Sensory analysis as a supporting method for marjoram breeding

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Summary: Marjoram is one of the most important spices in Hungary, which is utilized both in phytotherapy as well as in alimentary industry. Organoleptic quality of the drugs of two Hungarian varieties of *Majorana hortensis* /'Francia' and 'Magyar'/ were investigated by two different methods of sensory analysis. The chemical composition of the material was checked by GC analysis of the distilled essential oil. Although their main compounds and proportions showed similar patterns, both the human and instrumental sensory tests based on the complex odour and aroma of the drug, proved a significant differentiation of the two varieties.

On the basis of our results, sensory evaluation may be a useful tool in the practice in determination of complex aromatic values of marjoram, as a spice. At the same time it seems to be a suitable method in promotion of breeding efforts.

Introduction

Sweet marjoram (*Majorana hortensis* Mönch, syn.: *Origanum majorana* L.) is one of the most famous and popular spices, throughout the world. It has been known from the ancient times: in Egypt, marjoram was cultivated as a saint plant of Osiris and used as a spice. During the Greek-Roman times it was thought to be the symbol of happiness. The rich Roman women scented their hair with marjoram oil. In the cookery book of Apicius, marjoram was mentioned among spices having a multiple use (*Lenchés*, 1990).

Hungary is a traditional marjoram cultivating and exporting country, it has been cultivated here since the XVIth century (*Lenchés*, 1990).

Marjoram is cultivated under mediterranean and continental climate, throughout the world, main producer of marjoram products is Egypt. However, drug of its flowering shoots (*herba*) and essential oil are produced also in Hungary, Germany, France, Tunisia and Bulgaria, furthermore in Italy and Greece. Main marjoram consumers are Germany, the United States and Great Britain. (*Deans and Svoboda*, 1990).

In the field of sensory analysis research there are an increasing interest only in the recent years, new and specific equipments as for instance "Head space" and "Sniffing Detectors" (*Kaiser*, 1988, *Bicchi*, 1988) have been developed. Consequently, sensory aroma research remains an interesting area for innovative investigations also in plant breeding, plant production and processing. (*Franz*, 1990.)

In the flavour and fragrance industry a new instrument, a so-called "electronic nose" method has been tested to analyse complex odours. The term "electronic nose" is the general name for the analytical instrument that profiles the headspace volatiles over or around a sample. The technology is based on an array of chemical sensors whose outputs are integrated by advanced signal processing to identify complex aromatic mixtures. Besides the existing techniques such as organoleptic panels and gas chromatography, the "electronic nose" can be complementary to the above mentioned methods in quality control. (*Moy* et al., 1994.)

The standards in connection with the evaluation of spices were worked out in the fifties in Hungary. Descriptive methods were found useful for sensory evaluation of seasonings. Four groups of characteristics are evaluated with weight factors: smell, appearance, colour and taste. (*Molnár*, 1990.) According to the Hungarian Standard (MSZ 20621-72) the smell and taste of marjoram spice has to be healthy, typical and spicy, foreign smell and taste are not allowed.

In Hungary, there exist two registered varieties of marjoram, 'Francia' (1959) and 'Magyar' (2000). In abroad, several other varieties are known or are under announcement as result of the last years' accelerated breeding activity. Some of them are 'Marcelka' (Czech Republic), 'Miraz' (Poland) and 'Erfo' (Germany), (*Lenchés & Németh*, 2000). The most often applied breeding methods are the traditional ones: mass selection and single plant selection. Recently, hybrid breeding, by the help of male sterile lines, has also been started (*Pank* et al, 1999/b).

The market requires high and standard quality raw material, which should be assured – beside controlled agrotehnology, – by breeding of appropriate varieties. As most important breeding targets in establishing high quality drug, high essential oil content (more than 2 ml/100 g dry matter) with high cis-sabinene hydrate content, colour of leaves and organoleptic features such as odour, taste and aroma are often mentioned. Technological features form a second important group of characteristics to be improved: such as upright growth, leaf/stem ratio, quick development of young plants, resistance against pathogens and drought, reaching high biomass production (*Franz* & *Novak*, 1997).

The essential oil composition of marjoram showes a great variability according to published results (*Table 1*). The proportions, mentioned by different authors, include a considerable range of variation even for the main components (γ-terpinene, cis-sabinene hydrate and terpinen-4-ol).

For some years, it has been proved by *Fischer* et al. (1987), that cis-sabinene hydrate and its acetate represent the original flavour compounds of the intact leaf. The great number of monoterpenes described in marjoram essential oil - especially terpinene and related compounds, are arising from several hydration and deprotonation reactions from cis-sabinene hydrate acetate. Cis-sabinene hydrate acetate rearranges very easily to other compounds of marjoram essential oil when heated or acified, or when the cell material is damaged.

Although cis-sabinene hydrate has a characteristic marjoram smell, however the typical marjoram aroma is the result of the complexity of the components (*Loßner*, 1968). Confirming this statment, a sniffing detector parallel with FID was used for sensory analysis of the essential oil. Marjoram samples with different flavour profiles could be separated by the help of this equipment. Cis-sabinene hydrate was described as the one having tropical-fruity, sweet and marjoram flavour, while terpinene-4-ol was characterised having potatoe-like and herbacious flavour (*Franz*, 1990).

Pank et al. (1999/a) investigated the sensory quality of spice marjoram, especially the colour of leaves. A simple ranking test was carried out to evaluate the preference of the panels in connection with the colour of the herb. The intensive fresh green coloured sample was set mostly to the first place in the rank order which confirmed the importance of colour as a quality determining trait.

In a research project many characteristics of twenty different marjoram populations were assessed in order to determine their performance and their genetical variation. Among the sensory features colour component L* according to the CIELAB System was particulary suitable for the genetical differentation of the population. They ascertained that smell and taste are genetically controlled, but they seem to be less suitable for genotype differentiation due to the subjective assessment methods used in this experiment. These characteristics were evaluated on a scale from 0–5. On the base of examination of essential oil composition and

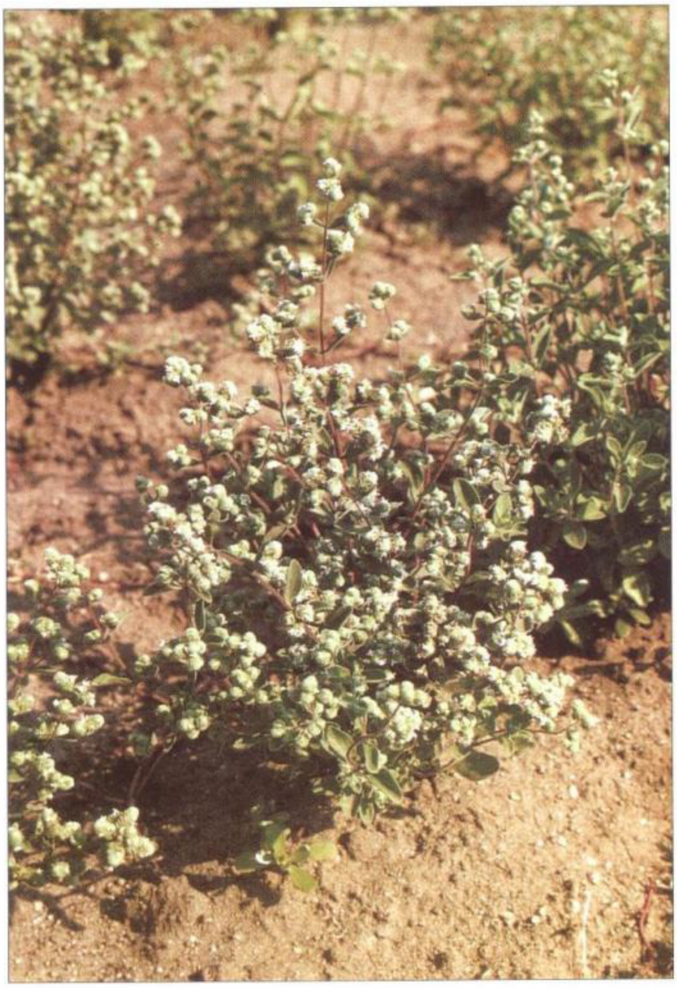
Table 1 Essential oil components of Majorana hortensis in the literature data

Essential oil component	Literature reference										
	Deans et al. (1990)	Fischer et al. (1987)	Hager (1993)	Mallvarapu et al. (1993)	Németh et al. (1998)	Pino et al. (1997)	Raghavan et al. (1997)	Ravid et al. (1987)	Schanz et al. (1987)	Schulz et al. (1990)	Taskinen (1974
α-Pinene	5			0.68	tr-2.8	0.64	0.65	1.1	2.2–4.4		
β-Pinene				0.41	7.2–11.6	4.60	0.61	0.3	tr-0.3		0.2
Sabinene	21	5.4	2–6	8.84			4.17		6.7–12.3		2.5
Myrcene	6	1.8		2.64		1.21	1.18	2.1			1.2***
α-Terpinene		6.3	1–7	5.51		3.32		9.2	3–7.4	0.7–8.7	6.1
Limonene		2.7		4.06		2.12	2.5*	2.0	1–2.1		0.6
β-Phellandrene									1.5–2.7		0.9
γ-Terpinene	12	10.4	2–12	10.75	tr-9.5	8.34	12.06	17	4.6–11.0	1.7–14.3	14.0
P-Cymene				0.48		4.98	8.34	2.9			0.7
1,8-Cineol					4.5–8.5						
Terpinolene		2.2		2.22		1.52	16.23**	3.8			2.5
Cis- Sabinene-hydrate		29.3	5–25	6.30	tr-4.7		3.52		6.7–20.0	12.5–68.2	
Cis –Sabinene hydrateacetat		3.5		0.14						0.9–6.9	
Trans- Sabinene-hydrate		6.6	2–6	7.87		2.26	3.59		3.7–5.9		4.0
Linalool	8			0.29	25.1–33.2	16.41	2.64	9.4	31.2–41.5		
Linalyl-acetate				2.94		0.25		2.5	0.1–8.1		0.1
Cis-p. menth-2-en-1-ol							1.98				2.0
Terpinene-4-ol	23	20.7	15–40	25.74	19.7–25.9	17.67	30.16	26.6	2.3–2.9	1.5–24.5	45.5
α–Terpineol		2.9		4.79		3.19	4.65		1.4–3.6		6.7

*: combined percentage of limonene and 1,8-cineole
**: combined percentage of cis-sabinene hydrate and terpinolene
***: combined percentage of alfa-phellandrene and myrcene

sensory analysis no relation was observed between smell and taste on the one hand and the essential oil or cis-sabinene hydrate content on the other. (*Pank* et al., 1999/b)

The Departement of Medicinal and Aromatic Plants has been engaged in breeding of marjoram about eight years. The variety 'Magyar' is the result of breeding efforts for establishment of a high yielding strain possessing also upright growth, bright green leaves and high essential oil content. Recently, examination of sensory quality of this new cultivar has been carried out parallel with the control material in order to test the organoleptic characteristics which have a growing importance in trade and culinary utilisation. Although sensory parameters has not been a goal previously during selection of this material, it seems to be important to evaluate differences of the two varieties from this respect. It should be cleared up if sensory evaluation methods applied in food industry are suitable for distinguishing of new strains and breeding materials.



Material and methods

Drugs of two variety of *Majorana hortensis* 'Francia' and 'Magyar' were investigated by two different sensory analytical methods as well as by chromatographic analysis (GC) of the essential oil. Considering the seasoning significance of marjoram, the sensory methods included aroma, flavour and scent characteristics and did not dealt with colour or other visual traits. The plant material was produced at the Experimental Station of the Faculty of Horticultural Sciences, Department of Medicinal and Aromatic Plants, situated in Soroksár, in Budapest, in 1998. Populations were harvested as usual, at the beginning of flowering, and dried afterwards under natural circumstances in shadow.

Dried and chopped (2 mm sieve) samples were water-distilled in a Clevenger-apparatus based on the standard method of Ph.Hg. VII. The essential oil content was calculated as a 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 × 0.25 mm) i.d. column 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.) The carrier gas was nitrogen, 1 ml/min at the starting temperature, 0.2 µl of essential oil of each sample was injected. The identification of the compounds was performed by comparison of their retention times with those of pure substances, by peak enrichment with standards. The relative percentage of the oil constituents was calculated based on the GC peak areas, as a percentage of the total area.

For the instrumental sensory analysis "SamSelect", an electronic nose developed by *DaimlerChrysler Aerospace* (Rostock) was used. This equipment 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. Samples were measured in standard headspace vials in nine replications. Headspace autosampling was used as a standard and reproducible sampling technique. For the evaluation of the sensor signal response of the sensor array, principal component analysis (PCA) was used.

Human sensory test was carried out by "simple difference test" as described by *Meilgaarg* (1991). Variety 'Francia' and variety 'Magyar' were compared by 36 panellists. Each of them got four coded sample-pairs, and pair-wise comparison of samples was accomplished in four different combinations (AA, AB, BA, BB). Samples were placed in non-transparent vials to assess only the flavour. Panellists were asked to test any observable difference between the flavour of sample-pairs. The results were evaluated by chi square test.

Results and discussion

Essential oil content of the 'Francia' population contained by 0.34% more essential oil in its shoots, than the other variety (*Table 2.*). However, this difference did not

Table 2 The identified compounds in essential oils of Hungarian marjoram varieties

Essential oil components	'Francia'	'Magyar'
α-Pinene	0.2	0.3
Camphene	0.3	0.3
β-Pinene	5.1	5.3
α – Terpinene	1.9	2.0
γ-Terpinene	5.6	4.4
1,8-Cineol	5.4	3.6
Cis-Sabinene-hydrate	6.7	7.4
Terpinolene	29.6	35.7
Sabinene	4.4	1.6
Terpinene-4-ol	19.5	15.8
α – Terpineol	3.8	3.9
β-Caryophyllene	2.4	3.0
Total essential oil content (%d.w.)	1.68	1.34

proved to be significant. Both values (1.68% and 1.34% respectively), can be considered as advantageous levels.

The main essential oil compounds of both varieties were terpinolene and terpinene-4-ol. While the former one showed by 6% higher proportions in variety 'Magyar', 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 these later ones can be considered as relatively low. The similarity of essential oil composition of the two samples may be originated also from the mentioned transformations during distillation. The varieties could not be distinguished based on their essential oils.

Dry shoot samples of marjoram varieties however, represented a well distinguishable sensory quality when evaluated by instrumental and human sensory tests.

Sensors of the "electronic nose" were capable to separate the samples of both cultivars (*Figure 1*). The co-ordinate system of PCA shows a clear division of the individual samples. Describing a statistical connection between individual essential oil compounds of the samples and evaluation of them by the sensors represent the next step of our investigations in the future.

Also 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).

On the base of the results the sensory analytical method (simple difference test), and the ''electronic nose'' technique seem to be suitable for distinguishing marjoram samples of different origin. Beside the traditional chemical analytical evaluations, the mentioned methods are appropriate tools in supporting breeding work. Their importance is especially considerable in food industrial, culinary or perfumery utilisation of marjoram products.

For further investigations we are planning to evaluate the marjoram spices with other sensory analysis methods (profile analysis, ranking tests) and to investigate the essential oil composition with GC-MS too.

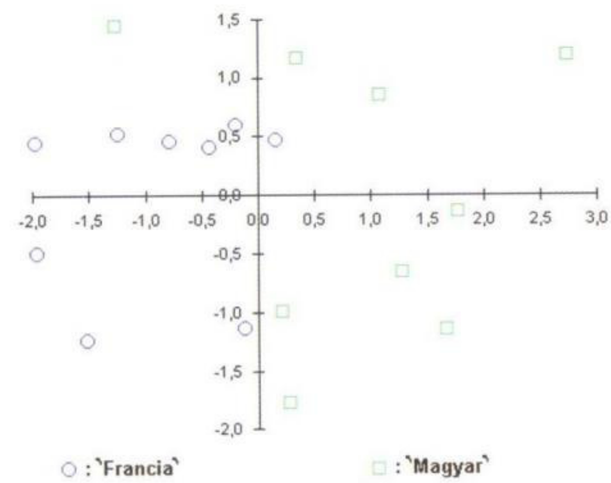


Figure 1 Distinguishing marjoram samples with ''electronic nose'' (result of principal component analysis)

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