Studies on the essential oil of different fennel *(Foeniculum vulgare* Mill.) populations during onthogeny

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Summary: In the recent studies two Foeniculum vulgare Mill. populations (Hungarian and Korean) had been studied in open field trials in 1997–98 in Budapest for the identification of their essential oil characteristics. The essential oil accumulation level as well as the composition of the oil were studied during ontogenesis and at 6 phases of the umbel development from budding to overripening.

In the vegetative phases, the accumulation level of the essential oil was higher in the Hungarian genotype. In the leaf rosette stage, anethole is the main compound in both genotypes (40–96%), except the root of the Korean populations which contains 54% dillapiol. The accumulation level of anethole is slightly decreasing, while dillapiol is slightly incresing during the ontogeny. α- and β-pinenes are characteristic compounds of the leaves, especially in the Korean genotype (10–11%). Before seed setting, fenchone was present in a considerable amount (7.5%) only in the umbels of the Hungarian genotype.

During the generative development, the maximum values of essential oil content are reached at the milky fruit stage (10,11%) in the Hungarian, and at the green fruit stage (7.1%) in the Korean type, while the composition of the essential oil changed only slightly. The ratio of fenchone is increasing after flowering and being stable during ripening. Anethole varies to a smaller extent in the umbels, only.

We proved, that the dinamics of essential oil accumulation and the oil composition may vary according to genotype. Based on our results, the Korean population is characterized in the system of Bernáth et al. (2) as a high anethol-low methyl chavicol chemoform of the anethol chemovariety (fenchone<15%; anethole>68%; methyl chavicol< 3,2%).

Introduction

Fennel (*Foeniculum vulgare* Mill.) is one of the most important medicinal plant species of the Apiaceae family. The bitter fennel – subsp. *capillaceum* var. *vulgare* – is widely used both in Hungary as well as in Korea. Main active constituent of this plant is the essential oil. Its level and composition varies widely according to origin, developmental phase, plant organ, etc.

It was proved, that among each organs, the highest essential oil accumulation level can be found in the green fruits, and its proportion is decreasing during the ripening process *(Gleisberg* and *Hartrott*, 1960; *Tóth* 1967/b; *Bernáth* et al., 1999). The actual maximum value however, is depending on genotype.

In the composition of the essential oil, anethole, fenchone and methyl chavicol are considered to represent the major compounds of fennel fruits. Among them, usually fenchone is mentioned, as the most important one in separating the bitter or sweet fennel types *(Karlsen* et al., 1969; *Lawrence*, 1994). *Krüger* and *Hammer* (1998) however, found no sharp chemotaxonomic borderline between fennel types with high and low fenchone contents, but they consider the levels of anethole and methyl-chavicol as most important ones for chemotype determination. *Bernáth* et al. (1996) distinguished different *chemoforms* within the var. *vulgare* according to the mentioned three main components of the fruits. Among further compounds of the fruits β-pinene, β-myrcene, p-cymol, γ-terpinene and terpinolene can be mentioned as the most general ones *(Karlsen* et al., 1969; *Krüger* and *Hammer* 1998; *Paukov* et al., 1971; *Trenkle*, 1972).

The qualitative composition of the oil in all of the overgound parts of fennel is almost uniform, however the quantitative composition of the fruits differs from the vegetative organs *(El-Hamidi* and *Ahmed*, 1966). In the vegetative parts (leaves, stems) α-pinene, limonene and α-phellandrene are found in higher levels, some of them taking eventually more, than 50% of the oil (*Tóth*, 1967/b; *Trenkle*, 1972). Changes during onthogenesis are also characteristic, and the last three components show a decreasing tendency in the juvenile parts (*Trenkle*, 1972). During fruit development, an increasing proportion could be registered for fenchone (up to 32% increase) and anethole (up to 27% increase), while methyl chavicol is mentioned as the most stable component from this point of view (*Bernáth* et al., 1996).

The root oil is characterized by qualitative differences in composition compared with other organs. Its main component is dillapiol (more, than 90% in the oil, *Tóth*, 1967/b), apiol, terpinolene, myristicine (*Stahl-Biskup* and *Witchmann*, 1991) and p-cymol (*Trenkle*, 1972). In juvenile stadium, the primary composition of the roots differs from the older ones, showing highest proportions for apiol, limonene and trans-anethol (*Stahl-Biskup* and *Witchmann*, 1991).

Although data on the level and composition of fennel essential oil are abundant, and it seems to be proved, that several factors influence this features, it still is not clear if the organic, onthogenic or ecological factors act similarly for each origin. Therefore, in recent studies we examined two different origins of *Foeniculum vulgare* Mill. with the aim, to describe parallelly the characteristic accumulation dinamics of their essential oils, and the distribution of its components according to phenological phases and plant organs. Furthermore, this examination contributes to the scientific evaluation of a practically unknown Far Eastern population of fennel.

Materials and methods

Two genotypes of *Foeniculum vulgare* subsp. *capillaceum* var. *vulgare* were drawn into the investigations. The Hungarian origin was represented by the "Soroksári" cultivar (genebank of the Department of Medicinal and Aromatic Plants, UHFI) and the Far Eastern origin by a common cultivated population from South Korea, (genebank of Gyeongnam Provincial Rural Development Administration).

The experiment was carried out at the experimental station of UHFI Department of Medicinal and Aromatic Plants, Budapest. Seeds were sown early March 1997 to open field, in plots of 20 m^2, in 3 replications. Row distance was 50 cm, seed dosis 8 kg/ha. The optimal plant development was assured by regular mechanical weed control and irrigation. The second year old stands had been investigated, in 1998. Measurements on both taxa were made in the following developmental stages:

I. The vegetative organs (roots, stems, leaves) were sampled:

a) at leaf rosette stadium
b) at the beginning of flowering (flowers in stages 2–3 according to *Bernáth* et al., 1999),
c) at waxy ripening stage

II. The generative organs, the umbels was collected in the following 6 phases

- yellow bud stage,
- at full flowering (individual flowers in stages 4–5 according to Bernáth et al., 1999),
- at green fruit stage,
- waxy fruit stage,
- full ripening stage (time of usual harvest), and
- overripening stage (after 3 weeks of the normal harvest).

Samples were dried under natural conditions, in shadow. They were distilled in Clevenger-type hydrodistillation apparatus for 1.5 h. (10 g fresh plant material in 400 mL water). The oil yield was calculated to the dry mass. The oil composition was analysed by GC (Shimadzu GC-B14 with Shimadzu Class -VP Chromatography Data System 4.2 computer system). An SE-30 25 m x 0,25 mm (film thickness 0,25 µm) column was used. The injector and detector temperatures were 220 °C and 250 °C respectively. Column temperature program: 110 °C (3 min.) 110 °C– 250 °C (8 °C/min.) 250 °C (5 min.). Carrier gas was nitrogen, 1 mL/min. The identification of compounds was performed by comparison of their retention times with those of pure substances, by peak enrichment with standards.

Results and discussions

Essential oil content and composition of the vegetative organs

Essential oil content

Examinations on the essential oil content of fennel plants revealed, that at the *leaf-rosette-stadium*, the Hungarian cultivar showed more, than two times higher accumulation levels of essential oil than the Korean material (*Table 1–3*). However, the levels at this stage in both of the experimental materials can be considered as low, compared with literature data (root: 0.7–1,2%), (aerial parts: 0.3–4.0%), (*Paupardin*, 1990; *Tóth*, 1967/b).

At the *fruit ripening stage* the essential oil accumulation level remained hardy unchanged in the roots (0.27 %), (*Table 1*). At the same time, the content of the leaves was quite different in case of the two taxa: nearly 1% in the leaves of the Korean genotype, while significantly lower amount (0.16%) was registered for the Hungarian genotype (*Table 2*). The stems showed very small amounts in case of both populations (about 0.05 %), (*Table 3*).

Essential oil composition

The studied populations showed considerable differences concerning the essential oil composition. The composition of the roots, the stems and the leaves proved to be different both quantitatively as well as qualitatively (*Table 1–3*). Characteristic differences could be observed during the developmental phases and between the two genotypes.

In the *leaf-rosette stage* in both genotypes and in each organ anethole proved to be the main component. The only divergence is shown by the root sampes from the Korean population, which contain a high amount of dillapiol (54%), (*Table 1*).

During the *flowering time* dillapiol can be observed also in the Hungarian population: 75% dillapiol is accumulating in the roots. At the *ripening stage* both genotypes' roots have dillapiol as the main component (90% in the Korean and 92% in the Hungarian material). In this phase even the leaves of the Korean type begin to accumulate this compound. This finding is new compared with the results of *Stahl-Biskup* and *Witchman* (1991), who detected myristicin and terpinolene as main compounds in fennel roots, but are similar to the data of *Tóth* (1967/a). Each identified compounds of our studies was described also by *Trenkle* (1972), but he did not gave quantitative data.

α- and β-pinenes are characteristic compounds of the leaves, especially in the Korean genotype (*Table 2*). However, their levels decrease during development and they almost disappear till the time of ripening both from the leaves as well as from the stems. It partly coincides with the results of *Tóth* (1967/a), who mentioned these compounds as the characteristic ones in the older leaves and stems, while decreasing in the younger, apical parts of the plant and almost missing in the umbels or fruits. Concerning this tendency, he detected considerable differences among fennel origins. Investigating three taxa of different origin, *Bernáth* et al. (1996) described a decreasing tendency of α-pinene from the leaves to the apical parts only in case of a single taxon. Similarly, our results reflect also a difference between the studied Korean and the Hungarian origins from this point of view.

In our samples fenchone was not present in considerable amounts in the vegetative organs. In the Hungarian genotype fenchone was detected in the leaves up to 12,3%, while in the Korean one only in traces. In former studies *Bernáth* et al. (1996) measured also varying fenchone levels in the leaves (from traces up to 12%) depending on genotype.

Although in each sample anethole remains a main component in the essential oil of both the Hungarian as well as the Korean population, its level is slightly decreasing during ontogenesis, which was detected in the vegetative organs also by *Tóth* (1967/a).

Essential oil content and composition in the reproductive organs

Essential oil content

During the development of reproductive organs, the essential oil accumulation shows a characteristic tendency (*Figure 1*). Its maximum value was reached in green fruit stage for the Korean, and in the milky fruit stage for the Hungarian genotype. In the recent publication of *Bernáth* et al. (1999) registered the maximum values for the same Hungarian cultivar at the beginning of fruit development, right after petals fall, presenting also the anatomical background for the phenomenon. The maximum essential

Table 1 The content and the main essential oil compounds of the roots of fennel genotypes

Compound	Leaf rosette stage		Flowering stage		Waxy ripening stage	
	Korean	Hung.	Korean	Hung.	Korean	Hung.
α-pinene	t	–	t	t	t	t
β-pinene	t	8.8	t	t	t	t
β-myrcene	0.4	2.3	0.2	0.3	0.4	0.5
fenchone	1.2	3.5	0.2	0.7	1.9	0.8
methyl chavicol	1.0	2.2	–	0.5	t	–
anethole	39.2	79.8	6.5	20.6	2.1	2.0
dillapiol	53.6	–	90.6	75.3	90.3	92.1
CONTENT (%)	0.157	0.347	n.m.	n.m.	0.268	0.277

Table 2 The content and the main essential oil compounds of the leaves of fennel genotypes

Compound	Leaf rosette stage		Flowering stage		Waxy ripening stage	
	Korean	Hung.	Korean	Hung.	Korean	Hung.
α-pinene	11.0	0.7	1.6	t	t	t
β-pinene	10.6	5.9	4.9	0.8	t	t
β-myrcene	2.7	2.2	1.6	0.5	t	1.0
fenchone	3.0	3.4	5.2	3.3	t	12.3
methyl chavicol	2.3	2.5	2.6	2.3	t	1.9
anethole	60.9	82.2	77.9	87.6	23.5	77.8
dillapiol	–	–	4.6	0.8	67.3	t
CONTENT (%)	0.215	0.538	n.m.	n.m.	0.930	0.161

Table 3 The content and the main essential oil compounds of the stems of fennel genotypes

Compound	Leaf rosette stage		Flowering stage		Waxy ripening stage	
	Korean	Hung.	Korean	Hung.	Korean	Hung.
α-pinene	2.5	t	t	t	0.1	t
β-pinene	2.6	t	t	t	0.6	0.4
β-myrcene	1.0	t	2.2	t	2.4	0.2
fenchone	1.0	1.0	t	t	0.8	0.7
methyl chavicol	2.1	2.0	2.5	1.3	1.0	1.3
anethole	81.2	95.5	90.1	93.3	78.2	72.3
dillapiol	–	–	3.3	1.3	3.2	39.0
CONTENT (%)	–	–	n.m.	n.m.	0.053	0.054

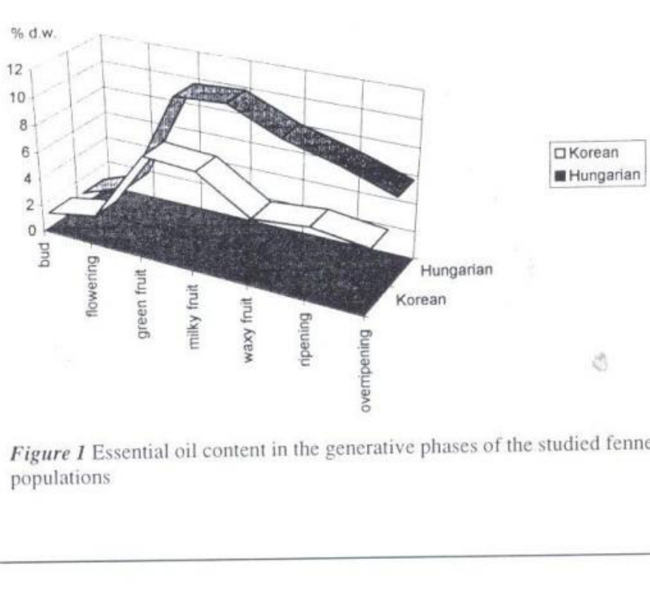

Figure 1 Essential oil content in the generative phases of the studied fennel populations

Table 4 Essential oil content and main comounds of of the fennel genotypes during the development of generative organs

K – Korean population
H – Hungarian cultivar

Compound	Bud stage		Flowering		Gren fruit		Milky fruit		Waxy fruit		Ripening		Over-ripening	
	K	H	K	H	K	H	K	H	K	H	K	H	K	H
α-pinene	0.6	1.7	–	2.4	1.9	3.2	1.9	3.6	0.1	2.6	2.0	1.9	0.7	2.1
β-pinene	–	0.7	0.1	0.9	0.2	1.5	0.2	1.7	0.4	1.4	1.0	1.2	0.6	1.4
β-myrcene	1.3	1.4	0.1	2.4	1.0	0.6	0.9	0.5	0.7	0.4	0.4	0.3	1.6	0.4
limonene	1.7	2.1	0.3	2.5	2.2	3.1	2.0	2.9	1.9	2.5	2.0	2.7	1.0	2.6
fenchone	7.7	13.4	3.9	12.1	14.6	21.9	11.6	19.9	12.2	21.5	13.1	18.1	1.0	18.7
methyl chavicol	2.6	3.5	2.7	4.2	2.9	5.4	2.9	4.5	3.2	2.6	2.6	2.8	9.7	4.7
anethole	73.8	74.0	89.4	69.7	67.9	61.0	76.5	64.1	76.4	66.2	76.2	70.5	3.3	68.0
dillapiol	0.2	–	–	–	0.4	–	–	–	0.2	0.2	–	0.2	81.1	–
CONTENT(%)	1.12	0.85	1.79	2.22	7.10	9.89	6.86	10.11	4.46	8.40	4.85	7.34	4.4	6.0

oil content of fennel fruits was detected in waxy stage by *Paukov* et al. (1971) in a Russian genotype, while *Marotti* et al. (1994) measured the highest oil percentages at ripe seed stage.

In our investigatons, the maximum value in the Hungarian taxon was found at milky fruit stage, while in the Korean one at green fruit stage. (*Table 4*). The difference between the two taxa is greatest however, at waxy seed stage, where the difference of the oil levels reaches 4% d.w. It was also proved, that even in the over-ripening stage a further decrease in essential oil accumulation level occurs in case of both populations.

Essential oil composition

The composition of the essential oil showed less change during the development of reproductive organs. α-pinene, limonene and methyl chavicol are rather stable compounds, whose level is about duble in the Hungarian cultivar compared with the Korean population (*Table 4*). As for the myrcene content, a very small, not significant change was found during flowering and ripening, which contradicts to the data of *Bernáth* et al. (1996), who described a considerable decrease during the flowering and ripening periods. The accumulation proportions of fenchone are characterized by an increasing curve after flowering, and being stable afterwards. Anethole however, shows a rate changing continously, but there was no firm direction of this change. The change might origin also from the fact, that umbels of different range may have also slightly different composition, especially concerning the anethole level (*Tsvetkov*, 1970).

Based on the above mentioned results, the Korean population can be characterized in the system of *Bernáth* et al. (1996) as a high anethole - low methyl chavicol chemoform of the anethole chemovariety (fenchon < 15 %; anethole > 68 %; methyl chavicol < 3.2%). Furthermore, our data emphasize, that changes in the essential oil content and composition of fennel are genotype specific, characteristic for the population. To assure a desired quality of the products, it should be taken into account even during the practical agricultural production of the species.

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