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STUDY OF ESSENTIAL OIL COMPONENTS IN DIFFERENT *ORIGANUM* SPECIES BY GC AND SENSORY ANALYSIS

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Oregano is used worldwide both as spice and crude drug, which is mainly provided by species of *Origanum* genus. The quality of the product is usually determined by chemical analysis, whereas in food industrial applications sensory tests are also practised. The aim of the present study was a comparison of parallel quality investigations of oregano samples by a new and effective instrumental sensory evaluation method, the "electronic nose", and by gas-chromatographic and human sensory analysis.

The GC analysis of essential oil components revealed mainly differences between plant species (*Origanum vulgare* subsp. *hirtum* and *Origanum majorana)*. Main components of the oil of the former taxon are carvacrol and thymol, while those of marjoram are terpinene-4-ol, γ-terpinene and terpinolene. A wholesale oregano sample showing considerable divergence from the other ones with respect to ratios of carvacrol, β-caryophyllene β-cubebene and thymol. It was assumed not to belong to ssp. *hirtum.*

The electronic nose analysis, evaluated by PCA, proved to be an appropriate, rapid, non-destructive, reagent-less method for the reliable separation of all of the oregano samples based on their complex aroma features. Assumptions could be made about correlations between separation of samples by the instrumental sensors and proportions of terpenoid compounds of the oil established by GC in some cases only. The varying essential oil content of the samples did not influence the success of instrumental evaluation.

The instrumental and human sensory analysis showed similar results: varieties of *O. majorana* could be well distinguished on the basis of their complex aroma, while their gas-chromatograms did not show characteristic differences.

The results call the attention that quality evaluation of drug items of aromatic plants should be oriented in different directions, considering the current utilisation area of the items.

Keywords: *Origanum vulgare* subsp. *hirtum, Origanum majorana*, gas-chromatography, sensory analysis, electronic nose

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In recent decades the importance of oregano has been considerably increased. It is mainly used as condiment for pizza dishes, it can be found in spaghetti sauces, in different tomato-based foods, seafood, and almost any garlic-flavoured dish moreover even in some kinds of cheese (OLIVIER, 1996).

Different literature searches cite at least 61 species of 17 genus belonging to six families, which are mentioned under the name oregano (BERNÁTH, 1996). Among them, the family *Lamiaceae* is considered to be the most important group containing the genus *Origanum*, which provides the source of well-known oregano spices, i.e. Turkish and Greek types.

The subspecies *Origanum vulgare* subsp. *hirtum* is widely used as a spice under the name 'Greek oregano'. Among different taxa of *Lamiaceae* and *Verbenaceae* used all over the world as spice named 'oregano', 'Greek oregano' is generally considered as the one having the highest quality (KOKKINI, 1996).

Carvacrol, thymol, p-cymene and γ-terpinene could be found as major components in the essential oil of *Origanum vulgare* subsp. *hirtum*. However, the subspecies cannot be characterised by homogeneous composition of oil. According to FLEISHER and SNEER (1982) it is divided into three intraspecific chemotypes: carvacrol-type, thymol-type and terpinene-4-ol-type.

At our Department a research project has been under way from 1996 investigating the chemical and morphological variability of this subspecies (SZABÓ, 2001). Starting from different gene-bank collections, we have developed several selected strains. Among them, a carvacrol-rich chemotype (with high or medium amount of carvacrol) and a thymol-carvacrol chemotype (with lower carvacrol and higher thymol contents) could be considered as the most valuable ones and are under propagation now.

As both the therapeutical applications and the utilisation in food industry are considerable, the quality of drug items should be checked from different aspects. In this respect, three main questions arise:

1. What are the chemical characteristics of the strains concerning the quantity and quality of their essential oil?

2. Is there any considerable difference in their complex sensorial aroma features?

3. Is there any significant correlation between the detected chemical composition and the complex aroma features?

The answer to these questions were provided by the help of a combination of objective instrumental analysis and traditional human sensory tests. For instrumental sensorial evaluation the "electronic nose" was used. It is the general name for the analytical instrument that profiles the headspace volatiles over or around the sample. The technology is based on an array of chemical sensors (e.g. conducting polymer sensors) whose outputs are integrated by advanced signal processing to identify complex aromatic mixtures (HODGINS & CONOVER, 1995).

In the current publication we summarised the results of the parallel evaluation by gas-chromatographic measurements and chemosensor-array (electronic nose) methods intended to answer the above questions.

1. Materials and methods

1.1. Plant material

The investigated plant material was the herb samples of different origins of *Origanum* spp. (Table 1), including our selected strains as well as commercial samples. The *O. vulgare* subsp. *hirtum* and *O. majorana* strains were produced at the Experimental Station of the Faculty of Horticultural Sciences in Soroksár, Budapest, in 1998. The oregano strains originated from botanical seed collections, of which these have been selected and examined since 1996 (SZABÓ, 2001). The two marjoram populations are registered Hungarian varieties, produced from elite seed stocks.

Table 1. The examined samples

Sample No.	Population/Origin
1	*Origanum vulgare* subsp. *hirtum* (Link) Ietswaart genebank population
2	*Origanum vulgare* subsp. *hirtum* (Link) Ietswaart selected strain (H5)[a]
3	*Origanum vulgare* subsp. *hirtum* (Link) Ietswaart selected strain (H2)[a]
4	*Origanum vulgare* subsp. *hirtum* (Link) Ietswaart selected strain (H3)[a]
5	Oregano spice retail sample (unknown taxon)
6	Origani herba wholesale sample (unknown taxon)
7	*Origanum majorana* L. (sweet marjoram) variety 'Francia'
8	*Origanum majorana* L. (sweet marjoram) variety 'Magyar'

[a] SZABÓ (2001)

1.2. Gas-chromatography

The dry, shelled herb samples were water-distilled in Clevenger-apparatus according to the standard method of PHARMACOPOEA HUNGARICA (1986). The content of essential oil was calculated as percentage of the dry mass. The main chemical compounds of the essential oil were determined by GC method in a capillary gas chromatograph (Shimadzu GC-B14 with Shimadzu Class – VP Chromatography Data System 4.2 equipped with FID). An SE-30 type 30 m × 0.25 mm i.d. column was used (film thickness 0.25 μm). The injector (1:100 split) and detector temperatures were 220 °C and 250 °C, respectively. Column temperature program was the following: 90 °C (3 min), 90–180 °C (6 °C min^{-1}), 180 °C (5 min), carrier gas was nitrogen (1 ml min^{-1}). The identification of compounds was performed by comparison of their retention times with those of pure substances by peak enrichment with standards. Relative percentage of the oil constituents was calculated from the GC peak areas. The results were evaluated by variance and principal component analysis, too.

1.3. Instrumental sensory analysis (electronic nose)

For the instrumental sensory analysis "SamSelect" electronic nose, produced by *DaimlerChrysler Aerospace* (Rostock), was used. This equipment works with a sensor array consisting of six individual quartz crystal sensors coated with six different gas

sensitive materials. The adsorption of the volatile molecules on the sensor surface causes changes in their masses, resulting in frequency modifications of the quartz oscillators. The basic frequency is 10 MHz±1 Hz, while this value is several hundred Hz during the measuring time. The changes in frequencies serve as sensor signals for the evaluation. The eight dry and shelled samples were measured in standard headspace vials in nine replications. Headspace autosampling was used as a standard and reproducible sampling technique. During the heating time, balance was established in the vial between the solid and gas phase and the sample can reach a constant temperature (70 °C in this occasion). Overpressure of 0.07 MPa was reached in the vial after the reference gas streamed in for 75 s. As a consequence of the overpressure the aroma of the sample streams into the measuring cell, which has 1.5 cm^3 cubic capacity. The measuring time was 60 s in this case. The last step is cleaning, when the reference gas is streamed through the sensor array for 75 s. For the evaluation of the sensor signal response of the sensor array, principal component analysis (PCA) was used, which is part of the SelectWare software and is attached to SamSelect chemo-sensor array.

1.4. Human sensory analysis

A traditional human sensory analysis, simple difference test according to MEILGAARG and co-workers (1991) was carried out in the Sensory Laboratory of Szent István University. Two samples of *O. majorana* Nos 7 and 8 were compared by 36 panellists. Each of them got four coded sample-pairs, and pairwise comparison of samples was carried out in four different combinations (AA, AB, BA, BB). Samples were placed in non-transparent vials to assess smell only. Panellists were asked to evaluate any difference between the aroma of the sample-pairs. The results were evaluated by Chi-square test.

2. Results

2.1. Results of the essential oil investigations

The accumulation level of essential oil proved to be a characteristic separation feature among the samples (Table 2). One of our selected oregano strains (No. 2) contained the highest volatile oil level (3.968%) followed by the other populations originating from Soroksár, while the lowest oil quantity appeared in the two commercial samples, Nos 5 and 6 (1.478% and 0.052%). These latter ones do not conform to the specification of the standard, while the other ones are over the required level (1.8%) (INTERNATIONAL STANDARD, 1985).

Essential oil content of the 'Francia' population (No. 7) contained by 0.34% more essential oil than the other marjoram variety (No. 8) (Table 2). However, this difference did not prove to be significant. Both values (1.68% and 1.34%, respectively), can be considered as advantageous levels compared to the standard specification (0.4%) (HUNGARIAN STANDARD, 1988).

Table 2. Essential oil content and compounds of the investigated samples

Component	Samples[a]							
	Amount of components in the essential oil (%)							
	1	2	3	4	5	6	7	8
α-Pinene	0.5	–	0.9	–	0.2	Traces	0.2	0.3
Camphene	0.4	–	–	–	–	1.1	0.3	0.3
β-Pinene	–	1.4	2.5	1.4	–	1.3	5.1	5.3
t_R 3.93	9.5	5.6	17.5	7.8	4.8	1.0	–	–
α-Terpinene	–	–	–	–	–	–	1.9	2.0
γ-Terpinene	4.2	5.9	7.0	9.1	5.1	1.2	5.6	4.4
t_R 4.02	0.4	–	–	–	0.4	3.8	–	–
t_R4.05	–	–	1.3	–	–	3.0	–	–
1,8-Cineol	–	–	–	–	–	–	5.4	3.6
Limonene	–	–	–	–	–	2.1	–	–
Cis-sabinene-hydrate	–	–	–	–	–	–	6.7	7.4
Terpinolene	–	–	11.0	–	–	–	29.6	35.7
Sabinene	–	–	–	–	–	–	4.4	1.6
Terpinene-4-ol	–	–	–	–	–	–	19.5	15.8
α-Terpineol	–	–	–	–	–	–	3.8	3.9
Thymol	2.2	5.0	–	25.6	2.3	6.1	–	–
Carvacrol	75.9	80.6	53.5	44.2	78.3	–	–	–
β-Caryophyllene	–	1.5	1.3	1.8	–	11.0	2.4	3.0
β-Cubebene	–	–	–	0.5	–	13.8	–	–
Total essential oil content (ml 100 g^{-1})	3.03	3.97	2.14	2.31	1.48	0.05	1.68	1.34

[a] For sample codes see Table 1

The GC analysis of essential oil components revealed mainly differences between species (*Origanum vulgare* subsp. *hirtum* and *Origanum majorana)*. Main components of the oil of the former taxon are carvacrol and thymol (FLEISHER & SNEER, 1982), while those of marjoram are terpinene-4-ol, γ-terpinene and terpinolene (RAVID et al., 1987). Besides, among oregano samples, No. 6 showed a considerable divergence from the other ones. It was free of carvacrol, contained characteristically high ratios of sesquiterpene components such as β-caryophyllene and β-cubebene (11.0% and 13.8%, respectively) and a low amount of thymol (6.1%). It can be assumed that this commercial sample represented another unknown subspecies of *O. vulgare*.

In oregano samples Nos 1–5, carvacrol proved to be the major oil component. Sample No. 2 contained the highest level of carvacrol (80.6%), however, samples 1 and 5 reached significantly similar levels (75.9% and 78.3%, respectively). Samples 3 and 4 showed significantly lower levels of carvacrol (53.5% and 44.2%, respectively), and in the latter sample, besides carvacrol, a considerable amount of thymol was detected (25.6%), as a characteristic feature of this selected strain. In other samples the proportion of thymol is significantly lower (2.2–6.1%) or it is not detectable at the current sensitivity level.

A further important separation feature was an unknown component with a retention time of 3.93 min. It can be found in the highest percentage in sample No. 3 (17.5%), while other oregano samples (Nos 1, 2, 4, 5 and 6) contain it in lower levels (1.0–9.5%). The marjoram samples are free of this compound.

The main essential oil compounds of both marjoram varieties were terpinolene and terpinene-4-ol. Terpinolene showed higher proportions by 6% in variety 'Magyar', while proportions of terpinene-4-ol were by 4% higher in variety 'Francia'. The terpinene-derivatives add up to 61.8 and 60.4% of the essential oil, respectively, which reflect a big similarity of composition. There has not been any considerable difference in contents of sabinene and cis-sabinene-hydrate, either. The proportions of the latter two can be considered as relatively low.

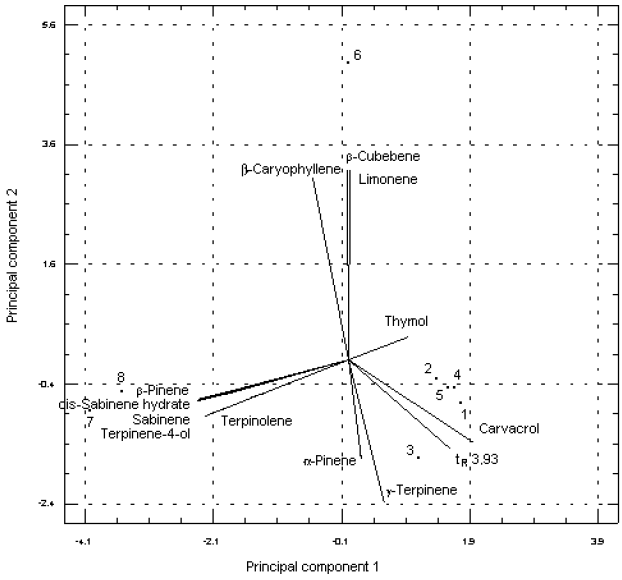

Fig. 1. Distinguishing the samples according to their essential oil composition (result of principal component analysis)

Multivariate evaluation (PCA) of the samples according to the composition of the essential oil is shown in Fig. 1. In the two-dimensional coordinate system of the first and the second principal component, almost all oregano samples can be differentiated from each other, however, samples 5 and 4 are very close to each other. Separation based on the first principal component is most likely due to differences in proportions of carvacrol and the unknown compound (t_R 3.93), which have the highest principal component weights (0.342 and 0.277). The second principal component consists of

mainly limonene, β-caryophyllene and β-cubebene with component weights 0.475; 0.454 and 0.475, respectively. According to them, especially sample 6 shows a distinct character.

2.2. Results of the instrumental sensory evaluation (electronic nose)

The instrumental sensory analysis assured a good separation of each of the investigated items. In our experiment, three PCA calculation steps were needed for reliable separation. Differences in the essential oil content did not influence the sensory evaluation.

Figure 2 shows the location of the quality points of the samples in the projection plane for the eight oregano samples determined by the first two principal components.

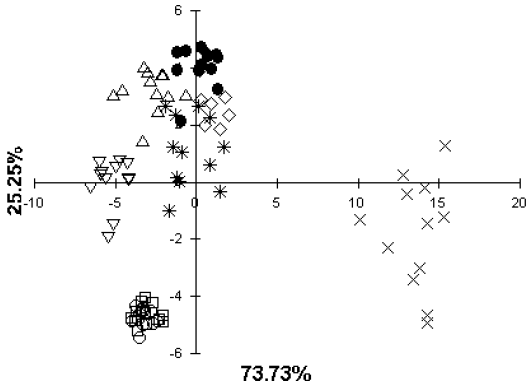

Fig. 2. Distinguishing the eight samples by electronic nose (result of principal component analysis). ●: 1; Δ: 2; ∇: 3; ∗: 4; ◊: 5; ×: 6; ○: 7; □: 8. For sample codes see Table 1

The first two principal components proved to be suitable for the appropriate separation of the marjoram populations, furthermore sample No. 6 (commercial oregano without any carvacrol in the essential oil) and also No. 3 (carvacrol chemotype oregano with lower carvacrol content) were different also from the remaining samples. Although the weights of principal components cannot be directly bound to any chemical compounds, because they represent sensibility characters of the instrumental sensors, – it can be established that this separation coincides well with the GC result, especially concerning the first three samples. Observing the instrumental separation, we can suppose that the main essential oil component, carvacrol, might have a considerable weight in principal component I, which describes 73.73% of the differences. In the other direction, component terpinolene might play a major role in separation according to principal component II. Besides, several minor compounds may effect the characteristic separation of sample No. 6.

In Fig. 2 the quality points of samples 1, 2, 4, 5 and on the other side points of samples 7 and 8 are overlapping each other. Therefore, a next step of separation proved to be necessary, which is illustrated in Fig. 3. Here, samples No. 1 (genebank population oregano sample) and No. 2 (strain of high carvacrol content) show a better separation from the remaining ones.

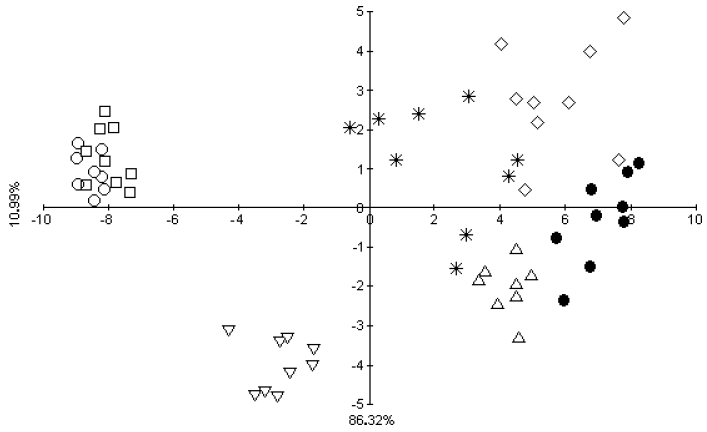

Fig. 3. Distinguishing the samples in an other projection plain by electronic nose (result of principal component analysis). ●: 1; Δ: 2; ∇: 3; *: 4; ◊: 5; ○: 7; □: 8. For sample codes see Table 1

For correct separation of marjoram samples, a further calculation step was necessary (Fig. 4). In this plot, separation of the two varieties could be carried out with high probability. Principal component II was the basis of this separation, however, it can hardly be related to the GC results.

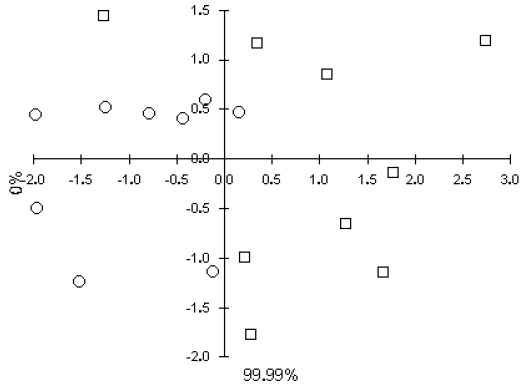

Fig. 4. Distinguishing marjoram samples by electronic nose (result of principal component analysis). ○: 7; □: 8

2.3. Results of the human sensory evaluation

The dried and shelled herb samples of two *O. majorana* varieties (Nos 7 and 8) were compared by simple difference test.

Similarly to the instrumental sensory evaluation in the human sensory test of complex odour and aroma, panellists declared a significant difference between these two crude drugs. (Chi-square = 29.26, critical value at 99.995, significance level =7.88.)

3. Conclusion

On the basis of GC analysis of essential oils, as the main method of pharmacopoeas, a firm differentiation between the two plant species (*Origanum vulgare* subsp. *hirtum* and *Origanum majorana*) was possible. This method also showed characteristic differences of the commercial sample No. 6 from other oreganos. Samples Nos 2 and 3 exhibited compositional characteristics of the carvacrol-chemotype, while sample 4 seems to belong to the thymol-carvacrol chemotype (SZABÓ, 2001). The marjoram varieties could not be distinguished on the basis of their essential oil composition. In the composition of marjoram oils biochemical transformations during distillation may play a considerable role (FISCHER et al., 1987), it might reflect the differences between terpenoid components of the intact plant and composition of distilled marjoram oil.

With the help of the "electronic nose" equipment, each examined *Origanum* samples could be distinguished. It was observed that items, separated in the first PCA step exhibited the highest compositional differences evaluated by GC. In our experiment it can be stated that the first step assured a separation on species and subspecies level. Thus, separation of the *O. majorana* cultivars, and the *O. vulgare* accession, which – in contrary to the remaining ones – was presumed not to be a subsp. *hirtum* taxon. Signals of the sensors of the instrument assured that in further calculation steps slighter intraspecific differences were also detected and described. However, in these further steps compositional results of the GC analysis are difficult to connect to the sensory signals.

Results of human sensory analysis seems to be in harmony with the results of instrumental sensory analysis: the samples of the two varieties could be distinguished by the above-mentioned two methods based on their complex aroma features.

The results call the attention that quality evaluation of crude drug items of aromatic plants should be oriented in different directions, considering the current utilisation area of the items. Data of gas-chromatographic instrumental analysis might be proper and primarily necessary in certain pharmacological applications, however they may not suit the special requirements of food industrial or perfumery processing. In the latter case, up-to-date instrumental sensory methods support and coincide with traditional human tests, while having all of the advantages of objective evaluation.

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