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Anethole and fenchone in the developing fruits of *Foeniculum vulgare* Mill.

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The anethole and fenchone content of the developing fruits of bitter and sweet varieties of *Foeniculum vulgare* Mill. (fennel) has been assayed by gas chromatography over three seasons. Anethole continuously increases in both varieties to about 22 mg/100 fruits. Fenchone is present at all stages of development of both varieties, and continuously increases to about 10 mg and 2 mg/100 fruits in the bitter and sweet varieties respectively. Greater oil yields from the bitter fruit are thus due to their higher fenchone content and lower weight.

THE principal flavouring constituents of *Foeniculum vulgare* Mill. (fennel) are the aromatic *trans*-anethole and the terpenoid (+)-fenchone, which may comprise up to 90% and 22% respectively of the essential oil of different specimens of this umbelliferous fruit (Tóth, 1967a). The product of these two dissimilar oil constituents in developing fennel fruits is compared in this communication with the situation previously observed for carvone (Betts, 1965), where a "specific level" was observed in fruits that produce it.

Experimental

Plant material. This was identified, grown, marked and sampled as previously described (Betts, 1965), samples being taken during three growing seasons at various stages of fruit development from "bitter" (var. *vulgare* (Mill.) Thelung) and "sweet" (var. *dulce*) varieties. Plants of the latter variety were raised from "seed" obtained from Italy, whilst the former variety was found growing in Myddelton House gardens, Enfield. The two varieties are distinct in appearance, the bitter variety being a taller, but less robust plant bearing smaller flowers and fruits than the sweet variety, and having a distinct camphoraceous taste and odour of fenchone overlaying the sweetness of anethole.

Extracts. Fruit collected were dropped as soon as possible into volumetric flasks (5, 10 or 20 ml according to the degree of development) containing enough absolute ethanol to cover them; the contents were then made up to volume with ethanol. After at least six months storage in the dark at room temperature (20°), the volume was again made up if necessary and aliquots (1 μl) were taken for qualitative and quantitative gas chromatography.

Gas-liquid chromatography. Pye 104 apparatus was used isothermally at 140° with a flame ionization detector at the oven temperature. A glass column (5 ft), internal diameter 4 mm was used, packed with 15% Carbowax 20M (a polyethylene glycol) on Chromosorb W (80–100 mesh), previously purged at 225°. Mobile phase nitrogen, with flow rate at column exit 40 ml/min; the hydrogen supply to the detector being at the

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same flow rate. Under these conditions the column contained approximately 2000 theoretical plates for anethole and 800 theoretical plates for fenchone.

Anethole and fenchone. These were obtained by measuring the heights of anethole and fenchone peaks from the extracts against those obtained on the same occasion from standard solutions. While the anethole standard was pure, the fenchone was of technical quality and contained an impurity of longer retention time with a peak area one-ninth that of the fenchone. Calculations of fenchone content were therefore made on the assumption that the fenchone was 90% pure. Peaks present in the extracts were identified by comparison with reference materials both on the Carbowax column and on a polyethylene glycol adipate column, and included a small peak of estragole, the allyl isomer of anethole, which was observed in all extracts. An internal standard was not used but the same operator made the injection and assays were repeated until results were consistent.

Results and discussion

Similar results were obtained for the developing fruits over three seasons, and illustrative figures are in Table 1. Results are given as

TABLE 1. OBSERVATIONS ON DEVELOPING FENNEL FRUITS 1966

Date marked i.e. in flower	Date collected	Days since marking	mg anethole in 100 fruits*	mg fenchone in 100 fruits*	% fenchone in essential oil†
		Bitter fennel			
2 Sept.	16 Sept	14	2·9	1·6	36
2 Sept.	26 Sept.	24	5·0	2·9	37
10 Aug.	16 Sept.	37	16·6	6·6	28
10 Aug.	26 Sept.	47	18·5	6·9	27
10 Aug.	20 Oct.	71	22·4	10·0	31
		Sweet fennel			
16 Sept.	26 Sept	10	2·5	trace	–
2 Sept.	16 Sept.	14	4·5	0·3	6
10 Aug.	16 Sept.	37	7·6	0·5	6
10 Aug.	26 Sept.	47	15·9	1·4	8
10 Aug.	20 Oct.	71	21·5	1·9	8

* Average of at least two determinations.
† Assuming the essential oil is composed only of anethole and fenchone.
Relative retention time to linalol: fenchone, 0·66; anethole 3·75.

content of substance assayed per 100 fruits (entire cremocarps) as this method is of more value in developmental studies than figures based on weight of tissue. Such values for carvone give misleading "peak" concentrations at early stages of fruit development due to the oil constituent increasing more rapidly than the dry weight (Betts, 1965). Fennel fruits were extracted by cold ethanolic maceration and not by steam distillation, for whilst the latter process rapidly completes the removal of carvone from whole fruits, the removal of anethole requires repeated co-distillations with water. Several months extraction was allowed to ensure that

the oil constituents were evenly distributed throughout the contents of the volumetric flask. That extraction was complete was shown by obtaining the same assay several months later. There was no gas chromatographic evidence of anethole decomposition, as shown by the appearance of an anisaldehyde peak for example, during storage of the extracts. Gas chromatography is preferable to thin-layer chromatography for qualitative examination of fennel as anethole is always partly decomposed to anisaldehyde when spotted on to the plate, and fenchone gives an unreliable response to visualizing sprays (Betts, 1964). In addition, estragole has the same Rf value as anethole and is superimposed on it. Zacskó-Szász & Szász (1965) claim that estragole has a much lower Rf value than anethole, but their spray, phloroglucinol and hydrochloric acid, only detects an impurity. Gas chromatography was also required for quantitative work, as there is no very sensitive spectrophotometric assay for anethole. Peak height ratios were used, being more rapidly obtained, and giving the same result as peak areas: it was found that on any one occasion the peak width for a substance at half the peak height was constant.

Luyendijk (1957) and Betts (1965) observed that in caraway and dill fruits, carvone seems to reach a fairly constant level for each species some weeks before ripening. Although in bitter fennel there appears to be a "plateau" of development of both anethole and fenchone from about the fifth to seventh weeks, there is a subsequent increase in both constituents. Thus the results for both fennels do not suggest that the concept of a "specific level" is valid for the essential oil constituents of umbelliferous fruits in general, or even for the terpenoids. However, fennel fruits do ripen later, and take longer to do so, than those of caraway or dill, and this may account for the difference.

The anethole content of the two varieties of fennel is similar, although the fruits of the sweet variety are about twice the weight of those of the bitter variety, and so give a lower percentage oil yield. Anethole increases continually during fruit development, there being about 22 mg/100 fruits when they are ripe. By comparison with anethole peak areas, estragole also increases, but to less than 1 mg/100 fruits in both varieties. The fenchone content of the two varieties is different at all stages of fruit development, being much greater in the bitter variety than the sweet, and further increasing the percentage oil yield of the bitter variety. Fenchone forms about one-third of the combined anethole and fenchone content of the bitter variety, but only one-tenth or less of the combined constituents of sweet fennel, although it is definitely present at all stages of development (Table 1). Tóth (1967a) found that the oil of European bitter fennel fruit contained 12·3–22·2% fenchone, whilst that of European sweet fennel contained only 0·4–0·8%. There appears to be some disparity between Tóth's figures and the results obtained in the present work, where the percentage fenchone of both varieties was much higher, assuming that the essential oils were mainly anethole and fenchone. This may be due to partial decomposition of the fenchone during steam distillation, or to preferential loss of fenchone from the fruits before Tóth received

them* The latter is likely, as preferential loss of limonene relative to carvone occurs on storage of dill fruits (Kalitzki, 1954).

Both varieties of fennel, at all stages of their fruit development, contain some fenchone and therefore comply with what appears to be a B.P.C. requirement. However, the essential oil may contain over 20% fenchone or a negligible amount.

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* Since completion of the present paper Tóth (1967b) has recorded a decrease in the fenchone content of fennel fruit stored for 10 months (along with an apparent (?) increase in anethole).

References

Betts, T. J. (1964). *J. Pharm. Pharmac.*, 16, *Suppl.*, 131*T*–135*T*.
Betts, T. J. (1965). *Ibid.*, 17, *Suppl.*, 41S–43S.
Kalitzki, M. (1954). *Pharmazie*, 9, 155–166.
Luyendijk, E. N. (1957). *Pharm. Weekbl. Ned.*, **92**, 349–374.
Tóth, L. (1967a). *Planta med.*, **15**, 157–172.
Tóth, L. (1967b). *Ibid.*, **15**, 371–389.
Zacskó-Szász, M. & Szász, G. (1965). *Fette Seifen Anstr-Mittel, Hamburg*, **67**, 332–334.