Production of New Greek Oregano Clones and Analysis of Their Essential Oils

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ABSTRACT. A gas liquid chromatographic analysis of the essential oils from four new Greek Oregano clones revealed that carvacrol was the predominant constituent in all clones, representing extremely high percentages of the oils (from 79.5% in a clone named Athos to 93% in a clone named Olympus). The thymol content ranged from 0.04 to 0.55 percent among the tested clones. Phenols (carvacrol and thymol) and γ-terpinene and p-cymene were main constituents of the oils. These new clones are considered of superior quality as compared with other oregano cultivars currently grown in Greece. The clone named Olympus was the most efficient of the clones tested, having a high dry weight yield and a crushed oregano product high in essential oil and carvacrol.

KEYWORDS. Carvacrol, essential oil, herb, spice, quality, yield

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INTRODUCTION

Species of oregano (*Origanum* spp.), widespread throughout the Mediterranean area, are also observed in many parts of the mild, temperate climates of Europe, Asia, North Africa, and America. The most commonly distributed species in Europe is *Origanum vulgare*, represented in Greece by three subspecies: ssp. *vulgare*, ssp. *hirtum* (Link) Ietswaart (synonym *O. hirtum* Link, and *O. heracleoticum*), and ssp. *viridulum* (Martin-Donos) Nyman (10). The ssp. *hirtum*, known as Greek oregano, is the most used commercial type of oregano and, in the wild form, considered in quality as the best (13).

Greek oregano (ssp. *hirtum*) grows wild throughout nearly all of Greece, particularly in mountain and semi-mountain districts. The plant, which is also cultivated in many areas, is widely used as spice, containing an essential oil, rich in the phenols carvacrol and thymol (5,18). The phenols are responsible for the antimicrobial and antifungal properties of the oil *in vitro* (1,4) and in animals (12,17). The yield and the quality of the essential oil is dependent on the plant origin and environmental variables associated with the growing site (18).

Greek oregano, the most exported aromatic plant from Greece, is primarily collected, as most spices of the Lamiaceae family, from wild populations. According to Kokkini (10), the ssp. *hirtum* has great variability in the essential oil content and carvacrol percentage, making the final quality of the collected plants inconsistent and not of superior value. Because of the commercial importance of Greek oregano (ssp. *hirtum*), clones of high yield and quality were developed for introduction into cultivation.

MATERIALS AND METHODS

Plant material. Oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) was used in this study. The plants represented selections from indigenous plant material collected from different districts of Greece and used to create four new oregano clones in our Institute (6,7). These new clones were selected, based on, plant shape, bloom time, yield, essential oil content, and dry plant color, from among nine initial clones grown for experimental purposes in fields established in North and Central Greece.

The four new clones, named for the Greek mountains where originally collected, were: Olympus, Vermio, Pelio, and Athos. Clone Olym-

pus is a late harvest selection, clone Vermio is an early harvest selection, and clone Pelio and Athos are a mid-early harvest selections. For analysis, samples were collected from the two middle rows (of four rows) of field plants during the 80 percent blooming period. The plant material was subsequently dried under shade (without air circulation) until crisp.

Essential oil. The essential oil yield was determined using the European Pharmacopoeia apparatus (Clevenger type) for three replicate samples of the dried oregano tissues. The samples, 50 g each, were crushed and the oil distilled for 1.5 h in the presence of 450 ml deionized water (7) at a distillation rate of 3 to 3.5 ml/min (9). The distilled, lighter than water, yellow oil was dried over anhydrous Na_2SO_4 and stored with 3 ml of n-pentane in sealed glass vials under refrigeration (−20°C) until analyzed.

The essential oils were analyzed by gas chromatography using a Hewlett Packard 5890 Series II gas chromatograph equipped with one injection port and a two channel system of columns and respective FIDs connected to a chromatographic integrator (Hewlett Packard 3396 Series II Dual Channel). Fused silica columns of three different polarities were used: a Durabond-DB 1 and a DB-Wax (both 60 m × 0.25 mm i.d., film thickness 0.25 μm, J & W Scientific Inc., Rancho Cordova, CA) and a CP-Sil 19 CB (25 m × 0.25 mm i.d., film thickness 0.20 μm, Chrompack Nederland, BV, Middleburg, The Netherlands). Oven temperature was programmed to rise from 45 to 220°C at 3.5°C/min. The carrier gas was nitrogen at 140 Kpa, the injection temperature was 220°C and the temperature of the detectors was 300°C. The composition of each oil sample was computed from the GC peak areas without correction factors.

Oil composition (peak identification) was confirmed by GC-MS, using a Packard 438 A chromatograph interfaced with a Finnigan MAT Ion Trap Detector (software version 3.0; Finnigan Mat, San Jose, CA) and a 60 m × 0.245 mm i.d. fused silica CP-Wax 52 CB column, film thickness 0.25 μm (Chrompack Nederland BV, Middleburg, The Netherlands). Oven temperature increased from 45 to 240°C at 3°C/min. The carrier gas was helium at 200 Kpa using a split ratio of 1:40 and the scan time was 1 sec.

RESULTS

The main identified constituents of the essential oils of the oregano clones represented 95.7 to 97.5 percent of the oils (Table 1). Carvacrol

TABLE 1. Composition of major essential oil constituents in oregano clones

Constituents[1]	Olympus	Vermio	Pelio	Athos
	(% of total oil)			
α-Thujene	0.07	0.81	0.12	0.52
α-Pinene	0.07	0.65	0.21	0.48
Camphene	0.06	0.15	0.05	0.09
Sabinene	0.50	0.80	1.12	0.66
Myrcene	0.13	1.70	0.40	1.09
α-Phellandrene	-	0.17	-	0.02
α-Terpinene	-	0.73	0.22	0.40
p-Cymene	0.74	4.25	4.05	8.10
Terpinolene	0.08	0.21	0.10	0.14
Limonene	-	0.17	0.08	0.16
γ-Terpinene	0.55	2.43	0.32	0.63
Trans-sabinen-hydrate	0.02	0.51	0.31	0.46
Terpinen-4-ol	0.42	0.42	0.49	0.43
α-Terpineol	0.31	0.26	0.12	0.19
Thymol	0.32	0.28	0.04	0.55
Carvacrol	92.90	82.68	86.97	79.45
α-Cubebene	0.08	0.08	0.09	0.06
β-Caryophyllene	0.30	0.70	1.58	1.29
Trans β-farnesene	0.11	0.12	0.09	0.14
α-Humulene	0.06	0.08	0.19	0.09
Muurolene	0.17	0.16	0.04	0.08
γ-Cadinene	0.24	0.08	0.60	0.70

[1] Based on chromatographic results obtained with DB-1 and DB-Wax columns; mean of three chromatographic samples.

was the major oil component in the four clones, varying from 79.4 percent (clone Athos) to 92.9 percent (clone Olympus). In addition to carvacrol, γ-terpinene, p-cymene, and thymol were predominant constituents of the oils. Qualitative differences consisted mainly of differences in the level of α-phellandrene, a constituent not present in the clones Olympus and Pelio, and differences in the level of α-terpinene and limonene, which were not present in the clone Olympus. Other noted differences in oil among the clones included a particularly low level of p-cymene in Olympus (0.74%) as opposed to 4.00 percent in Pelio, 4.25 percent in Vermio, and 8.10 percent in the Athos clone. In contrast, the Olympus had a higher content of carvacrol (92.90%), than

the other clones, while Athos, which contained the highest amount of p-cymene of the tested clones, had the lowest carvacrol content (79.45%). The thymol content was low in all the studied clones, ranging between 0.04 and 0.55 percent.

Of the monoterpene hydrocarbons, excepting p-cymene and γ-terpinene, only the levels of sabinene and myrcene (1.12 and 1.70%, respectively) were notable. Sesquiterpenes, as a group, represented only by a low percentage of the total volatile constituents, 0.96 percent in clone Olympus, 1.22 percent in clone Vermio, 2.39 percent in clone Athos and 2.59 percent in clone Pelio. The main sesquiterpene compound, β-caryophyllene, was 1.58 percent in the clone Pelio.

The production of dry oregano varied from 2.25 and 2.85 kg/10 m^2 in the clones, particularly high yields as a common, mean yield in dry, crushed oregano is 1.50 kg/10 m^2 (Table 2). Moreover, the essential oil content of the clones ranged between 3.80 and 4.35 percent.

DISCUSSION

The relatively high amounts of carvacrol in the essential oil in *Origanum vulgare* ssp. *hirtum* characterize this plant as a carvacrol-type oregano (10). The new oregano clones evaluated in this study were clearly of the carvacrol type, having an essential oil high in carvacrol (> 79.45%) and low in thymol (< 0.55%). Such high contents of carvacrol in oregano have been observed only in a few populations in Greece (10,18). Indeed, the highest yield of carvacrol in wild oregano plants of other Mediterranean areas have been 56.63 percent in a sample of Italian origin (15) and 78.73 percent in a sample of Turkish origin (3). Most oregano plants from Turkey have recorded carvacrol contents ranging from 58.71 percent to 70.47 percent (2,16). The essential oil yields of the four investigated clones (mean 4.10%) are within the ranges recorded for *O. vulgare* ssp. *hirtum* plants from other Mediterranean

TABLE 2. Yield of selected Greek oregano clones

Yield	Olympus	Vermio	Pelio	Athos
Essential oil (% v/w)	4.35	3.80	4.05	4.35
Dry wt (kg/10 m^2)[1]	2.85	2.37	2.25	2.35

[1] Data of Goliaris (8).

countries–2.25-5.73% for Italy and 5.50, 3.40, and 1.3-6.5% for different populations from Turkey (2,3,15,16).

The presence of γ-terpinene and p-cymene in the essential oil of the tested clones indicates a biosynthetic relationship of these constituents with thymol and carvacrol. According to Poulose and Croteau (14), γ-terpinene is converted to p-cymene and then by hydroxylation to thymol and carvacrol. Our data demonstrate that the higher level of γ-terpinene and p-cymene observed in clones is correlated with lower levels of carvacrol and thymol. For example, in clone Athos where the sum of the yield of γ-terpinene and p-cymene is highest (8.7%), the corresponding sum of the yield of carvacrol and thymol is lowest (80%). Similarly the reverse is true for the clone Olympus. The Olympus clone is the most efficient as compared with the other clones because of the high dry weight yield, the high essential oil content, and the very high content of carvacrol.

The introduction of new clones into cultivation has greatly improved the income of the growers (6). All tested clones could be considered superior in yield and quality to all other currently grown oregano cultivars in Greece.

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