Technical paper

Chemical Composition of the Essential Oils Obtained From Some Spices Widely Used in Mediterranean Region

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Abstract

Spices are widely used in the countries of Southern Europe and North Africa where they play a central role in the Mediterranean diet. Spices are used for their flavour and aroma and also for the sensations that they produce; they can be used as colouring as for their nutritional and antioxidant properties.

The aim of this work was to determine the chemical composition of the essential oil of six spices widely used in the Murcia Region (Spain): oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cumin (*Cuminum cyminum*) and clove (*Syzygium aromaticum* L). Essential oils were chemically analysed and identified by GC-MS.

The principal components of sage essential oil were camphor (24.95%), 1,8-cineole (24.75%) and camphene (7.63%). Major oil components of oregano included carvacrol (61.21%) and p-cymene (15.12%). The essential oil of thyme was characterized by a high content of terpinen-4-ol (13.15%), γ-terpinene (9.21%) and cis-sabinene hydrate (7.65%). The predominant compounds in clove essential oil were eugenol (85.5%), β-caryophyllene (10.54%) and α-humulene (3.12%) while β-pinene (12.75%), α-pinene (36.42%) and camphor (15.65%) were the main constituents of rosemary essential oil. Cumin essential oil was manly composed of γ-pinene (27.4%), p-cymene (20.49%) and cuminal (20.39%).

Keywords: Essential oil, spices, chemical composition, GC/MS.

1. Introduction

Spice production in Mediterranean countries is approximately 38 million tonnes per year, with Turkey being the highest producer. Other countries, too, show impressive production figures but are normally dedicated to one specific spice; for example, in Spain, paprika is the most important spice crop.[1]

Spices are aromatic plant products which are frequently used to enhance food palatability. Most spices were originally indigenous to the tropics; for instance, cinnamon, pepper, clove and nutmeg. However, Mediterranean countries have also provided a number of aromatic seeds (coriander, mustard) and other spices such as bay leaf, thyme and oregano.[2] At present, about 44000 ha are dedicated to the production of spices in countries bordering the Mediterranean Sea.

Many essential oils and extracts obtained from spices and plants have recently gained in interest both or the general population and for the scientific community.[1] Many plants are used for different purposes, for example, in the food, drugs and perfumery sectors. Several researchers have shown interest in biologically active compounds isolated from plants and spices for eliminating pathogenic microorganisms because of the resistance that many microorganisms have built up to antibiotics.[3]

Culinary spices and herbs contain a wide variety of active phytochemicals (including flavonoids, terpenes, polyphenols, curcumins, coumarins) and may fulfil more that one function in any food to which they are added.[4] Spices also contain fibre, proteins, sugars, cations and pigments (carotenoids, chlorophylls, etc.). Phenolic compounds, as are vanillin, gallic acid, caffeic acid, etc. are involved in olfactory, taste and tactile sensations and volatile compounds such as essentials oils.[5]

The aim of this work was to determine the chemical composition of the essentials oils from several spices widely used in Mediterranean countries: oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cumin (*Cuminum cyminum*) and clove (*Syzygium aromaticum*).

2. Experimental

2. 1. Essential Oils

The essential oils of thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), clove (*Syzygium aromaticum* L.), rosemary (*Rosmarinus officinalis* L.), cumin (*Cuminum ciminum* L.*)* and oregano (*Origanum vulgare* L.) were obtanined by steam distillation, and were purchased from Ravetllat Aromatics, (Barcelona, Spain). The following authentic compounds were employed as standards in the gas-chromatography analyses: camphene, p-cymene, eugenol, (+)-limonene, α-pinene, β-pinene, α-terpinene, 1,8-cineole, thymol, linalool and carvacrol (Extrasynthese, Lyons, France).

2. 2. Analysis Conditions

2. 2. 1. Gas Chromatography Analysis(GC)

The essentials oils were analysed using a Shimadzu GC-17A equipped with FID detector and HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 250 and 270 °C, respectively. Oven temperature was kept at 50 °C for 3 min, then gradually raised to 240 °C at 3 °C/min. Helium was the carrier gas, at a flow rate of 0.8 mL/min. Diluted samples (1/10 acetone, v/v) of 0.2 μL were injected manually in the split mode (split ratio 1/44). Quantitative data were obtained electronically from FID area data without using correction factors. All the tests were performed in triplicate.

2. 2. 2. Gas Chromatography/mass Spectrometry Analysis (GC/MS)

Analysis of the essentials oils was performed using a Shimadzu GC-17A equipped with a Shimadzu GCMS-QP5050A mass selective detector and a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium at a flow rate of 0.8 mL/min was used as carrier gas. Injector and MS transfer line temperatures were set at 250 and 270 °C, respectively. Oven program temperatures was the same as for the GC analysis. Diluted samples (1/10 acetone, v/v) of 0.2 μL were injected automatically in the split mode (split ratio 1/44). The components were identified by comparing their relative retention times and mass spectra with those of standards (for the main components), Wiley 229 library data of the GC/MS system, Kovats Index and literature data.[6] All the tests were performed in triplicate.

3. Results and Discussion

3. 1. Chemical Composition of the Essential Oil

The chemical composition of the essential oil of *Thymus vulgaris* L., *Salvia officinalis* L., *Syzygium aromaticum* L., *Rosmarinus officinalis* L., *Cuminum cyminum* L. and *Origanum vulgare* L. was studied. The main constituents of each oil, their relative percentage of the total chromatogram area, Kovats index and retention times are summarized in Table 1.

The six essential oils analyzed varied greatly in composition. Some components were common to several oils, but were present in large amounts in only a few oils, whereas other components were found just in one oil. For example, α-pinene was present in all the essential oils except clove, but its percentage only exceeded 35% in rosemary. In the essential oil of thyme, 52 compounds were identified, representing 91.4% of the total oil, the major constituents being terpinen-4-ol (13.15%), γ-terpinene (9.21%), cis-sabinene hydrate (7.65%), linalool (7.12%) and p-cymene (5.75%). Tomaino et al.[7] reported that the major constituents of thyme essential oil were thymol (45.3%), p-cymene (26.1%) and linalool (6.17%). Several early studies on *Thymus* spices suggested that the main components of the oils were terpinen-4-ol, γ-terpinene, p-cymene in *T. baeticus*,[8] carvacrol, γ-terpinene and p-cymene in *T. revolutus*,[9] 1,8 cineole and linalool in *T. mastichina*,[10] p-cymene and carvacrol in *T. capitatus*,[11] thymol and p-cymene in *T. daenensis* and thymol, carvacrol and *p*-cymene in *T. kotschyanus*,[12] thymol, carvacrol and *p*-cymene in *T. spathulifolius*.[13]

This great variability and diversity observed, in the chemical composition of the essential oils of *Thymus* species and subspecies can be attributed to climatic and soil variations, stage of the vegetative cycle, seasonal variation, etc.[14] In some cases, two different varieties may provide the same essential oil yield and quality, even though the plants are morphologically different.[15] Some studies have reported that thyme essential oil possesses a high level of the phenolic precursors, p-cymene and γ-terpinene, probably due to its early flowering time.[16]

When the essential oil of oregano was analyzed by GC-MS 32 compounds were identified, representing 88.5% of the total oil, the major constituent being carvacrol (61.21%). Other important compounds were p-cymene (15.12%) and γ-terpinene (4.80%). Sezik et al.[17] investigated the essential oil composition of four subspecies of

Table 1. Constituents of sage, thyme, rosemary, cumin, clove and oregano and their relative percentages of total chromatogram area, Kovats Index and retention time.

		Essentials oils					
Compounds	**KI**[a]	**Sage (% area)**	**Thyme (% area)**	**Rosemary (% area)**	**Cumin (% area)**	**Clove (% area)**	**Oregano (% area)**
α-thujene	928	Tr.	2.10	0.15	0.30	–	0.07
α-pinene	936	6.75	5.16	36.42	0.76	–	2.34
camphene	951	7.63	1.30	11.08	Tr.	–	0.30
sabinene	974	0.09	1.66	–	0.26	–	–
β-pinene	977	5.19	0.65	3.67	12.75	–	0.44
β-myrcene	992	1.58	2.69	2.19	0.67	–	1.45
α-terpinene	1019	–	4.05	Tr.	0.16	–	0.56
p-cymene	1026	1.60	5.79	2.14	20.49	–	15.12
limonene	1031	–	5.09	–	0.56	–	1.41
1,8-cineole	1034	24.74	2.93	12.02	0.23	–	1.11
γ-terpinene	1060	1.04	9.21	0.18	27.44	–	4.80
cis-sabinene hydrate	1070	–	7.65	Tr.	Tr.	–	–
terpinolene	1089	0.67	1.56	0.27	0.08	–	3.63
linalool	1104	1.84	7.12	0.66	0.09	–	–
1-terpineol	1125	0.14	0.95	–	–	–	0.08
dihydrocarveol	1144	–	0.89	–	–	–	Tr.
verbenol	1148	–	1.15	–	–	–	–
camphor	1151	24.95	–	15.05	–	–	–
isoborneol	1162	1.11	–	0.44	–	–	0.08
borneol	1172	2.29	4.07	4.00	–	–	0.58
terpinen-4-ol	1181	0.19	13.15	0.38	0.43	–	–
α-terpineol	1195	3.60	5.84	1.14	0.44	–	0.15
verbenone	1211	Tr.	5.69	0.12	–	–	–
cuminal	1226	–	–	–	20.39	–	–
bornyl acetate	1288	3.38	0.38	2.13	–	–	0.31
2-caren-10-al	1289	–	–	–	7.85	–	–
carbicol	1293	–	–	–	4.35	–	–
thymol	1296	–	2.27	–	–	–	0.48
carvacrol	1304	–	0.13	–	Tr.	–	61.21
α-terpinyl acetate	1353	5.95	0.84	0.07	–	–	Tr.
eugenol	1370	–	–	–	–	85.85	–
β-caryophyllene	1426	1.80	0.71	1.64	–	10.54	2.62
α-humulene	1460	Tr.	Tr.	0.16	–	3.12	0.24
cyclogermacrene	1501	–	0.13	–	–	–	–
δ-cadinene	1528	–	0.35	0.09	–	0.29	Tr.
SEM		0.76	0.65	0.35	0.24	0.12	0.46

–: Not detected. Tr: Trace (Area ≤ 0.06%). [a]: Kovats Index in DB-5 column in reference to n-alkanes (C_8–C_{32})
SEM: Standard Error of the means

Origanum vulgare, subsp. *hirtum* (Link) Ietswaart, subsp. *gracile* (C. Koch) Ietswaart, subsp. *vulgare* and subsp. *viride* (Boiss.) Hayek, all of which grow wild in Turkey, and identified more than 80 constituents. *Oringanum vulgare* subsp. *hirtum* was rich in carvacrol (70.47%), while subsp. *gracile* contained β-caryophyllene (17.54%) and germacrene-D (12.75%) as dominant components. The major components of subsp. *viride* were terpinen-4-ol (16.82%) and germacrene-D (15.87%) and of subsp. *vulgare* terpinen-4-ol + β-caryophyllene (20.94%) and germacrene-D (17.80%). In *Origanum calcaratum* and *Origanum scabrum* essential oils, 22 and 28 components, respectively, were identified by Demetzos et al.[18] The major components of *Origanum calcaratum* were thymol (42.8%), p-cymene (18.1%), carvacrol (12.9%), γ-terpinene (9.6%) and isocaryophyllene (4.7%), while carvacrol (66.7%) was predominant in *Origanum scabrum* oil, which also contained *p*-cymene (7.8%), γ-terpinene (3.6%) and caryophyllene oxide (2.1%). Kokkini et al.[19] reported that the four major components of the essential oil of *Origanum vulgare* subsp. *hirtum* from autumn-collected plants were γ-terpinene, 0.6–3.6%; p-cymene, 17.3–51.3%; thymol, 0.2–42.8%; and carvacrol 1.7–69.6%. The essential oils of oregano spp., *Origanum vulgare* L. subsp. *viridulum* (Martin-Donos) Nyman from Greece, and *Origanum libanoticum* Boiss. and *O.*

syriacum L. from Lebanon were investigated by Arnold et al.[20] the first contained thymol (61.0–69.1%), the second contained methyl thymol (32.8%) and the last oil contained carvacrol (88.3%) as the major flavour compounds. Veres et al.[21] investigated the composition of *Origanum vulgare* subsp. *hirtum* oil and found it to contain carvacrol (76.4%), γ-terpinene (6.6%), thymol (0.23%), and p-cymene (4.7%) as the main constituents while the major compounds in *Origanum vulgare* subsp. *vulgare* oil were p-cymene (22.3%), caryophyllene oxide (10.2%), sabinene (7.9%), γ-terpinene (5.1%), thymol (0.34%) and spathulenol (4.8%).

GC-MS analyses of sage essential oils identified 37 constituents, representing 90.0% of the total oil. The main components were camphor (24.95%), 1,8-cineole (24.75%) and camphene (7.63%). There are many reports on the chemical composition of the oils isolated from the plants belonging to the genus *Salvia*,[22,23] most of which indicate that 1,8-cineole and borneol are the main constituents. These variations in the essential oil composition might have arisen from several differences (climatic, seasonal, geographical, geological).[22]

Asllani[24] investigated the composition of essential oils obtained from wild Albanian sage, from detected about 30 were identified. The major components identified were α-thujene (12.2–49.3%), β-thujene (3.1–10.5%), camphor (13.7–37.8%) and 1,8-cineole (3.9–23.4%). These results agree with the results presented here, except that α-thujene and β-thujene were not detected. Pino et al.[25] analysed the essential oil of sage (*Salvia officinalis* subsp. *altissima*) grown in Cuba. Among the 43 compounds identified, germacrene-D (32.9%), β-caryophyllene (31.8%) and caryophyllene oxide (23.2%) were the major constituents. Lorenzo et al.[26] investigated essential oils obtained by steam distillation from the leaves and inflorescences of *Salvia sclarea* plants cultivated in Uruguay identifying 27 components. The essential oil was found to contain high levels of linalool (8–22%), linalyl acetate (39–48%) germacrene-D (8–20%) and β-caryophyllene (3–5%). Lima et al.[27] investigated the essential oil of sage isolated from the air-dried aerial parts of the plants, and identified more than 50 compounds. The major compounds were cis-thujene (17.4%), α-humulene (13.3%), 1,8-cineole (12.7%), caryophyllene (8.5%) and borneol (8.3%). When Vera et al.[28] analysed the steam-distilled essential oil from the flowering parts of sage grown on Reunion Island, it was found to contain 51 compounds, the major ones being α-thujene (43.3–45.5%) and β-thujene (8.4–8.8%), camphor (15.9–16.2%) and 1,8-cineole (5.8–8.3%). Carruba et al.[29] reported that they were qualitative and quantitative di-fferences between the essential oils from the inflorescences and leaves, the former being characterized by a high content of linalool (26–29%) and linalyl acetate (35–53%) with germacrene-D as the main compound (68–69%). The inflorescences at full flowering stage were richer in linalool, α-terpineol and germacrene-D, but showed a lower content in linalyl acetate compared with those collected at early stags. Latifeh and Mehdi[30] reported, the development stage did not influence the oil composition of leaves. The quality and quantity of the compounds in different parts of the plant were not the same (e.g. α-thujene and β-thujene contents were lowest in the leaves collected at the flowering stage, 1.2% and 3%, respectively). The quantities of camphor (2.9%), 1,8-cineole (2%), α-thujene (6.4%) and β-thujene (1.6%) in the essential oil of aerial parts of the plant were lower than the international standards (33%, 10%, 16% and 2%, respectively).

In clove essential oil, five compounds were identified as representing 98.5% of the total. The predominant compounds were eugenol (85.5%), β-caryophyllene (10.54%), α-humulene (3.12%), δ-cadinene (0.29%) and caryophyllene oxide (0.20%). Raina et al.[31] found 16 compounds, the main components being eugenol (94%) and β-caryophyllene (2.9%), while Zapata and Meireles[32] reported that eugenol (58.62%) eugenyl acetate (19.58%), β-caryophyllene (19.87%) and α-humulene (1.60%) were the major constituents. Kwang and Shibamoto[33] reported that eugenol and eugenyl acetate were the major constituents of clove essential oils made from buds which agree with the results obtained in this study, except in the case of eugenyl acetate, which was not found. The yield and quality of the essential oil depends on the part of the plant used, the predominant compounds in essential oil from clove stems being eugenol (83–92%), β-caryophyllene (4–12%), eugenyl acetate (0.5–4%), while the predominant compounds in the essential oil obtained from leaves are eugenol (80–92%), β-caryophyllene (4–17%) and eugenyl acetate (0.2–4%). The predominant compounds in the essential oil of clove buds are eugenol (75–87%), β-caryophyllene (2–7%) and eugenyl acetate (8–15%).[34]

Turning on attention to rosemary, 39 components were identified in the essential oil, representing 89.5% of the total, the major constituents being, α-pinene (36.42%), camphor (15.65%), 1,8-cineole (12.02%) and camphene (11.08%). Dellacassa et al.[35] investigated the composition of the essential oils obtained from the leaves of *Rosmarinus officinalis* cultivars growing in different areas of Uruguay and southern Brazil. Finding that the former were rich in α-pinene (37.8–46.2%) and 1,8-cineole (13.4–13.8%), while the essential oil from cultivated Brazilian plants contained α-pinene (32.2%) and 1,8-cineole (14.7%), and that from wild Brazilian plants contained α-pinene (12.4%), myrcene (22.7%) and 1,8-cineole (15.3%). The essential oil from the fresh leaves of *Rosmarinus officinalis* L. grown in Rio de Janeiro, Brazil, was isolated and analysed by Porte et al.[36] 45 constituents were identified, the major constituents of the oil being camphor (26.0%), 1,8-cineole (22.1%), myrcene (12.4%) and α-pinene (11.5%). That the geographical location of where the plant grows can also contribute to the content and quality of essential oil was confirmed by Guillem et al.[37] who reported that rosemary essential oil appeared to be more complex and richer in flavour notes than other previously studied Spanish rosemary oils, and had intermediate proportions of α-pinene and 1,8-cineole compared with rosemary essential oils from different geographical origins, but higher proportions of camphor, verbenone and linalool. Tomei et al.[38] investigated the essential oils from flowers and leaves of *Rosmarinus officinalis* (collected from the wild in southern Spain), and found the main components to be camphor (32.33%), 1,8-cineole (14.41%) and α-pinene (11.56%). The essential oils of *Rosmarinus officinalis* from Spain, were analysed by Chalchat el al.[39] who found them to be rich in α-pinene (24.7%), 1,8-cineole (21.8%), and camphor (18.9%), although they also contained some borneol (4.5%), findings that are in agreement with the results presented here. Soliman et al.[40] carried out a comparative study of the essential oils prepared from the fresh leaves of plants collected from Siani and Giza. Analysis of the oils by GC-MS led to the identification of 43 components in the sample from Siani, with verbenone (12.3%), camphor (11.3%), bornyl acetate (7.6%) and limonene (7.1%) being the major constituents. 37 components were identified in the sample from Giza but in this case camphor (14.9%), α-pinene (9.3%) and 1,8-cineole (9.0%) were the main constituents. Boutekedjiret et al.[41] investigated the essential oil from flowering aerial parts of *Rosmarinus officinalis* collected in Algeria. More than 90% of the components were identified, with 1,8-cineole (52.4%) and camphor (12.6%) being the major components. Benhabiles et al.[42] also investigated the essential oil, from flowering aerial parts of *Rosmarinus officinalis* collected in Algeria observing the major components to be camphor, borneol, α-terpineol, bornyl acetate, β-caryophyllene, δ-cadinene, muurolene and α-humulene.

The effect of harvest time on oil production and chemical composition is very important. The highest oil yields were recorded during the fruiting period (summer), while the lowest concentrations of camphor and maximum concentrations of α-pinene were observed in winter. Concentration of 1,8-cineole was proximally constant throughout the year, though other oil constituent levels varied randomly with the plant life cycle. It is suggested that seasonal and geographical variations in the content of the most representative components help in the quality control of rosemary oils and, consequently, for deducing the best period for processing.[43]

GC-MS analyses of cumin essential oils identified 26 constituents, representing the 80.0% of the total oil. The main components of cumin essential oil were γ-pinene (27.4%), p-cymene (20.49%), cuminal (20.39%) and β-pinene (12.75%). In a study carried out by Ia-cobellis et al.[44] p-mentha-1,4-dien-7-al, cuminal, γ-pinene and β-pinene were seen to be the major constituents of cumin oil. These findings agree with those results presented here, except for p-mentha-1,4-dien-7-al, which was not found, and p-cymene, which represented 20.49% of the total. The composition of cumin essential oil of Turkish origin was investigated by Baser[45] who found it to be characterized by high amounts of cuminal, p-mentha-1,4-dien-7-al, γ-pinene, β-pinene, perilla aldehyde and p-mentha-1,3-dien-7-al. Anon[46] reported that the main constituents of Egyptian cumin essential oil were cuminal, β-pinene, γ-pinene, p-mentha-1,3-dien-7-al, p-mentha-1,4-dien-7-al and p-cymene. Atta et al.[47] investigated the composition of cumin seeds obtained from di-fferent localities in Turkey with Cuminal (19.6–27.0%), p-mentha-1,3-dien-7-al (4.3–12.3%), p-mentha-1,4-dien-7-al (24.5–44.9%), γ-terpinene (7.1–14.1%), p-cymene (4.6–12.0%) and β-pinene (2.9–8.9%) identified as the major components.

4. Conclusions

The major components of oregano essential oil, included carvacrol (61.21%) and p-cymene (15.12%). Sage essential oil mainly contained camphor (24.95%) and 1,8-cineole (24.75%). The essential oil from thyme is characterized by a high content of terpinen-4-ol (13.15%) and γ-terpinene (9.21%). The predominant compounds in clove essential oil are eugenol (85.5%) and β-caryophyllene (10.54%). The main constituents of rosemary essential oil are β-pinene (12.75%) and α-pinene (36.42%), and the major constituents of cumin essential oil are γ-pinene (27.4%) and p-cymene (20.49%).

There is then, great variability in the chemical composition of essential oils obtained from spices. Such variability depends on several factors including climatic, season, geographical location, geology, part of the plant and the method used to obtain the essential oil.

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Povzetek

Začimbe so v državah južne Evrope in severne Afrike široko v uporabi zaradi okusa, arome in barve. Cilj tega dela je bila določitev kemijske sestave eteričnih olj šestih dišavnic in začimbnic iz španske regije Murcia: origano (*Origanum vulgare*), timijan (*Thymus vulgaris*), rožmarin (*Rosmarinus officinalis*), žajbelj (*Salvia officinalis*), kumina (*Cuminum cyminum*) in nageljnove žbice (*Syzygium aromaticum*). Analize smo opravili z uporabo GC-MS.
Glavne sestavine eteričnega olja žajblja so bile kafra (24,95 %), 1,8-cineol (24,75 %) in kamfen (7,63 %); origana: karvakrol (61,21 %) in *p*-kimen (15,12 %); timijana: terpinen-4-ol (13,15 %), γ-terpinen (9,21 %) in cis-sabinen hidrat (7,65 %); nageljnovih žbic: eugenol (85,5 %), β-kariofilen (10,54 %) in α-humulen (3,12 %); rožmarina: β-pinen (12,75 %), α-pinen (36,42 %) in kafra (15,65 %), ter kumine: γ-pinen (27,4 %), *p*-kimen (20,49 %) in kuminal (20,39 %).