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Phytochemical characterization of different varieties of thyme

Ts. S. Valev, K. Zh. Dobreva*, M. D. Dimov

Trakia University, Faculty of Engineering and Technology, 38 Graf Ignatiev Street, 8600 Yambol, Bulgaria

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For centuries, thyme has been used as a spice and medicinal plant. Of the more than 350 established species of plants of Thymus genus, garden thyme (*Thymus vulgaris* L.) and common thyme (*Thymus serpyllum* L.) have economic importance. Three chemotypes of thyme are the object of this study - thymol, citral and geraniol. The main physical and chemical characteristics of the studied thymes are determined - humidity, proteins, fibers, lipids, essential oil and mineral composition. The chemical composition of organic substances in the essential oils of the different chemotypes was determined by GC – MS analysis. The distribution of the components by groups has been established. The yield of essential oil is highest in "French" and "Pagane" varieties. "French" and "German Winter" thyme oils are characterized by a high thymol content (36.98 % and 55.49 %, respectively), which determines their classification as thymol chemotype. In the essential oil of "Slava" variety, the main components are geraniol (28.89%), nerol (3.84%) and geranyl acetate (34.35%), which define it as a citral chemotype. Main components in "Pagane" thyme oil are geraniol (55.56%) and geranyl acetate (27.72%), defining it as a geraniol chemotype. The determination of the phytochemical characteristics of thyme aims to provide information on its condition, its suitability for storage and processing, as well as to determine the type and technological parameters for its processing.

Keywords: *Thymus vulgaris* L., chemical composition, essential oil, GC/MS.

INTRODUCTION

Today, about 250 species of thyme are known, 214 of which are species and 36 are subspecies divided into eight sections: *Mikantes, Mastihcina, Piperella, Teucrioides, Pseudothymbra, Thymus, Hyphodromy* and *Serpyllum* [1–4]. It is distributed mainly in the Mediterranean, where it originates. Today it is cultivated in Spain, France, Italy, Portugal, Germany, Algeria, Egypt, England, the Caribbean, the USA [5, 6]. The composition of essential oil in thyme is influenced by many factors such as: genotype (species, genus, family, order, class), agro-meteorological (geographical origin, climatic conditions, soil composition, etc.) and technological factors (cultivation, species - collection, storage, processing technology, etc.) [4, 7–11].

In Bulgaria, thyme can be found in diverse soil and climatic conditions, semi-mountainous terrains at different altitudes, along roads, meadows and sunny places, together with other heat-loving plants. Likes sandy, stony and drained soils: brown forest, cinnamon forest, neutral and slightly alkaline soils [10, 12].

The varieties grown in our country: "German Winter", (*Thymus vulgaris* sv "German"), "French" (*Thymus vulgaris* cv. 'French'), "Slava" (*Thymus marshalianus*) and "Pagane" (*Thymus siptorpii* L.) differ in both morphological and aroma-flavor characteristics, as well as in the composition of essential oil [12].

"Pagane" variety was selected from the population of *Thymus marshalianus*. It has upright, highly branched tufts and a subtle, fresh, pleasant rose fragrance. The yield of essential oil is 0.55-0.85%, which is rich in geraniol – 74-76% and nerol 15-17%.

"Slava" variety was created from species *Thymus siptorpii* L. The plants have upright stems, the variety is suitable for mechanized cultivation, high yield. It contains essential oil in the fresh aerial mass 0.5-0.7%, with the main ingredient citral - up to 23%, citronellol - up to 10% and geraniol - up to 36%. The aroma is fresh, subtle with a citral note, reminiscent of lemon balm, suitable for the food industry. Both varieties are product of Bulgarian selection [12].

The variety "German Winter" was introduced from Germany and belongs to the species *Thymus vulgaris* L. The tufts are more compact, with erect flower-bearing stems, equal in height. The variety successfully winters under our conditions. The content of essential oil is 0.28% with the main ingredient thymol – 39.71% - 61.44%, carvacrol – up to 6.04% [12, 13].

The purpose of the research is to determine the phytochemical characteristics of different types and chemotypes of thyme grown in Bulgaria, which provides information on its condition, its suitability for storage and processing, the method and technological parameters of its processing, and the possibilities for the application of aromatic products, obtained from it as well.

EXPERIMENTALS

Plant material

The aerial part of thyme from "French", "German Winter", "Slava" and "Pagane" varieties was used, delivered from the experimental field of the Institute of Roses, Essential and Medical Cultures - Kazanlak, Bulgaria during the months of May-June 2023.

In order to reduce a significant part of the humidity

* To whom all correspondence should be sent:
E-mail: *krasimira.dobreva@trakia-uni.bg*

and improve the storage of the raw material, the aerial part of the plant was dried in a dry, shaded and ventilated place for 20 days, immediately after harvesting. It was packed in paper bags and stored in a dark, dry and cool place.

Chemical analyses

The raw materials (air-dried) were analyzed for moisture content by azeotropic distillation using a Dien – Stark laboratory device, % [14]; for protein content, by Kjeldahl, results are expressed as % w/w (BDS - ISO 5983-1: 2006); for fat content, by Soxhlet, results are expressed as % w/w (BDS - ISO 6492: 2007); for fiber content, by Henberg and Stoman, results are expressed as % w/w (BDS - AOAC, 2007) and for ash content, by incineration in a muffle furnace at 650 °C, results are expressed as % w/w (BDS - ISO 5984: 2007).

Mineral analysis

The mineral composition (except for phosphorus) was determined by flame AAS on a Perkin Elmer spectrometer according to BDS EN 15510**:** 2017. The sample was dried in a dry or wet manner and dissolved in acid to obtain a solution with an optimum concentration of the elements. It was atomized in air-acetylene flame at a temperature of 2000 - 3000°C. The absorption (optical density) was determined and the concentration was calculated using a standard curve.

The phosphorus content was determined as the air-dry sample was burned at 500 - 550 °C until gray-white ash was obtained. Phosphorus was determined by the method of Gerike and Kurmis, measuring the optical density at a wavelength of 470 nm [15].

Essential oil extraction

Prior to the technological processing the raw material has been cut into pieces measuring 1.5 - 2 cm.

The essential oil was extracted by hydrodistillation in a Clevenger-type laboratory apparatus, % w/v. The distillation begins with the separation of the first drop of distillate into a container. Distillation is complete when two consecutive measurements in 30 min do not mark an increase in the amount of essential oil [14]. The obtained essential oils were dehydrated over anhydrous Na_2SO_4 and stored in glass vials at 4 - 6 °C until analysed. The yields of the essential oils were converted to absolutely dry mass.

Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) analyses

The chemical composition of the essential oils was determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) by direct headspace analysis according to ISO standards (ISO 11024-1: 1998, ISO 11024-2: 1998).

GC analysis: Agilent 7890 A device with flame ionization detector; HP-INNOWax polyethylene glycol column (60 m × 0.25 mm; 0.25 μm film thickness); temperature conditions: 70 °C for 10 min, 70 - 240 °C at 5 °C/min, 240 °C for 5 min; 240-250 °C at 10 °C/min, 250 °C for 15 min; helium carrier gas, 1 cm^3/min constant velocity; injector: split, 250 °C, split ratio 50:1.

GC-MS analysis: Agilent 5975 C device, helium carrier gas, column and temperature conditions as in the GC assay; detectors: FID, 280 °C, MSD, 280 °C transfer line.

The flavor components were identified by comparison with the witness retention index and mass spectra (MS), stacked at retention time, the amount was given in percentage.

All experiments were performed in triplicate, with averaged values in the tables and graphs, and represented with their mean and standard deviation. The measurements and calculations were processed in MS Excel 2016 (Microsoft Corporation Inc.) at a level of significance α=0.05.

RESULTS AND DISCUSSION

The studied plants have low humidity, which is an indicator of good storage of the raw material. The composition of the dried above-ground part of the raw material is shown in Table 1, and the mineral composition in Table 2.

Table 1. Physical and chemical characteristics of various varieties thyme

Components	French	German Winter	Slava	Pagane
Moisture, %	6.74	6.46	6.47	6.38
Proteins, %	12.96	8	8.09	8.91
Fats, %	1.93	1.99	1.23	1.75
Ash, %	7.31	10.01	8.75	9.75
Fibers, %	22	26.47	26.3	19.06
Essential oil, % w/v	0.18	0.1	0.1	0.18

Table 2. Mineral composition of various varieties of thyme

№	Ca %	P %	K mg/kg	Mg mg/kg	Mn mg/kg	Zn mg/kg	Cu mg/kg	Fe mg/kg
German Winter	0.52	0.27	8 942.92	2 515.27	81.30	39.95	11.99	3703.66
Slava	0.66	0.10	9 791.58	2 156.78	59.28	49.10	13.71	1996.44
Pagane	0.41	0.13	10 551.06	2 393.39	67.75	48.83	11.86	1853.57
French	0.55	0.14	9 908.12	1 949.56	40.02	31.93	8.82	993.67

The amount of proteins in the studied raw materials is comparable to the data on the content of proteins in other herbaceous plants of the *Lamiaceae* family - common oregano, mint, sage, savory, rosemary, basil (4.88 - 22.98%). The fat and fiber content is lower compared to the raw materials (from 4.07 to 15.22% for fat and from 37.00 to 45.70% for fiber) due to their different botanical affiliation [16].

There is a lack of data in the literature on the mineral composition of thyme grown in Bulgaria, which makes comparison difficult. All essential oils are pale yellow liquids except "Pagane" oil, which is almost transparent due to the high content of geraniol determining its color. The chemical composition of the essential oils from the different varieties of thyme grown in Bulgaria is shown in Table 3.

Table 3. Chemical composition of essential oils, %.

№	RI	Compound	Content, %			
			French	German Winter	Slava	Pagane
1	929	α-Thujene	0.55	0.47	nd*	nd
2	935	α-Pinene	0.57	0.43	nd	nd
3	948	Camphene	0.72	0.55	nd	nd
4	972	β-Pinene	0.20	0.23	nd	nd
5	977	1-Octen-3-ol	1.60	0.26	0.29	0.19
6	990	Myrcene	0.62	0.73	nd	nd
7	994	3-Octanol	0.33	nd	0.22	nd
8	1016	α-Terpinene	0.92	1.05	nd	nd
9	1022	p-Cimene	29.43	17.68	0.46	nd
10	1024	Limonene	0.35	0.25	nd	nd
11	1026	Eucalyptol	1.03	1.40	nd	nd
12	1057	γ-Terpinene	5.23	6.45	0.27	nd
13	1071	Camphenilone	1.50	1.17	nd	nd
14	1098	β-Linalool	4.46	3.16	0.52	0.53
15	1105	1-Octen-3-yl-acetate	nd	nd	nd	1.33
16	1146	Camphor	0.52	nd	0.60	nd
17	1157	Benzyl acetate	0.76	nd	nd	nd
18	1169	Borneol	2.97	1.83	0.28	nd
19	1178	1-Terpinen-4-ol	0.43	0.87	nd	nd
20	1190	α-Terpineol	0.19	0.30	nd	1.40
21	1226	Nerol	nd	nd	3.84	2.80
22	1233	Methyl thymyl ether	0.29	0.34	nd	nd
23	1237	Neral	nd	nd	8.53	1.51
24	1250	Geraniol	nd	nd	29.89	55.56
25	1266	Geranial	nd	nd	7.86	0.75
26	1291	Thymol	36.98	55.49	3.97	0.23
27	1300	Carvacrol	3.59	3.02	nd	nd
28	1344	α-Terpinyl acetate	nd	nd	0.35	3.22
29	1350	Thymyl acetate	0.12	0.10	nd	nd
30	1361	Neryl acetate	nd	nd	1.69	0.86
31	1382	Geranyl acetate	nd	nd	34.35	27.72
32	1395	β-Elemene	0.14	0.21	nd	nd
33	1420	β-Caryophyllene	3.21	1.54	1.65	0.22
34	1455	α-Caryophyllene	0.11	0.15	1.26	0.14
35	1468	9-epi-(E)-Caryophyllene	0.24	nd	nd	nd
36	1484	Germacrene D	0.30	nd	0.65	0.77
37	1514	γ-Cadinene	0.17	0.11	0.20	0.15
38	1525	β-Cadinene	0.25	0.14	0.37	1.64
39	1560	Elemicin	nd	nd	0.92	0.13
40	1590	Caryophyllene oxide	1.65	1.34	1.07	0.37
41	1624	10-epi-γ-Eudesmol	0.24	0.42	0.53	0.28
		Essential oil, % (v/w)	0.18±0.98	0.1±0.04	0.1±0.01	0.18±0.04

* nd - not determined

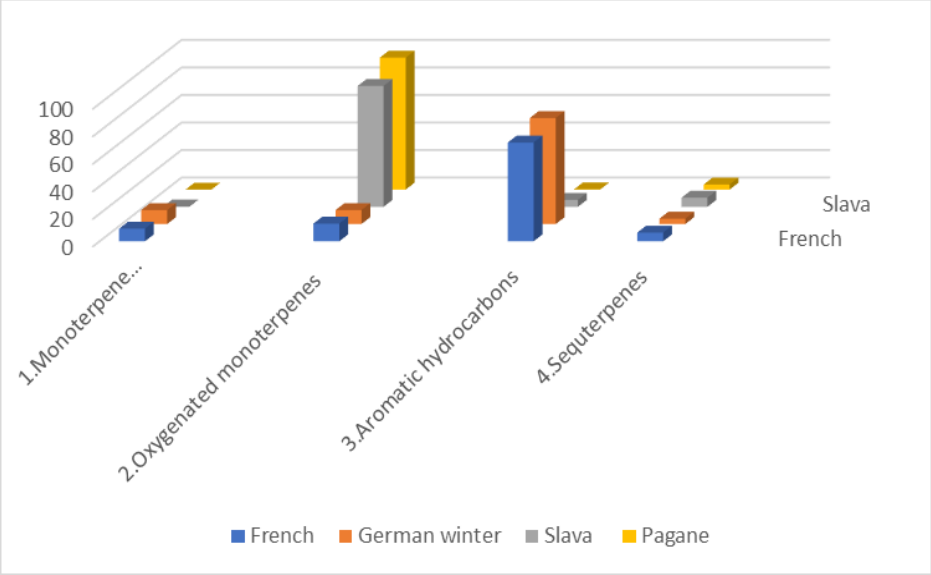

Fig. 1. Groups of components in thyme oils, %: 1 – monoterpene hydrocarbons; 2 – oxygenated monoterpenes; 3-aromatic hydrocarbons; 4 – sesquiterpene hydrocarbons. In the essential oil of "French" variety (*Thymus vulgaris* cv. 'French'), 32 compounds were identified, representing 99.66% of the oil. The main components (over 3%) in oil from the aerial part are: thymol (36.98%), p-cimene (29.43%), γ-terpinene (5.23%), β-linalool (4.46%). carvacrol (3.59%). β-caryophyllene (3.21%).

In the oil of "German Winter" variety collected during the mass flowering phase, 27 components were identified constituting (99.72%) of the essential oil. The main compounds (over 3%) are: phenolic monoterpene thymol (55.49%), p-cimene (17.68%), monoterpene γ-terpinene (6.45%), oxygenated monoterpene β-linalool (3.16%), of these 27.

In the thyme oil of "Slava" variety, 23 components are observed, making up 99.76% of the total amount of oil with main compounds (over 3%) oxygen-containing monoterpenes: geranyl acetate (34.35%), geraniol (29.89%), neral (8.53%), geranial (7.86%), nerol (3.84%) and the aromatic monoterpene thymol (3.97%) as well.

20 components were found in the essential oil of "Pagane" variety, which are 99.8% of the total amount. The main compounds in the composition of the essential oil (over 3%) are the oxygen-containing monoterpenes: geraniol (55.56%), geranyl acetate (27.72%) and α-terpinyl acetate (3.22%).

The distribution of the identified aromatic compounds based on functional groups in these four essential oils is presented in Fig. 1.

In the essential oils of "French" and "German Winter" varieties, aromatic hydrocarbons predominate (71.72% and 77.07%, respectively), and in those of "Slava" and "Pagane" varieties, the oxygen-containing monoterpene hydrocarbons (87.81% and 95.87%, respectively)). The content of sesquiterpenes in the essential oils of these four varieties is as follows: "French" - 6.3%; "German Winter" - 3.92%; "Slava" - 6.64% and "Pagane" - 3.7%.

CONCLUSION

Physical and chemical parameters of different varieties and chemotypes of thyme grown in Bulgaria were determined: variety "French", variety "German Winter", variety "Slava" and variety "Pagane". The different varieties are characterized by a different chemical composition of the essential oils, which also characterizes them as different chemotypes. The main component in the thyme oils of “French” and “German Winter” varieties is the aromatic monoterpene thymol (36.98% and 55.49%, respectively), which is decisive for their classification as a thymol chemotype. In "Slava" and "Pagane" varieties, oxygen-containing monoterpenes dominate. In the essential oil of "Slava" variety, the main components are geraniol (28.89%), nerol (3.84%) and geranyl acetate (34.35%), which define it as a citral chemotype. Main components in "Pagane" thyme oil are geraniol (55.56%) and geranyl acetate (27.72%), defining it as a geraniol chemotype. Both varieties of thyme have monoterpene hydrocarbons below 1%, which is positive for the perfumery industry.

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