hold of 2 min at 150°C. Linolenate (18:3) and eicosenoate (20:1) are resolved under these conditions.
[c]See Table 1 for seed source and variety by National Center for Agricultural Utilization Research (NCAUR) Number.

acid content (21.1%) at the expense of erucic acid (68.1%). In contrast to *T. speciosum*, little nervonic acid (24:1) is found in *T. majus* (<3%). Significant variability is expressed in the fatty acid distributions of this species. The extent of contributions to this variability from genetic and environmental factors is not known. However, because the seed suppliers are all located in a small coastal geographic area of California, environmental factors may have played a negligible role in the observed variability in fatty acid distribution.

Figure 1 provides a visual comparison of the fatty acid distributions in three erucic acid-containing oils, from *T. speciosum* (NCAUR 64155), *T. majus* (NCAUR 64471, variety Primrose Jewel) and high-erucic acid rapeseed (*Brassica napus*). This rapeseed contains a typical 50% erucic acid (Fig. 1A). Attempts to breed higher-erucic acid rapeseed have met with some success, raising the erucic acid level to 55–60% (16). Fatty acids of *T. majus* are dominated by erucic acid at 80% (Fig. 1B). On the other hand, the breadth of the fatty acid distribution is apparent in the *T. speciosum* curve (Fig. 1C).

Figure 2 shows the triglyceride distribution of oils from *Crambe abyssinica* (57% erucic acid, 66% CN 62), *T. majus* (53% CN 66) and *T. speciosum* (39% CN 66). The dominant triglycerides of both crambe (Fig. 2A) and rapeseed oils have CN 62, *i.e.*, mainly triglycerides containing one C_{18} and two C_{22} acyl groups. Only a trace of CN 66 is found in these two oils, and little, if any, of this is expected to be trierucin (3). *Tropaeolum majus* oil, however, is dominated by triglycerides with CN 66 (Fig. 2B), which must be largely trierucin (3,4). This particular variety (Jewel Mix, NCAUR 64472) contains 52.6% CN 66 by GC integration, and is representative of the highest-erucic acid varieties in Table 1. Other significant peaks are CN 62 (9.0%), CN 64 (30.8%) and CN 68 (3.3%), triglyceride combinations from the pool of C_{20}, C_{22} and C_{24} acids. This distribution closely matches that reported by Harlow *et al.* (4). A strong correlation (r = 0.9529; Table 2) is observed between the integrated area of the CN 66 peak in *T. majus* oil and the erucic acid content of the oil (14 samples). Erucic acid content is also positively correlated with the CN 64 triglyceride peak area (r = 0.6571), and is negatively correlated with the CN 62 triglyceride peak area (r = −0.6385).

Tropaeolum speciosum also exhibits a large CN 66 peak (39.0%), along with significant CN 64 (29.1%) and CN 68 (14.6%) peaks (Fig 2C). Litchfield (5) has shown that the long-chain monoenes (22:1, 24:1 and 26:1) in this species are essentially esterified only at the 1,3-positions of glycerol. Therefore, CN 66 apparently arises largely from C_{18} combinations with long-chain fatty acids (*e.g.*, C-24,18,24 and C-22,18,26; 1,3-glycerol positions not implied). Reasonable triglyceride postulations for CN 64, 68, 70 and 72 peaks can be made based on combinations from the available pool of C_{18} and C_{22} to C_{28} fatty acids (Table 3).

Mass spectra were obtained by GC–MS of the FAME prepared from *T. speciosum* seed oil. The MS of methyl nervonate (24:1, methyl *cis*-15-tetracosenoate), the predominant (42.5%) long-chain acid in the oil of this species, had characteristic ions at *m/z* 380 (parent ion, M^+), 348 (M − 32, loss of methanol) and 306 (M − 74, McLafferty rearrangement loss from M^+). The MS of methyl *cis*-17-hexacosenoate (26:1), which makes up 9.8% of the fatty acids in *T. speciosum*, had characteristic ions at *m/z* 408 (parent ion, M^+), 376 (M − 32, loss of methanol) and 334 (M − 74, McLafferty rearrangement loss from M^+). Both spectra were characteristic of monounsaturated long-chain FAME, showing many ions attributable to systematic loss of saturated and monounsaturated hydrocarbon fragments after electron impact. Litchfield (5) previously characterized these monoenes by GC of their reductive ozonolysis products.

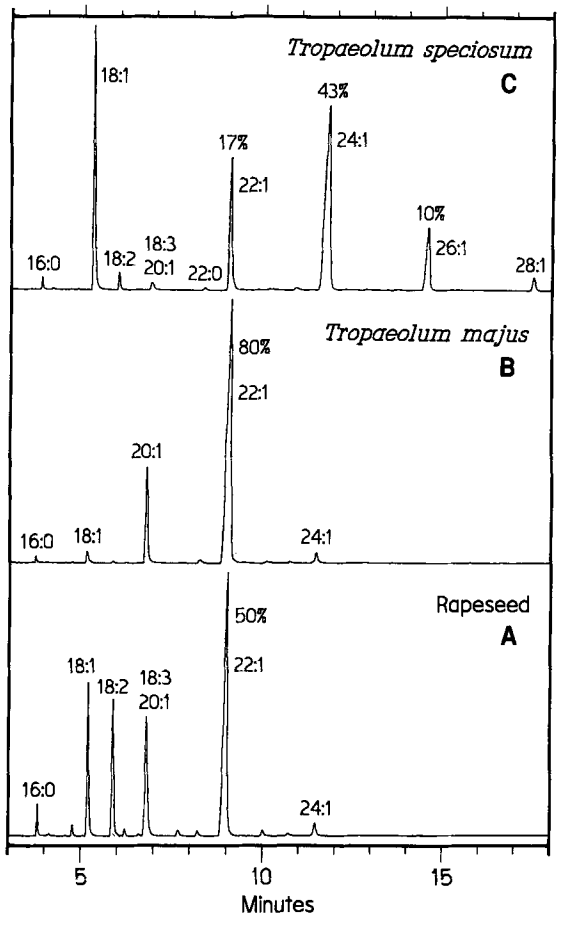

FIG. 1. Gas chromatographic curves for methyl esters of erucic acid oils. A. Esters from high-erucic acid rapeseed oil (50% 22:1). B. Esters from *Tropaeolum majus* L. oil (80% 22:1). C. Esters from *T. speciosum* oil (17% 22:1, 43% 24:1). A 30 m × 0.32 mm capillary column was used; oven temperature was programmed from 180 to 250°C at 3°C/min with an initial 1-min hold at 180°C, and final hold of 5 min at 250°C. Linolenate (18:3) and eicosenoate (20:1) are not resolved under these conditions.

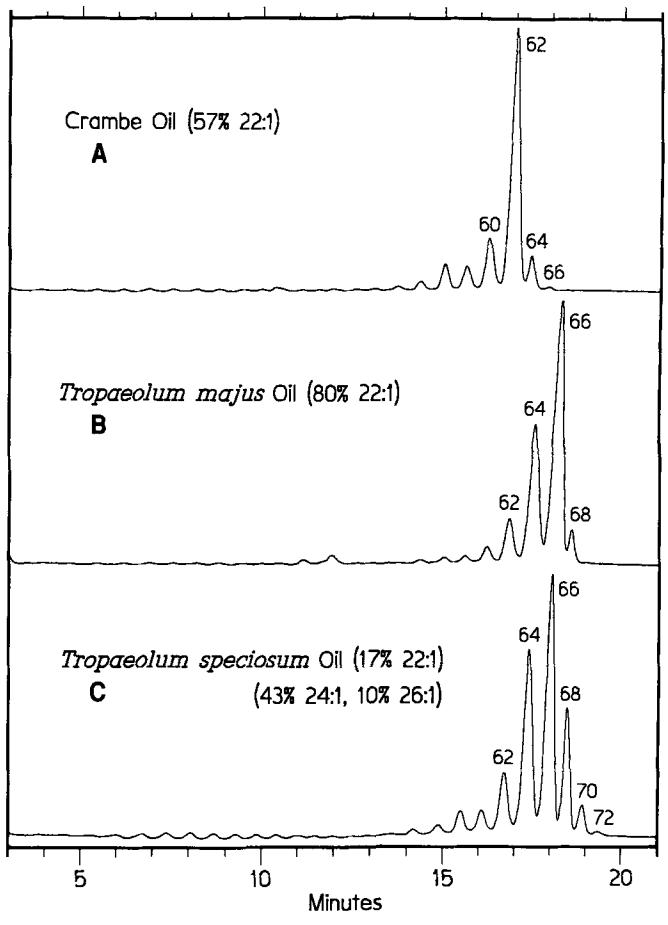

FIG. 2. Gas chromatographic curves for erucic acid containing triglyceride oils. A. *Crambe abyssinica* oil (66% CN 62). B. *Tropaeolum majus* L. oil (53% CN 66). C. *T. speciosum* oil (39% CN 66). A 2.5 m × 0.32 mm GB-1 capillary column was used; oven temperature was programmed from 170 to 350°C at 10°C/min with an initial 1-min hold at 170°C, and a final hold of 6 min at 350°C. CN, carbon number.

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