Evaluation of essential oils by gas-chromatography and a new method: "electronic nose"

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Summary: The chemical quality of the essential oils is determined by their composition as well as by the complex aroma features. For the evaluation of odour and aroma, sensory tests are practised, especially for applications such as aromatherapy, food industry or perfumery. In the recent studies we investigated eight *Mentha* origins (populations of *Mentha iperita, M. spicata* and *M. arvensis)* of our genebank collection using a new and effective instrumental sensory evaluation method parallelly with the usual GC analysis.

The results show that the examined mint clones possess different essential oil accumulation levels and special flavour characteristics, too. The GC analysis of the essential oil revealed characteristic differences at both inter- and intraspecific level in the proportion of mentol, menthon, carvon, limonene, menthyl acetate and in two not identified monoterpenes.

In the sensory tests the special complex aroma enables a reliable distinction with a single exception among the populations by the help of the "electronic nose" equipment. The distinction among the samples based on the sensor signals of the instrument – evaluated by multivariate methods – shows a close relation with the detected monoterpene components of the essential oil.

Introduction

Mentha species have been used and cultivated for more than 2000 years. One of the most important species is the peppermint. The botanical origin of peppermint drugs is *Mentha* x *piperita* L., which is a natural hybrid between *Mentha spicata* and *Mentha aquatica* (*Lawrence*, 1991.) The hybrid was discovered in England in the XVII. century and since then it is cultivated almost in each European and in several other countries of the world. The main producer countries are the US, the former Soviet Union, Bulgaria, Brazil and Japan. In Hungary it has been cultivated since the beginning of XX. century (*Hornok*, 1990.) Peppermint is widely used in pharmaceutical-, food- and cosmetic industry. It is used as a spasmolytic and carminative agent in the phytotherapy. The essential oil and its main component, menthol is used in dermatology, laringology and otolaringology. Ointments and tinctures are also made from peppermint for external application (*Rácz*, 1992). In the cosmetic and food industry it's used in alcoholic beverages, chewing gums, toothpaste, candies, dentifrices and in perfumes (*Praszna*, 1993).

Spearmint (*Mentha spicata* var. *crispa*) has been known in agriculture since the IX. century. The main spearmint producers are the US, Brazil and Spain (*Hornok*, 1990). In phytotherapeutical preparations its drugs have digestive and spasmolytic effects. Beside the therapeutical application, this species has an outstanding role in culinary. Its aromatic compounds are processed in chewing gums and toothpaste, too.

Currently, mint flavour (both peppermint and spearmint) is the third one in the world top list of the most favoured

tastes. Approximately 10 000 tonnes of extracts are used yearly all over the world, 65% of it in oral hygiene (primarily in toothpastes). The turnover of minty flavour extracts in the mouthwash application is 20 million dollar alone in Germany and in the UK (*Schumacher*, 1991). Chewing gum and confections take part with 20% and 10% of the total world mint flavour market, respectively (*Lawrence*, 1991).

Mentha arvensis is mainly cultivated in the Far East, in Japan as well as in Brazil and Argentina. Cultivation is oriented exclusively for essential oil production. This essential oil contains 80–85% menthol, the main consumer of which is the pharmaceutical industry (*Hornok*, 1990).

Concerning the economical and scientific value of mint species, maintenance and evaluation of their biological diversity is of basic importance. In order to evaluate of our genebank collection of mint populations, a complex research program has been started in 1998. Beside a detailed morphological and phenological description of different mint origins, the investigations included the chemical and sensory analysis of the essential oil properties. In this respect three main questions arised:

1. What is the chemical composition of the experimental population concerning the quantity and quality of the essential oil?
2. Is there any considerable difference in their complex flavour which can be detected by objective instrumental sensory tests?
3. Is there any significant correlation between the measurable chemical composition and the complex aroma features of the drugs?

The term "electronic nose" is a general name for analytical instrument that profiles the headspace volatiles over or around the sample. The technology is based on an array of chemical sensors whose outputs are integrated by advanced signal processing to identify complex aromatic mixtures. Different sensors can be used to analyse complex vapours: e.g. conducting polymer sensors (*Hodgins, Conover,* 1995.); combination of metal oxide semiconducting and conducting polymer sensor (*Moy* et al., 1994.)

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 of the above questions.

Material and methods

The plant materials were produced at the Experimental Station of the Faculty of Horticultural Sciences, Department of Medicinal and Aromatic Plants, in Soroksár, Budapest, in 1998. They had been maintained by clonal propagation during several years, however the origin of the majority of them is not known exactly (*Table 1*). Samples were taken at budding stadium. After natural drying the leaves were divided from the stems and water-distilled in Clevenger-apparatus according to the standard method of Ph.Hg. VII. 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 30 m x 0.25 mm i.d. coloumn was used (film thickness 0.25 µm). The injector and detector temperatures were 220 °C and 250°C respectively. Column temperature program: 90 °C (3 min.), 90- 180 °C (6 °C /min), 180 °C (5 min.) Carrier gas was nitrogen, 1 ml/min at the starting temperature, 0.2 µl of essential oil of each samples were injected. The identification of compounds was performed by comparison of their retention times (marked with t_R in the following) with those of pure substances, by peak enrichment with standards. Relative percentage of the oil constituents was calculated from the GC peak areas in percent of the total area.

For the instrumental sensory analysis "SamSelect" electronic nose developed by *DaimlerChrysler Aerospace* (Rostock) was used. This instrument works with 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 cause changes in their masses, resulting in frequency modifications of the quartz oscillators. The changes in frequencies serve as sensor signals for the evaluation.

The eight samples were measured in standard headspace vials in nine replications. Headspace autosampling was used as a standard and reproducible sampling technique. The biometrical evaluation of the sensor signal responses of the sensor array was carried out by principal component analysis (PCA, which is part of the SelectWare software and attached to SamSelect chemo-sensor array), and the PQS (Polar qualification system) method developed at the Department

Table 1 The examined mint populations

Population	Origin	Main morphological features
1. *Mentha x piperita* clone "A"	unknown	It has strongly crisped leaves and dark reddish colour, it is late flowering.
2. *Mentha x piperita* clone "I"	farm collection	In the production under the name 'Mitcham', middle-early flowering with strong reddish colour and medium size leaves.
3. *Mentha x piperita* clone "C"	unknown	Middle-early flowering clone with big leaves and dark reddish colour.
4. *Mentha x piperita* clone "H"	USA	Middle-early flowering strain, the leaves are medium size and blistered among the veins.
5. *Mentha x piperita* cultivar 'Mexian'	China	Possess light green, big and lance shaped leaves, it is early flowering.
6. *Mentha spicata* var. *crispa* clone "D"	unknown	Early flowering material with green crisped leaves.
7. *Mentha spicata* var. *crispa* clone "B"	from farm collection	Early flowering clone of similar habit to the clone "D".
8. *Mentha arvensis* clone "O"	China	It has huge leaves, they are mild hairy and light green.

of Refrigeration and Livestock Products Technology using sequence optimisation (*Van der Vlies* et al., 1995). For the evaluation of analytical data Statgraph 5.1 statistical software was used.

Results and discussion

Results of the analytical measurements

The accumulation level of essential oil proved to be a characteristic separation feature among the species (*Table 2*). *Mentha arvensis* (sample 8) contained almost half as much essential oil content than the samples of peppermint origins (sample 1, 2, 3, 4), as has been found in former investigations, too (*Németh* et al., 1997). The lowest oil quantity appeared in the two spearmint samples (6 and 7) (0.95% and 0.79%). Among the peppermint samples clone "I" (sample 2) gave the highest volatile oil quantity (3.11 %), besides, peppermint clone "H" (sample 4) and peppermint clone "A" (sample 1) also contained relatively high levels (2.78 and 2.83%, respectively). The other peppermint samples (3 and 5) tended to accumulate lower amount of volatile oil.

In the essential oil composition, main and characteristic differences were proved in contents of menthol, menthone, carvone, limonene, menthyl acetate and in two not identified compound (t_R 6.78 and t_R 7.05), (*Table 2*).

The menthol content is significantly outstanding in the *M. arvensis* sample (8) (73.7%), while it could not be detected at all in the *M. spicata* samples (6 and 7). Among peppermint samples the 'Mexian' (5) proved to have almost half as much menthol proportion as the other populations. The difference appears also in the distribution between l-menthol and isomenthol: while 'Mexian' (5) contains mainly the former one, the other populations (1, 2, 3, 4) contain the isomers in an approximately equal proportion.

The menthol content of peppermint oil samples shows an opposite tendency, than menthol. It was the highest in the clone "I" (2), (54.7%) and the lowest in 'Mexian' (5), (29.8%).

The main component of essential oil of spearmint is carvone. Both samples (6 and 7) have similarly high carvone contents (64.2 and 67.0%). However, all of the other clones contain it only as a minor component, in proportions under 2%.

Limonene accumulates in peppermints at a level of 4–5%, except 'Mexian' (5), whose content is similar to *M. spicata* samples (6 and 7) and exceeds 10%. This monoterpene compound shows the lowest level in *M. arvensis* (sample 8).

The proportion of menthyl acetate was characteristic mainly for peppermint, while the other mint species contain it only in traces. The unidentified compound (t_R 6.87) accumulates mainly in clone "A" (sample 1) and cultivar 'Mexian' (sample 5), (8.0% and 8.7%, respectively); it is much less in the other peppermints and *M. arvensis* (sample 8), and is fully missing in spearmint samples (6 and 7). These latter ones accumulate however another unidentified compound (t_R 7.05) in considearble quanitities.

The result of statistical evaluation (PCA) of analytical data is shown on *Figure 1*. In the two-dimensional coordinate system of the first and the second main component, almost all mint samples can be differentiated from each other, except two ones, peppermint clone "I" and clone "H" (samples 2 and 4). These two samples are very close to each other according to their GC-component spectra, too (*Table 2*). Separation based at the level of the first main component is most likely on the base of carvone and a not identified compound (t_R 7.05) which have the highest principal component weights (0.359 and 0.358). The second principal component consist of mainly menthone, isomenthone and menthyl acetate with component weights 0.27; 0.45 and 0.35, respectively. According to them, especially sample 8 (*M. arvensis*) shows a distinct character. L-menthol, limonene and the not identified compound (t_R 6.78) have considerable component weights (0.354; 0.454; 0.770, respectively) in the third and fourth principal components.

Table 2 Essential oil content and composition of the examined mint samples (%)

Components	Samples (according Table 1.)							
	1	2	3	4	5	6	7	8
α-Pinene	0.5	0.4	0.3	0.4	0.3	0.4	0.3	0.5
β – Pinene	1.1	1.1	1.0	1.0	1.1	1.7	1.6	1.7
Limonene	5.8	4.4	4.2	4.2	10.4	13.2	13.7	2.2
Menthone	36.5	54.7	50.6	50.0	29.8	5.5	4.4	12.8
Isomenthol	15.0	10.0	9.6	9.1	3.4	1.0	–	–
Isomenthone	Traces	3.4	2.4	3.1	3.2	2.7	1.6	–
l – Menthol	22.2	15.2	18.8	17.9	31.2	–	–	73.7
t_R 6.78	8.0	2.3	1.6	3.1	8.7	–	–	4.9
Carvone	1.6	0.6	0.6	0.6	1.8	64.2	67.0	0.6
t_R 7.05	–	–	–	–	–	0.9	0.8	–
Menthyl acetate	3.6	5.3	6.5	6.9	4.2	0.5	0.6	0.2
t_R 9.60	0.3	–	0.2	0.2	0.4	1.1	1.0	0.2
β – Caryophyllene	1.0	0.9	1.0	1.0	0.8	2.4	2.4	0.5
t_R 11.14	1.0	1.0	1.1	1.2	–	1.1	1.0	–
Total essential oil content (ml/100g)	2.83	3.11	2.56	2.78	2.35	0.95	0.79	3.61

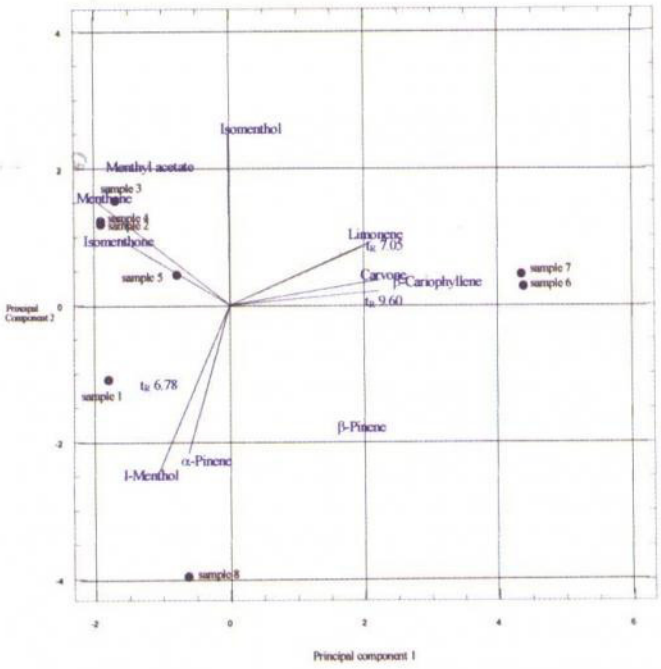


Figure 1 PCA evaluation of the analytical data

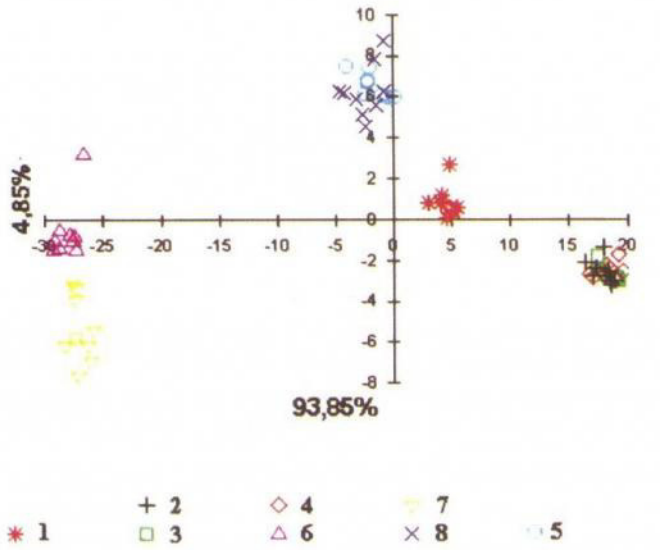


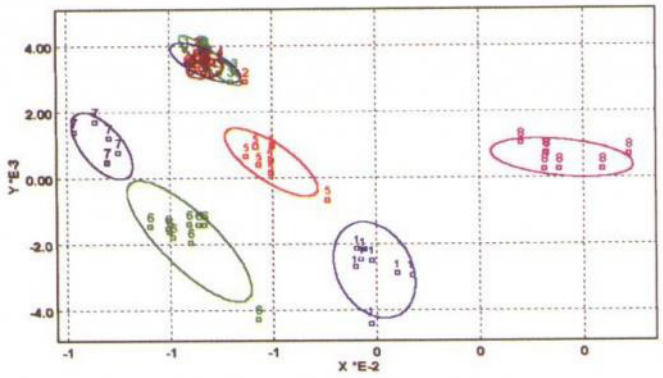
Figure 2 PCA evaluation of electronic nose sensor signals of the eight mint samples

Results of the instrumental sensory evaluation

The sensors of the "electronic nose" evaluated the experimental materials as being different according to their complex flavour characters. The PCA calculating with the signals of the six sensors of the equipment proved an accurate separation of six ones among the eight samples.

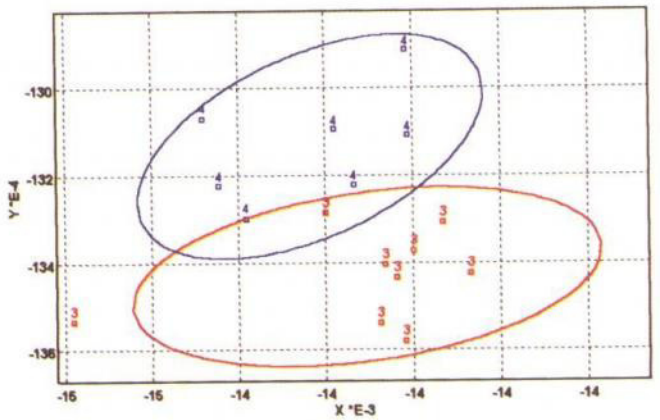
Figure 2 shows the location of the quality points of the volatile oil samples in the projection plane for the eight mint populations determined by the first two principal components. Using the co-ordinate system of the first two main components (consisting 93.85% and 4.85% of the total variance, respectively) the appropriate separation of the spearmint samples (6 and 7) as well as of the peppermint clone "A" (sample 1) from the other ones was only possible. However, under these circumstances, the samples of variety 'Mexian' (sample 5) and the *M. arvensis* (sample 8) could not be distinguished from each other. Similarly, the quality points of samples 2, 3 and 4 (peppermint clones "I"; "C" and "H") are overlapping each other. In a further evaluation step, determining the projection plane (based on the first two principal components) for the critical samples only, the overlapping samples 8 (*M. arvensis*) and 5 ('Mexian') could already be well separated, while it was not possible for the peppermint samples 2, 3 and 4. It means, that these three samples exhibit very closely related scents.

Nevertheless, the ''sequence optimisation'' of the PQS technique allowed us to set more adequate results partly even for these closely related samples. *Figure 3* shows the location of quality points (center of their normalised data sets drawn as polar spectra) of the measured volatile oils in original data sequence coming directly from the instrument. The normalised distances and sensitivities are high, the



1:sample 1 2:sample 2 3:sample 3 4: sample 4
5: sample 5 6: sample 6 7: sample 7 8: sample 8

Figure 3 PQS evaluation of eight mint samples



3: sample 3 4: sample 4

Figure 4 PQS evaluation of peppermint clone "C" (sample 3) and "H" (sample 4) in second projection plane

distances are considerable higher among the groups compared to their standard deviations. It provides an acceptable separation of each tested mint population, only the formerly mentioned related ones (peppermint samples 2, 3 and 4) remained unseparated. However, in the course of the further data processing, an almost complete distinction between samples 3 and 4 proved to be possible (*Figure 4*). According to this, only sample 2 (clone "I") can not be confidentially identified in the instrumental sensory evaluation according to its complex aroma.

Our results show, that beside the morphological and phenological differences, the examined mint clones possess different essential oil accumulation levels and special flavour characteristics, too. Only two clones showed great similarity from this respect. The special aroma enables a reliable distinction among them by the help of the "electronic nose" equipment. The "electronic nose" proved to be an appropriate, rapid, non-destructive, reagent-less method in complex evaluation and comparison of aroma profiles of essential oils. The accumulation rate of the essential oil does not seem to have an important role in the separation of the samples according their flavours. However, the distinction among the samples based on the sensor signals of the instrument – evaluated by multivariate methods – shows a close relation with the detected monoterpene components of the essential oil. The establishment of the exact correlation between the individual chemical compounds and the sensors registrating them, is the task of further experiments.

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