

Volatile Constituents of Moroccan Thyme Oil

J. D. Miquel, H. M. J. Richard,* and F. G. Sandret

Moroccan thyme, *Thymus satureioides*, was extracted by steam distillation in a modified Clevenger apparatus, and the constituents of the essential oil were separated and purified by column and gas chromatography. Twenty-one volatile constituents were characterized on the basis of their infrared spectra: tricyclene, α -pinene, camphene, β -pinene, myrcene, α -terpinene, limonene, 1,8-cineole, γ -terpinene, *p*-cymene, *trans*-4-thujanol, linalool, camphor, bornyl acetate, carvacrol methyl ether, 1-terpinen-4-ol, β -caryophyllene, α -terpineol, borneol, thymol, and carvacrol. Moroccan thyme essential oil is very different from the other thyme essences, and possesses a characteristic gas chromatographic pattern.

Thyme is a wild perennial shrub that originated in the mediterranean region. Lagriffe (1968) mentions that its name comes from the Greek word "thumos", which means odor. Harvest takes place just after blooming. According to Debuigne (1972), the plant is well known for its bactericidal properties, and is used in some tooth pastes and cough syrups. In Morocco, olive oil containing macerated thyme is used as a dressing for wounds.

According to Granger and Passet (1973), the widespread species, *Thymus vulgaris* L., exhibits tremendous variations in its essential oil composition. On the basis of their results, they define several chemotypes. Because of this diversity, much confusion exists in the literature. Guenther (1972) mentions that Spanish thyme consists of two varieties, *T. vulgaris* and *T. zygis*, which must not be confused with the French wild thyme, *T. serpyllum*. Another example concerns Moroccan thyme, termed *T. broussonetii* Boissier by some people and *T. satureioides* by others.

While the composition of the essential oil of *T. vulgaris* L. is well known (Derdiles, 1967; Granger and Passet, 1973; Guenther, 1972; Messerschmidt, 1964, 1965; Naves, 1959; Russell and Olson, 1972; and Schratz and Horster, 1970), few works deal with *T. satureioides*. Herisset et al. (1973a,b) recently analyzed different samples of thyme and reported that Moroccan thyme oil was rich in borneol. They identified several compounds on the basis of retention time alone, and suggested differentiation of this species from others by means of infrared spectroscopy.

Because the variety, *T. vulgaris*, sold in France under the label "Thym de Provence", is much more expensive than the Moroccan thyme, *T. satureioides*, interest has developed in the composition of the essential oil with a view to detection of possible adulteration.

EXPERIMENTAL SECTION

Essential Oil Extraction. Moroccan thyme (150 g), *T. satureioides*, provided by the French organization "Répression des Fraudes et Contrôle de la Qualité", was placed with 1 l. of distilled water in a 2-l. flask and connected to a modifier Clevenger apparatus. This device prevents siphoning, eliminates almost completely the clinging of oil droplets to the glass wall, minimizes splashing of condensed liquid, permits recovery of the essential oil without water, and facilitates cleaning of the apparatus (Figure 1). After 2 h of refluxing, the extraction was stopped. The essential oil was recovered, dried over anhydrous sodium sulfate, and stored at 4 °C until use.

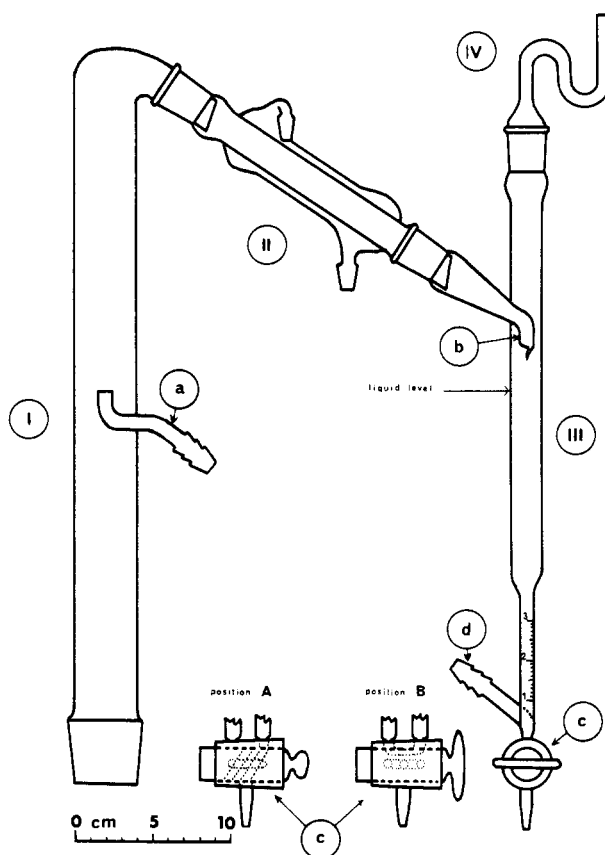


Figure 1. Modified Clevenger apparatus: (I) bent glass tube with an outside diameter of 4 cm; (II) West condenser; (III) separator for oils lighter than water; (IV) S-shaped glass tube to prevent dust contamination; (a) water-flushed pipe with upward opening to prevent siphoning; (b) drip tip; (c) three-way stopcock; (d) glass tube connected to (a) by means of Rhodorsil tubing. Position A allows recovery of essential oil. Position B permits water to flush back to the reactor flask.

Separation by Column Chromatography. A column, 70 cm high \times 3 cm in diameter, filled with 130 g of 60–80 mesh Florisil, was prewashed with 100 ml of distilled anhydrous petroleum ether. Thyme essential oil (5 g) was poured at the top of the column and eluted first by 500 ml of petroleum ether, then by 9 fractions of 500 ml each containing petroleum ether–benzene with ratios of 9:1 to 1:9. The next elution utilized 500 ml of benzene, then three portions of benzene–diethyl ether with respective ratios of 95:5, 90:10, and 80:20, and finally 500 ml of diethyl ether. The 15 fractions collected were relieved of solvents by a rotatory evaporator, recovered in 2 ml of diethyl ether, and

Ecole Nationale Supérieure des Industries Agricoles et Alimentaires, C.E.R.D.I.A., 91305 Massy, France.

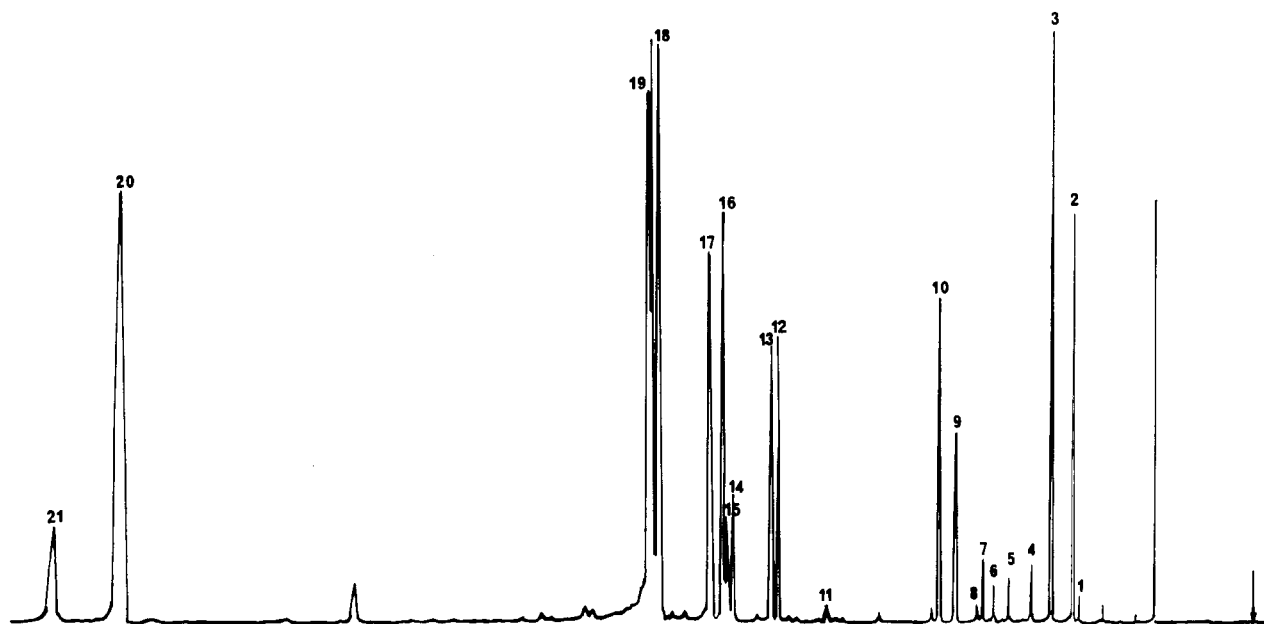


Figure 2. Chromatogram of Moroccan thyme oil on a glass capillary column (86 m \times 0.5 mm i.d.) coated with Carbowax 20M: (1) tricyclene, 0.19%; (2) α -pinene, 3.27%; (3) camphene, 5.43%; (4) β -pinene, 0.44%; (5) myrcene, 0.33%; (6) α -terpinene, 0.28%; (7) limonene, 0.51%; (8) 1,8-cineole, 0.16%; (9) γ -terpinene, 1.70%; (10) *p*-cymene, 2.96%; (11) *trans*-4-thujanol, 0.14%; (12) linalool, 3.43%; (13) camphor, 2.65%; (14) bornyl acetate, 1.71%; (15) carvacrol methyl ether, 1.43%; (16) 1-terpinen-4-ol, 4.81%; (17) β -caryophyllene, 6.44%; (18) α -terpineol, 12.24%; (19) borneol, 26.22%; (20) thymol, 19.23%; (21) carvacrol, 4.25%.

analyzed by gas chromatography. Fractions having the same patterns were combined to produce seven final fractions.

Separation by Gas-Liquid Chromatography. Initial separation of the different fractions was achieved with an Intersmat Model 12 ML gas chromatograph, equipped with a 4 m \times 7 mm o.d. glass column packed with 4% (w/w) F.F.A.P. on 80-100 mesh AW-DMCS Chromosorb G. A thermal conductivity detector was used and the flow rate of helium was maintained at 60 ml/min. The column was programmed from 60 to 230 $^{\circ}$ C at 6 $^{\circ}$ C/min.

Purification was achieved on the same instrument using another 4 m \times 7 mm o.d. glass column packed with either 4% (w/w) Apiezon L on 80-100 mesh AW-DMCS Chromosorb G, or 4% (w/w) OV-101 on the same support. The helium flow rate was the same as above.

The components were trapped in thin-walled glass capillaries, according to the procedure described by Jennings (1970).

Identification. Identifications were based on retention data, physical properties, and ultraviolet and infrared spectroscopy.

Infrared spectra of the purified liquid compounds were taken on thin films between NaCl plates on a Perkin-Elmer Model 21 infrared spectrophotometer. Infrared spectra of purified solid compounds were taken in pressed pellets of KBr. Spectra were compared with those reported in the literature, or with spectra obtained from synthesized compounds.

Bornyl acetate was obtained by the reaction of acetyl chloride on borneol in pyridine; carvacrol methyl ether was synthesized as described by Russell and Olson (1972) from carvacrol and methyl sulfate in the presence of concentrated KOH; and *trans*-4-thujanol was made using the oxymercuration method as described by Brown and Geoghegan (1967) and modified by Russell and Jennings (1970).

Quantitative Analysis of Moroccan Thyme Oil. Quantitative analyses of Moroccan thyme oil utilized a glass capillary column, 86 m \times 0.5 mm i.d., made in the

laboratory on a Hupe glass capillary drawing machine from a glass tube 1.50 m \times 7.8 mm o.d. (4 mm i.d.) and coated with Carbowax 20M following the static coating procedure described by Bouche and Verzele (1971). The column was filled by suction with a degassed 5 mg/ml Carbowax 20M and 0.05 mg/ml Igepal solution in dichloromethane. The solvent was evaporated by pulling vacuum at one end of the column while the other side was carefully sealed with a Scotch 3M glue.

The column was connected to a Varian Aerograph Model 204 gas chromatograph equipped with a special capillary column injection splitter. Thyme essential oil (0.5 μ l) was injected into the injector port with a split ratio of 1:15; nitrogen carrier gas flow rate in the column was 3 ml/min. The detector and injector temperatures were 250 $^{\circ}$ C and column temperature was programmed from 60 to 200 $^{\circ}$ C at 2.7 $^{\circ}$ C/min.

The chromatograph was connected to a digital integrator (Varian Model 475) which determined the retention time and surface area of each peak. Differences in response factors were ignored in calculating percentage composition from the integrator data.

RESULTS AND DISCUSSION

The essential oil yields of the different extractions were between 1.1 and 1.3% of the dried material.

From the seven fractions separated by column chromatography, fraction no. 1 contained 12 main compounds which were characterized by infrared spectra, except for tricyclene, the identification of which was obtained on the basis of retention data on three different columns. Ten of the compounds were terpene hydrocarbons (tricyclene, α -pinene, camphene, β -pinene, myrcene, α -terpinene, limonene, γ -terpinene, *p*-cymene, and β -caryophyllene) and two ethers (1,8-cineole and carvacrol methyl ether).

In fraction no. 2, three principal peaks were separated, and their structures determined by comparison of their infrared spectra with spectra reported in the literature. Again, carvacrol methyl ether was found, accompanied by thymol and carvacrol.

Fraction no. 3 consisted of the alcohols, thymol and carvacrol, already identified in fraction no. 2, and four new compounds: linalool, α -terpineol, borneol, and 1-terpinen-4-ol.

Borneol represents the main constituent of the essential oil. This is an interesting fact because in the literature no other variety of thyme was reported to contain more than 8% of borneol, and generally the level is around 2 to 3% of the total essential oil. Adulteration of Provence thyme by Moroccan thyme might be detectable by determining the quantity of borneol. We are now studying *T. satureioides* to establish whether the borneol content of all samples is consistently higher than 20%.

In fraction no. 4, two new compounds were identified: *trans*-4-thujanol and bornyl acetate.

Fraction no. 5 revealed peaks already known, and from fraction no. 6, camphor was characterized by its infrared spectrum. The presence of this compound was assumed by several workers, but to date, no evidence has been presented. It is noteworthy that this particular variety of thyme contains a large amount of camphor, 2.6%, giving a noticeable note of camphor to the essential oil.

In fraction no. 7, no new components were identified.

The chromatogram of the whole essential oil on a Carbowax 20M glass capillary column (Figure 2) shows that all the main constituents were identified. Analysis of several other samples of *T. satureioides* revealed this

same chromatographic pattern.

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Volatile Flavor Components of Beef Boiled Conventionally and by Microwave Radiation

Glesni MacLeod* and Belinda M. Coppock

Using a modified Likens and Nickerson extraction procedure followed by low temperature/high vacuum distillation, representative samples of aroma volatiles were obtained from beef both while boiling by microwave radiation and by conventional means. Separation of the components of the isolates was achieved by gas chromatography and the majority of the components identified using combined gas chromatography-mass spectrometry. Odor assessments were made of the separated volatile components. A comparative study was undertaken of the effect on the volatile components of boiling for different periods of time conventionally and by microwave heating.

The flavor of meat has been extensively studied and the literature frequently reviewed (Dwivedi, 1975; Herz and Chang, 1970; Patterson, 1974; Rhodes, 1974; Wasserman, 1972). Many factors pre-slaughter, at slaughter, and post-mortem (including cooking procedure) may influence the final flavor (Herz and Chang, 1970; Patterson, 1974). Uncooked meat has little odor and only a blood-like, metallic taste and it is generally agreed that desirable meat flavor is developed during cooking. Two types of cooked meat flavor have been distinguished, a "meaty" flavor believed to originate from the lean and a "species" flavor which characterizes different animal species and which derives from the fat tissue. Nevertheless, lean meat contains 4-6% intramuscular fat which is sufficient to give rise to the characteristic species flavor (Patterson, 1974).

The flavor of cooked beef, in particular, has been comprehensively researched. Numerous workers, using gas chromatographic techniques, have studied and characterized the volatile flavor components (Hirai et al., 1973; Liebich et al., 1972; Mussinan et al., 1973; Persson and von Sydow, 1973; Watanabe and Sato, 1971a, 1972; Wilson et al., 1973) and lists of over 300 volatile constituents of the aroma of heated beef have been compiled (Coppock, 1975; Dwivedi, 1975; van Straten and de Vrijer, 1973). These include members of at least 18 different chemical classes. Heating methods used for flavor development have included conventional boiling and roasting at atmospheric pressure, frying, pressure cooking, and retort heating.

In contrast to this wealth of literature, only two references can be traced to the use of modern methods (e.g., gas chromatography and/or mass spectrometry) in the flavor analysis of any food cooked in the microwave oven (MacLeod and MacLeod, 1970; Walradt et al., 1970), neither of which relates to meat.

*Department of Food Science and Nutrition, Queen Elizabeth College, London W8 7AH, England.