Evaluation of Yield and Chemical Composition of Fennel Seed from Different Planting Dates and Row-Spacings

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Abstract

In an evaluation of potential oilseed crops, fennel (*Foeniculum vulgare* L.) was planted at Lincoln, Nebraska, on three dates and at three row-spacings. Yields from the two earliest dates averaged 518 lb/acre, compared with 102 lb/acre for the latest date. Differences in yields for the row-spacings were not statistically significant, nor was there interaction between dates and row-spacings.

Oil content of the seed varied from 10.7 to 19.0% for all plantings, the significantly higher amounts occurring in seed from the earliest plantings. The amount of petroselinic acid present as C_{18} monoenes was found by ozonization of the mixed methyl esters, reduction to aldehydes, and consequent GLC analysis; the amount ranged from 70.0–74.5% of the oil and was not significantly affected by either planting date or row-spacing.

Introduction

FENNEL (*Foeniculum vulgare* L.) was one of the plants investigated in the New Oilseed Crops program of the USDA. Although fennel is a short-lived perennial who seed yields are greater after the first year (1,2), this paper reports its evaluation as an annual oilseed crop in a climate too cold to permit it to survive the winter. The research was designed to determine if planting date and row-spacing influence the yield, oil content, and fatty acid distribution of fennel seed, as they are known to do in some oilseed crops.

The predominant fatty acid in the glycerides of fennel seed oil, as in many other members of the *Umbelliferae* family, is petroselinic (*cis*-6-octadecenoic) acid. However, the determination of petroselinic acid is complicated by the presence of its positional isomer, oleic acid. In 1962, Privett and Nickell (3) reported an improved method for the determination of the structure of unsaturated fatty acids, including petroselinic and oleic acids. In the present research, their method of ozonization and reduction proved to be applicable for differentiating petroselinic from oleic acid in the presence of other fatty acids.

Agronomic Evaluation

Pilot Experiment

Fennel grown at Lincoln, Nebraska, was evaluated for its potential as an oilseed crop. In 1960 fennel was seeded in duplicate plots on April 26 and on June 1. The four-row plots were 25 ft long and 36 in. between rows. Although seed from the June 1 planting did not mature by time of killing freeze, yields of cleaned seed from the April 26 planting averaged 1,368 lb per acre. Since the favorable growth and seed yields from this planting suggested that fennel showed promise as a potential oilseed crop, an experiment was planned for 1961 and 1962 to permit study of the influence of date of seeding and row-spacing on the yield and chemical composition of fennel seed.

Investigation of Planting Variables

In 1961 seedings were made on April 20, May 10, and May 23 in a silty clay loam high in available phosphorus and potassium with 80 lb nitrogen added per acre. On each date, three replicated plots were established for each row-spacing of 12, 24, and 36 in. Plots were 33 ft long and 8 or 9 ft wide, depending upon the row-spacing treatment. Plants were thinned to about 10 in. apart.

Data on plant growth and development are recorded in Table I. Plant growth was excellent. Plant height and date of bloom varied with date of planting but were constant for the row-spacing treatments within each date. Individual plants branched more and produced more flowers as the distance between rows increased.

After frost, the plots were harvested with an experimental plot harvester, the seed was cleaned and weighed, and samples within the border were taken for chemical analyses. Seed yields in 1961 (Table I) were lower than in 1960 but were based on more extensive planting. Seed yields from the April 20 and May 10 plantings were significantly higher than those from the May 23 planting. In the late May planting, flowering was so delayed that insufficient time was available for seed to mature before frost. The 36-in. spacing produced the highest average yield; however, the differences in yields for the various spacings were not statistically significant. Wider spacings appeared to be more advantageous in the later dates of planting, although the interaction of dates by row-spacings was not significant.

In addition to the experimental plots, an adjacent 1/10-acre plot was planted in 24-in. rows on May 10; it was combine-harvested to determine potential seed yield under field conditions. The yield from this plot was 595 lb of cleaned seed per acre. Since fennel produces flowers during an indeterminate period, all the seed does not mature at the same time. Seed from umbels that matured early shattered before harvest and thus reduced potential yields.

None of the plants established in either 1960 or 1961 survived the winter. In 1962, similar plots were

TABLE I

Seed Yields and Plant Development of Fennel Seeded at Three Dates and Three Row-Spacings at Lincoln, Nebraska, in 1961

Date planted	April 20	May 10	May 23
Days to emergence	16	14	6
Days to bloom	98	101	122
Height at bloom (inches)	32	31	28
Days to freeze	196	176	163
Seed yield (lb/A)			
12 in. rows	547	446	58
24 in. rows	519	425	118
36 in. rows	483	691	129
Mean [a]	516	520	*102*

[a] Means printed in different type are significant different at $p = .01$.

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planted, but extremely dry, unfavorable weather in April and May contributed to heavy weed infestation and prevented good stand establishment. These experimental plots finally had to be abandoned.

The limited information obtained indicates that in Nebraska fennel must be considered an annual crop. Good seed bed conditions and early planting are essential to success. Addition research is needed on influence of row-spacing and other cultural practices on yields of seed. Economic considerations, omitted from this study, also need to be assessed.

Chemical Evaluation

Materials and Equipment

The column supports, the boron trifluoride-methanol reagent, and the pure methyl esters, with the exception of methyl petroselinate and methyl decanoate, were obtained from Applied Science Laboratories. Methyl decanoate was prepared from commercial acid in the usual manner and distilled through a Podbielniak 4-ft Heli-Grid packed column. Petroselinic acid was prepared by the method of Fore et al. (4).

A Welsbach Ozonator Model T-23 was used for ozonization. Two gas chromatographs were used: a) Barber-Colman Model 20, containing a β-ionization detector with a tritiated titanium foil and equipped with a 12 ft (1/8 in. O.D.) column (2% DEGS on 80/100 Gas-Chrom A) using argon at an inlet pressure of 50 psi, and b) Aerograph Autoprep Model A-700, containing a thermal conductivity detector and equipped with a 7.5 ft (1/4 in. O.D.) column (20% stabilized DEGS on 80/100 Gas-Chrom P) using helium at an inlet pressure of 50 psi.

Experimental Procedures and Results

Nine samples of fennel seed from 1961 were obtained by compositing seed from the 3 replications of each combination of planting date and row-spacing in the experimental plots. Each sample was passed through a 6 mesh hardware cloth screen to remove twigs, and the seeds ground on a Wiley Mill through a 1-mm screen.

Seed Composition. Part of the ground sample was used to obtain the percent moisture-volatiles, determined in duplicate by AOCS Method Ad 2-52 (5), and the percent oil, determined in duplicate by AOCS Method Ad 5-52 (6). After being extracted for 4 hr, each sample was ground with 1 g of fine sand in a mortar. The samples were rewrapped and the extraction continued for an additional 2 hr to ensure complete extraction.

The percentages of moisture-volatiles and of oil in the ground samples are shown in Table II. The oil content of the seeds of the first two plantings (groups A and B) is higher than that of the third planting (group C). Furthermore, except for one sample (B-24), there appears to be a slight trend toward lower oil content with wider spacings. However, a definitive conclusion cannot be based on a few samples from one crop-year.

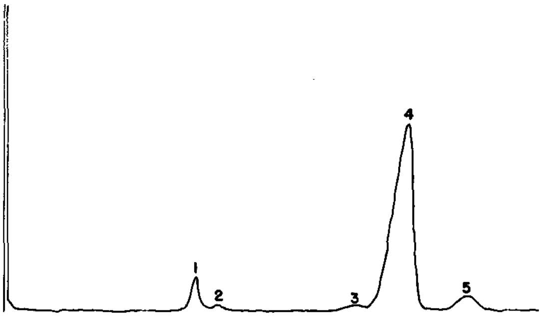

FIG. 1. Distribution of methyl esters from fennel seed fatty acids on GLC analyses: 1) palmitate; 2) palmitoleate; 3) stearate; 4) petroselinate and oleate; and 5) linoleate.

Oil Composition. Oil was extracted from the remainder of each ground sample in large Soxhlet extractors. Approximately 10 g of oil from each sample was saponified and the uncorrected percent unsaponifiables determined by AOCS Method Ca 6a-40 (7). The saponifiable material was treated with HCl, then extracted several times with Skellysolve F. The combined extracts were washed to neutrality with distilled water and then dried over sodium sulfate; the solvent was removed under vacuum; and the uncorrected percent acids in each sample was determined. The unsaponifiable material was titrated with NaOH, and the small amount of acids still present was calculated as percent oleic acid. This figure was then subtracted from the uncorrected percent of unsaponifiables and added to the uncorrected percent of acids to give the corrected percentages that appear in Table II.

Acid Composition. A 500-mg sample of each of the mixed acids was esterified with BF_3-methanol reagent (8). The composition of the mixed fennel seed acids in each sample was determined by GLC analyses of these methyl esters. The Barber-Colman chromatograph was used at a column temperature of 180C. A typical chromatogram is shown in Figure 1. Correction factors compensating for differences in detector response of the component methyl esters were obtained from known mixtures of palmitate, C_{18} monoenes, and linoleate. They were 1.37, 0.96, and 1.26, respectively. No correction was made for the small amounts of palmitoleate and stearate present. The composition of the mixed methyl esters of each sample is given in Table II.

Separation of C_{18} Monoenes. Since petroselinic and oleic acids are positional isomers, their methyl esters

TABLE II

Chemical Composition of Nine Samples of Fennel Seed

Sample No.	Seed composition %		Oil composition, %		Acid composition, %					
	Moisture and volatiles	Oil (dry basis)	Unsaponifiables (cor.)	Acids (cor.)	Palmitate	Palmitoleate	Stearate	Linoleate	C_{18} Monoenes	
									Petroselinate	Oleate
A-12[a]	10.69	19.03	11.21	85.08	6.3	....	....	13.1	71.4	9.2
A-24	10.97	17.69	11.32	81.64	5.8	0.3	0.6	12.3	74.5	6.5
A-36	10.68	16.30	13.47	79.98	6.2	0.4	0.7	13.0	70.0	9.7
B-12	10.03	17.23	12.05	81.18	6.3	0.4	0.4	13.4	70.7	8.8
B-24	10.16	17.75	11.89	83.16	5.9	0.2	0.5	12.3	74.1	7.0
B-36	10.53	15.62	12.74	83.20	6.9	0.4	0.5	13.2	71.1	7.9
C-12	10.54	11.57	12.05	76.79	6.6	0.3	0.6	13.4	72.3	6.8
C-24	10.23	11.08	15.24	77.22	6.2	0.5	0.8	13.5	70.4	8.6
C-36	10.87	10.72	15.26	76.73	6.5	0.5	0.7	14.4	70.1	7.8

[a] The numerals indicate the spacing between rows in inches. The letter A designates seed from a planting date of April 20, 1961; B, from May 10; and C, from May 23.

cannot be resolved on the GLC columns available in most laboratories. GLC analysis through the use of capillary columns was not tried because of the many difficulties encountered in the preparation of good capillary columns. Though other positional isomers have been separated on highly efficient capillary columns, separation of methyl esters of oleic, elaidic, and petroselinic acids has been reported only recently using a capillary column of 150,000 theoretical plates (9).

The role of ozonization in rupturing double bonds has recently been reviewed by Stein (10). The fission accomplished by ozonization may be followed by either oxidation or reduction. Analyses of the C_{18} monoenes in the mixed acids by ozonization and oxidation, followed by the elution of the resulting dibasic acids through a silicic acid column (11), gave inconsistent results. Ozonization and reduction by the procedure of Privett and Nickell (3), followed by GLC analysis of the aedehydes, was found to be the better method and was therefore used in this research.

A 50-mg sample of the mixed esters was subjected to ozonization. Ten milliliters of purified pentane was saturated with ozone to a dark blue color at −70C. The sample, dissolved in 2 ml of pentane, was poured into the solution and the excess ozone immediately removed by house vacuum. Solvent was removed at 0C and the ozonides were dissolved in 0.5 ml of methyl decanoate. The ozonides were reduced at 0C with approximately 25 mg of Lindlar catalyst (12) dispersed in 1 ml of methyl decanoate under a slight hydrogen pressure. The methyl decanoate solution was separated from the catalyst by centrifuging, then analyzed by GLC on the Barber-Colman instrument at a column temperature of 150C. For each sample, the weights of dodecanal and nonanal were obtained by integrating the corresponding GLC curves; only these two peaks were used in computing percentages. The mole percent of dodecanal times the percent C_{18} monoenes in the original sample gave the percent petroselinic acid in the total acids. Similarly, the percent oleic acid was calculated from the mole percent of nonanal.

TABLE III

Analyses of Snythetic Mixtures of Pure Methyl Esters

Sample	Actual		Found	
	A	B	A	B
Palmitate	5.4	5.9	5.6	6.2
Linoleate	9.7	10.1	8.9	9.2
Oleate	5.7	5.4	4.9	5.5
Petroselinate	79.2	78.6	80.6	79.1

Corroborating Analyses. To further test the method of Privett and Nickell (3), two synthetic mixtures of pure methyl esters, whose compositions were similar to fennel seed acid esters, were analyzed by GLC on an Aerograph chromatograph that became available. The column temperature was 210C and the flow rate was 100 ml/min. The percent C_{18} monoenes in each mixture was determined from an average of 5 runs. Duplicate samples were subjected to ozonization and reduction, and then analyzed by GLC at a column temperature of 170C and a flow rate of 75 ml/min. The weights of dodecanal and nonanal were determined from an average of at least 3 runs on each sample. The percent methyl petroselinate and percent methyl oleate in the original samples were determined as previously described. As shown in Table III, the percentages found are equal to the actual percentages within experimental error.

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REFERENCES

1. Williams, L. O., Agriculture Handbook 172, USDA (1960).
2. Kolachov, P. J., Nat'l. Farm Chemurgic Council Bull. (1940).
3. Privett, O. S., and C. Nickell, JAOCS *39*, 414–419 (1962).
4. Fore, Sara P., R .L. Holmes and W. G. Bickford, Ibid. *37*, 490–491 (1960).
5. American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed., Chicago, Ad 2-52, p. 2.
6. Ibid., Ad 5-52.
7. Ibid., Ca 6a-40.
8. Metcalfe, L. D., and A. A. Schmitz, Anal. Chem. *33*, 363–364 (1961).
9. Riva, M., F. Poy and P. Gagliardi, Rev. Ital. Sostanze Grasse *41*, 267–268 (1964).
10. Stein, R. A., JAOCS *42*, 326–329 (1965).
11. Higuchi, T., N. C. Hill and G. B. Corcoran, Anal. Chem. *24*, 491–493 (1952).
12. Lindlar, H., Helv. Chim. Acta 35, 446–450 (1952).

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