Characteristics of the *Foeniculum vulgare* Mill. collection by content and component composition of essential oil

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Abstract. The purpose of this study was to analyze the *Foeniculum vulgare* Mill. collection by the content and component composition of essential oil. The study was carried out in 2017-2019. The collection is located in the experimental site of the Research Institute of Agriculture of Crimea, located in the Crimea Foothill (Krymskaya Roza village) in accordance with the developed methods. The component composition of *F. vulgare* essential oil was determined by the method of gas chromatography on the "Crystal 5000.2". It was found that the essential oil content in mature *F. vulgare* fruits amounted to the average of 5,88± 0.10%, which was higher than in the green plants' material - 2,36± 0.06%. The main component of the essential oil is anethole. The anethole content in essential oil from fruits was higher than from the green plants' material: 64.9-84.3% and 33.5-74.6%, respectively. 66.0% of the analyzed samples corresponded to ISO 17412:2007 requirements. Essential oil from the green plants' material contained more α-phellandrene compared to essential oil from fruits: 1.5-19.9% and 0.1-0.4%, respectively. According to the research results, 9 promising samples with a high content of essential oil in fruits - from 6,52 ± 0.88 to 8,10± 0.91% were distinguished.

1 Introduction

Foeniculum vulgare Mill. (Apiaceae family) is known as a medicinal herb since ancient Rome. Fennel essential oil and "fennel water" started to be used in medicine since the beginning of the 16th century. *F. vulgare* fruits and essential oil are used as a carminative, diuretic, lactogenic, aromatic stimulating and strengthening gastric agent [1-3]. Studies have shown that *F. vulgare* essential oil possesses antimicrobial and antibacterial activity [4]. There is evidence that *F. vulgare* essential oil can be seen as a promising remedy opening new opportunities for antimicrobial and anticancer therapy [5].

Valuable qualities of *F. vulgare* are primarily due to the essential oil accumulating in its raw materials, the main component of which is anethole [6]. The components' ratio in essential oil is unstable and depends on many factors: processed raw materials, which can be both whole plants in the flowering phase and fruits; growing conditions, sample genotype, etc. [6,7]. Thus, essential oil from whole plants contains less anethole, but more

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α-phellandrene than in essential oil from fruits [6]. All these factors should be considered when cultivating *F. vulgare*, knowing the direction of further use of the received raw materials, as well as when creating new varieties of this crop.

The main source of valuable initial material in selection is collections including wild specimens from different regions, historical and cultivated varieties, local populations [8]. The efficiency of sampling characterized by individual or a set of indicators valuable for the varieties creation of a certain direction is increased in the presence of a preliminary collection's assessment.

In the Research Institute of Agriculture of Crimea, the gene pool collection of spicy aromatic, essential-oil-bearing and medicinal plants is supported, replenished and studied.The collection is registered in the Russian Federation as a unique scientific installation (USI No.507515 (http://www.ckp-rf.ru/usu/507515/). It includes a specialized *F. vulgare* collection, which contains 75 specimens of various ecological and geographical origin (from 28 countries around the world). For three years (2017-2019), the study of *F. vulgare* collection samples was conducted on morphobiological features and productivity indicators [9,10].

The purpose of this study is to analyze the *F. vulgare* collection on the content and component composition of essential oil.

2 Materials and methods

The analysis of the *F. vulgare* collection was carried out in 2017-2019 at the experimental site of the Department of Essential-oil-bearing and Medicinal Crops of the Research Institute of Agriculture of Crimea, located in the Crimea Foothills (Krymskaya Roza village, Belogorsky district). Climate of this region is moderately continental. This territory belongs to one of the five agroclimatic regions – the upper foothill, warm, not humid enough; to the northern subarea with moderately mild winters [11]. Average annual temperature is 10^0C. Period with positive air temperature lasts for 292 days a year. Average temperature of the warmest month, July, is $+21^0$C; that of the coldest month, January, is – 0.8^0C. Maximum possible temperature increase in summer is up to 40^0C and minimum decrease in winter – down to $-30\text{-}35^0$C. Average long-term amount of precipitation is 498 mm, during vegetation – 280 mm. Average annual air humidity is 70%, hydrothermal coefficient is 0.91 what indicates the arid nature of weather conditions. Soil at the study site is southern carbonate, heavy loamy chernozem (pH – 7,0-7,2)

The collection nursery was laid in 2016. Specimens were placed on plots 1 m long with row spacing of 0.6 m. The area of the accounting plot was 0.6 m^2. The samples were analyzed in two replicates. The study was carried out on plants of 2-4 years of vegetation. For all three years, the essential oil content and its component composition in the fruits of 50 out of 75 specimens of the collection and in the green mass of plants in 57 samples have been estimated. The remaining samples were evaluated for 1-2 years due to raw materials' insufficiency and were not included in the comparative analysis. The assessment of essential oil content in the green mass of plants was carried out in the mass flowering phase.

The analysis of essential oil content in raw materials was carried out in accordance with the developed methods [12,13]. The component composition of *F. vulgare* essential oil was determined by the method of gas chromatography on the "Crystal 5000.2" device with flame ionization detector [14]. The following chromatography conditions were selected for identification and complete separation of essential oil components: 30 m fused silica column with an internal diameter of 0.32 mm and 0.5-micron phase thickness. Stationary phase CR-WAXms. The temperature of the column's thermostat was programmed in the following mode: 75° C with an exposure of 1 min., then programming at a speed of 4 °C/min to 195° C. Injector temperature 230 °C, detector temperature 250 °C. The carrier gas flow 1.9 ml/min, the flow separation of the carrier gas - 1/20. The carrier gas - helium. Total analysis time - 31 minutes.

Most of the components were identified by comparing their Kovach's retention indices with literary data. Kovach's retention indices were determined in relation to a homologous series of n-alkanes (C8 — C40) in the same working conditions [15].

Statistical processing of the obtained data was performed using the Microsoft Office Excel 2010 software package 2010 [16].

3 Results

One of the most important indicators for essential oil plants is the content of essential oil in raw materials. For comparison correctness of this indicator in the *F. vulgare* collection, the content of essential oil in terms of an absolutely dry weight of raw materials was compared. It should be noted that the intensity of the oil-forming process significantly depends on the weather conditions during its passage. Obtaining essential oil from the green above-ground part of plants is carried out during their mass flowering. In the foothill zone of Crimea, *F. vulgare* flowering normally takes place in the third decade of June - the first decade of July. Active essential oil accumulation in fruits occurs after flowering and before their ripening, i.e., until late August - early September. The optimal conditions for the oil-forming process are high air temperatures with moderate precipitation.

During the research years, the wettest was the spring-summer period of 2017, and the hottest and driest - this period in 2018. The exception in 2018 was July, when precipitation during flowering did not favor pollination and fruit formation. Compared to the previous 2019, it was generally more favorable. However, the temperature regime in June was higher, in July - lower than in previous years. In addition, there was heavy rainfall in July.

In 2017 and 2018, the content of essential oil in plants on average in the collection did not differ significantly and was 2.51±0.09 and 2.40± 0.07%, respectively (Table 1).

Table 1. Characteristics of *F. vulgare* collection samples according to the mass fraction of essential oil and the content of the main components.

Indicator	Year	Essential oil from fruits			Essential oil from the above-ground part of plants		
		average content, %	limits of variation, %	coefficient of variation, Cv%	average content, %	limits of variation, %	coefficient of variation, Cv%
Mass fraction of essential oil*	2017	5.89±0.13	3.76-8.20	16.3	2.51±0.09	1.46-4.34	28.7
	2018	4.67±0.10	3.09-5.84	15.0	2.40±0.07	1.30-3.50	22.1
	2019	7.04±0.14	4.5-10.11	14.7	2.15±0.08	0.98-3.86	29.3
	average	**5.88±0.10**	**4.64-7.34**	**11.9**	**2.36±0.06**	**1.49-3.86**	**18.6**
Content of the main components in essential oil, %							
anethole	2017	73.5±0.8	48.0-83.6	7.8	66.4±1.0	35.5-79.1	11.9
	2018	75.9±0.7	64.1-85.7	6.7	60.5±1.5	3.8-84.6	19.0
	2019	73.2±0.7	62.5-88.4	7.2	66.0±1.0	47.8-83.3	12.0
	average	**74.1±0.6**	**64.9-84.3**	**5.9**	**63.9±0.9**	**33.5-74.6**	**10.7**
fenchone	2017	11.1±0.5	3.4-19.2	30.6	3.7±0.3	0.2-9.9	64.6
	2018	10.4±0.6	1.5-19.6	43.2	4.1±0.4	0.2-21.4	79.1
	2019	12.7±0.6	1.5-22.4	34.7	2.1±0.2	0.1-5.1	58.8
	average	**11.4±0.5**	**3.1-18.8**	**31.8**	**3.3±0.2**	**0.5-10.2**	**56.4**
methylchavicol	2017	3.5±0.45	1.5-25.4	92.5	3.0±0.4	1.7-24.1	95.7
	2018	3.2±0.04	2.6-4.1	9.3	3.3±0.8	1.4-45.5	181.8
	2019	3.0±0.03	2.6-3.6	8.1	2.5±0.03	1.8-2.9	10.2
	average	**3.2±0.2**	**2.6-10.5**	**34.0**	**2.9±0.4**	**1.9-24.0**	**100.0**
α-phellandrene	2017	0.06±0.01	0.0-0.5	216.7	7.0±0.5	0.7-15.4	49.9

	2018	0.35±0.02	0.1-0.6	31.4	8.4±0.6	0.7-18.6	52.5
	2019	0.39±0.01	0.2-0.6	25.6	9.8±0.6	1.1-24.1	46.1
	average	**0.27±0.01**	**0.1-0.4**	**29.6**	**8.3±0.5**	**1.5-19.9**	**44.8**

Note: * in terms of absolutely dry weight of raw material

This is due to close temperature and rainfall rates during the mass plants' flowering period when the biochemical analysis was carried out. The accumulation of essential oil was less intense in lower temperatures in the same period of 2019, which is reflected by the mean value - 2.15± 0.08%. The variability of the essential oil content in the collection by year was quite high: 1.46-4.34; 1.30-3.50 and 0.98 -3.86%, respectively. The coefficient of variation for this indicator in the collection was within 22.1 — 29.3%.

Essential oil content in mature *F. vulgare* fruits was significantly higher than in whole plants. First of all, this is due to the fact that occupying a significant proportion in the total raw materials' weight, the massive stem practically does not contain glandular receptacles in which essential oil accumulates, and is, in fact, a ballast. Average values of essential oil content in the collection also varied significantly by year: in 2017 — 5,891± 0.13% (3.76 - 8.20%), in 2018 — 4.67± 0.10% (3.09 -5.84%) and in 2019 — 7.04± 0.14% (4.50 -10.11%) (Table 1). The conditions of 2019 were the most favorable for the accumulation of essential oil in fruits. The coefficient of variation in the essential oil content in the collection samples' fruits was lower than such for the green mass of plants— 14.9 — 16.3%.

In general, data on the essential oil content in the collectible samples' raw materials indicate the possibility of high-oil forms' sampling for selection.

An extremely important indicator of essential oil is its component composition. The main component of essential oil *F. vulgare* is anethole, the content of which can reach 90% [17].

There are standard requirements (GOST 3902-82) for the component composition of *F. vulgare* essential oil obtained from fruits. According to the standard, it should contain at least 60% anethole and 15% fenchone (Table 2) [18].

Table 2. Standards for the content of the main components in essential oil from the fruits of *F. vulgare.*

Component	ISO 17412:2007 Oil of bitter fennel (*Foeniculum vulgare* Mill. *ssp. vulgare var. vulgare*)		GOST 3902-82 Fennel essential oil Technical conditions
	min, %	max, %	not less than, %
α-phellandrene	traces	8.5	not indicated
fenchone	10.0	25.0	15.0
methyl chavicol	1.0	6.0	not indicated
anethole	50.0	78.0	60.0

The content in essential oil from the fruits of 10 components, primarily anethole, fenchone, α-phellandrene and methylchavicol must comply to the ISO (17412:2007) standards (table 2) [19].

For three years, chromatographic component composition analysis of essential oil from the fruits of 50 out of 75 *F. vulgare* collection samples was carried out. For other samples, the number of fruits in different years was insufficient to produce essential oil for analyzing purposes. The content of anethole as the main component in essential oil from the fruits of the analyzed samples remained relatively stable on average through the collection, regardless of the differences in weather conditions of the 2017-2019 seasons and amounted to 73.5± 0.8%; 75.9± 0.7% and 73.2± 0.7%, respectively (Table 1). The high stability of this indicator is also evidenced by the coefficients of variation: 6.7 -7.8%. The variability range of this indicator by years amounted to 48.0 -83.6%, 64.1 -85.7% and 62.5 -88.4%, respectively.

The fenchone content in essential oil and *F. vulgare* fruits was much lower than anethole (Table 1). The average fenchone content in the essential oil of collectible samples by year was 11.1± 0.5%, 10.4± 0.6% and 12.7± 0.6%, respectively. As can be seen from the data, the differences were not so large. The variability range of the indicator in the collection, respectively by year - 3.4 -19.2%, 1.5 -19.6% and 1.5 -22.4%. Variability in the collection for this indicator was high: 30.6 — 43.2%. When selecting, attention should be paid to the stability of the components' content in the essential oil of specific samples.

The content of α-phellandrene in essential oil from the fruits in the collection ranged from 0 to 0.6% and methyl chavicol - from 1.5 to 25.4%.

The essential oil component composition from the fruits of 6 samples (16.0%) from the analyzed samples of all three study years of 50 samples corresponded to the requirements of GOST 3902-82, and 33 samples (66.0%) corresponded to the requirements of ISO 17412:2007.

An exception was the essential oil from the fruits of the K16 Iran sample, which differed from other samples in the main components' content and differed significantly during the study years. At the same time, the average content of anethole averaged to 65.8± 10.1% (by years: 48.0 -83.1%), fenchone — 10.9± 2.4% (by years: 6.4 -14.4%), methylchavicol — 10.5± 7.5% (by year: 2.7 -25.4%) and α- phellandrene - 0,1± 0.1% (by year: 0 -0.2%). This explains the extremely high coefficients of variation in methylchavicol and α-phellandrene content in essential oil from both fruits and green mass of plants.

It should be noted that anethole is not always the main component in the composition of *F. vulgare* essential oil. Thus, in the essential oil of wild samples collected in the regions of Greater Caucasus and Lesser Caucasus (Azerbaijan) in 2016-2017, methylchavicol (24.1%), trans-anethole (23.9%), α-pinene (19.9%), and β-pinene (12.3%) were identified as the main components [20]. 91 specimens of *F. vulgare* have been collected in different geographical points of India and studied. Essential oil analysis showed that anethole was an ever-present component with its content ranging from 9.15 to 96.64%. The second component, predominant in some cases, was methylchavicol, which was part of the essential oil in quantities from 2.04 to 83.01% [21]. Comparative analysis of the Tunisian and French *F. vulgare* varieties allowed to distinguish two chemotypes. The main component of the first chemotype essential oil to which Tunisian varieties were attributed was trans-anethole in the amount of 63.41 -78.26%. French varieties were represented by the second chemotype with the main component being estragole (methylchavicol) in the amount of44.72% -88.92% [22]. Based on this division to chemotypes, all samples of the collection studied should be attributed to the first chemotype.

There are no regulatory documents for *F. vulgare* essential oil obtained during the processing of whole plants. In terms of the components' ratio, it is significantly different from essential oil from fruits. However, such oil is also in demand in production depending on the direction of use. In this regard, an analysis of the essential oil component composition of 57 collection samples obtained during plant processing in the phase of full flowering was carried out.

The average anethole content in essential oil from plant raw materials of collection samples was the same in 2017 and 2019 and amounted to 66.4± 1.0% and 66.0± 1.0%, respectively (Table 1). The fluctuations of this indicator in the collection were in the range of 35.5 -79.1% and 47.8 -83.3%, respectively. In 2018, anethole content was lower and the collection average was 60.5± 1.4% with a range of 3.8 -84.6%.

In general, the anethole content in essential oil from plants (on average 63.9± 0.9%) was significantly lower than in essential oil from fruits (on average 74.1± 0.6%).

Being the main component in the essential oil of both the green plants' material and the fruits of *F. vulgare* studied samples, anethole is quite stable and is characterized by the least variability within the collection.

The average fenchone content in essential oil from plants in the collection (3.3± 0.2%) was significantly less than in essential oil from fruits (11.4± 0.5%) and amounted to 3.7± 0.3% and 4.1± 0.4% in 2017 and 2018 at a range of 0.2 -9.9% and 0.2 -21.4%, respectively. In 2019, fenchone content was lower and averaged to 2.1± 0.2% (at a range of 0.1 -5.1%).

The content of methylchavicol in essential oil from the green mass of collectible samples over the years of study averaged to 3.0±0.4; 3.3±0.8 and 2.5± 0.03% at a range of 1.4 to 45.5%. As it was already noted, the wide range is due to the non-standard components' composition of the K16 Iran sample. The content of this component in essential oil from fruits and green plants did not differ.

The content of α-phellandrene in essential oil from plants was much higher than in essential oil from fennel fruits, averaging to 8.3±0.5 and 0.27± 0.01%, respectively. The lowest indicator in 2017 amounted to a collection average of 7.0± 0.5% (0.7 -15.4% in different samples), and the highest in 2019 — 9.8± 0.6% (1.1 -21.1%).

The analysis results of the essential oil component composition showed a fairly high variability in the content of its main components through collection samples, depending on both the genotype and on weather conditions of the season. This indicates the prospect of selection research, the ultimate goal of which is to create new high-oil *F. vulgare* varieties with a given essential oil component composition.

According to the research results of the *F. vulgare* collection, 9 selection-promising samples with a high content of essential oil in fruits - from 6,52 ± 0.88 to 8,10± 0.91% were distinguished. For comparison, the content of essential oil in the fruits of Marcishor and Oxamite of Crimea varieties (selection of the Research Institute of Agriculture of Crimea) is 7.25± 1.46 and 6.30± 0.55%, respectively.

Stably high anethole content (75-78%) in essential oil from fruits with the corresponding ratio of other significant components was noted in 8 samples.

The high content of essential oil in the green mass of plantswas noted in 3 samples — 3,11±0.67 — 3,86± 0,2%. In varieties Mertsishor and Oxamite of Crimea, the average indices amounted to 2,32±0.34 and 2.72± 0.03%, respectively.

Dedicated *F. vulgare* collection samples will be incorporated into further selection process.

The study was conducted based on the gene pool collection of spicy aromatic, aromatic, and medicinal plants RIAC registered in the Russian Federation as a unique scientific installation USI No. 507515 (http://www.ckp-rf.ru/usu/507515/).

4 Conclusions

1. As a result of the comparative analysis of *F. vulgare* collection samples, it was found that the content of essential oil in mature fruits averages to 5,88± 0.10% in the collection, which is significantly higher than in the green mass of plants - 2.36 ± 0.06%.
2. The main component of the studied essential oil samples is anethole. The average anethole content in essential oil from the fruits of collectible samples was higher than from the green mass of plants: 64.9-84.3 and 33.5 -74.6%, respectively.
3. 16.0% of the component composition of essential oil from fruits corresponded to the requirements of GOST 3902-82, and 66.0% of the analyzed samples corresponded to ISO 17412:2007 requirements.
4. Essential oil from the green mass of plants contains significantly more α-phellandrene compared to essential oil from fruits: 1.5 -19.9% and 0.1 -0.4%, respectively.
5. According to the research results of the *F. vulgare* collection, 9 selection-promising samples with a high content of essential oil in fruits - from 6,52 ± 0.88 to 8,10± 0.91%, were distinguished.

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