CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

# PHYTOCHEMICAL AND BIOLOGICAL PROFILES OF FENNEL FRUITS (*FOENICULUM VULGARE* MILL. VAR. *DULCE* MILL.)

**Martina Pencheva[1], Zhana Petkova[2], Ivayla Dincheva[3], Iliana Kostova[4], Stanka Damyanova[4], Albena Stoyanova[5], Liviu Gaceu[6,7,8] ✉**

[1]*University of Russe "A. Kanchev", Russe, Bulgaria*
[2]*University of Plovdiv "P. Hilendarski", Plovdiv, Bulgaria*
[3]*AgroBioInstitute, Agricultural Academy, Sofia, Bulgaria*
[4]*University of Ruse "A. Kanchev", Branch-Razgrad, Razgrad, Bulgaria*
[5]*University of Food Technologies, Plovdiv, Bulgaria*
[6]*Transilvania University of Brasov, Brasov, Romania*
[7]*CSCBAS &CE-MONT Centre / INCE - Romanian Academy*
[8]*Assoc. m. Academy of Romanian Scientists*
✉*gaceul@unitbv.ro*

https://doi.org/10.34302/crpjfst/2022.14.4.3

**Article history:**
Received
9 August 2022
Accepted
7 September 2022
Published
December 2022

**Keywords:**
*Fennel fruits;*
*Chemical composition;*
*Antimicrobial activity.*

**ABSTRACT**

The aim of this article is to determine the phytochemical composition and biological activity of sweet fennel fruits, cultivated during two harvest years in Bulgaria (2020 and 2021). First, we researched the chemical composition of fennel fruits and determined their protein content (15.74% and 14.30%), crude fiber (32.88% and 31.50%), ash (6.49% and 7.99%), and mineral elements (58373.22 mg/kg and 102825.96 mg/kg), respectively for 2020 and 2021 harvest years. Then, the fruits were subjected to hydrodistillation and the essential oil was isolated (8.38% and 7.57%), respectively for 2020 and 2021 harvest years. At the end, the lipid fraction was extracted from the ground fruits (9.07% and 5.58%), respectively for 2020 and 2021 harvest years. Polar and unpolar fractions were isolated from the fruits. The results reveal that the fruits of the 2020 harvest have a higher content of protein, essential oil and lipid fraction. Some significant differences were also found in the amounts of macro elements, while the content of micro and toxic elements was comparable. Regarding the chemical composition, both essential oils were high in anethole and low in fenchone. The essential oils of the fennel fruits have antimicrobial activity, which is more pronounced against molds (*Aspergillus brasiliensis* and *Fusarium moliniforme*) and yeasts (*Candida albicans*). The main compounds of polar fraction were saccharides (mono- and di-), followed by sugar alcohols, organic acids, sugar acids, amino acids, and others. In both lipid fractions the main sitosterols are *β*-sitosterol and stigmasterol, and major tocopherol is *γ*-tocotrienol. Overall, the obtained results demonstrate that with its balanced composition of protein, crude fiber, essential oil, polar and lipid fraction, and mineral elements, the fennel fruits can be used as a supplement to the food of humans and animals.

## 1. Introduction

Fennel (*Foeniculum officinale* All. = *F. vulgare* Mill. = *F. capillaceum Gilib*) belongs to the Apiaceae family. The main species are dulce (var. *dulce* (Mill.) Thell.) and bitter (var. *vulgare* (Mill.) Thell). They differ both in the shape of the fruits and in chemical composition. The fruits of the fennel are mainly used in the traditional medicine and in the food industry (Anka *et al.*, 2020). The fruits are

processed in order to obtain essential oil and lipid fraction.

The main component of the sweet fennel essential oil is *trans*-anethole, and its content in essential oils from different countries ranges from 65 to 91% (most often 78-88%). The composition of the oil also contains fenchone (1.0-12.0%), limonene (up to 22.4%, most often 2-7%), methyl chavicol (2.3-7.3 and up to 67%, most often 2.3-4.0%) and many other components, the amount of which varies depending on the origin of the raw material (Conforti *et al*., 2006; Diaz-Maroto *et al*., 2006; Coşge *et al*., 2008; Renjie *et al*., 2010; He and Huang, 2011; Zheljazkov *et al*., 2013; Najdoska-Bogdanov *et al.,* 2015; Akhatou *et al*., 2016; Ali *et al*., 2016; Bahmani *et al*., 2016; Ahmad *et al*., 2018; Wodnicka *et al*., 2019). The essential oil is characterized by antimicrobial activity (Dadalioglu and Evrendilek, 2004; Damianova and Stoyanova, 2007; Mohsenzadeh, 2007; Kaur and Arora, 2010; Renjie *et al*., 2010; He and Huang, 2011; Jamwal *et al*., 2013; Ali *et al*., 2016; Balouiri *et al*., 2016; Wodnicka *et al*., 2019; Anka *et al*., 2020), antioxidant effect (Conforti *et al*., 2006; He and Huang, 2011; Jamwal *et al*., 2013; Zheljazkov *et al*., 2013; Anka *et al*., 2020) and other biological properties (He and Huang, 2011; Rather *et al.*, 2012; Jamwal *et al*., 2013; Dikova, 2014; Dikova *et al*., 2017; Anka *et al*., 2020). The pharmacological activities of the essential oil are attributed to its main compound – anethole (Baser and Buchbauer, 2010).

The main fatty acids in the lipid fraction are petroselinic acid (67.0-83.3%), oleic acid (12.0-16.4%), linoleic acid (6.50-8.97%), and palmitic acid (3.25-6.80%). Their amount varies depending on the origin of the raw material and the method of obtaining the lipid fraction (Coşge *et al*., 2008; Morales *et al*., 2012; Najdoska-Bogdanov *et al*., 2015; Ahmad *et al*., 2018).

The fennel fruits contain about 20% protein, phenolic compounds, flavonoids, minerals, vitamins, *etc*., which explains its biological properties and application (Jamwal *et al*., 2013; Anka *et al*., 2020).

In Bulgaria, the fennel fruits are processed only to obtain essential oil, and the remaining raw material after distillation is used as an additive to feed mixtures.

The studies on the composition of fruits and their biological activity are scarce. Therefore, the aim of the present work is to determine the phytochemical composition and biological activity of sweet fennel fruits cultivated during two harvest years – 2020 and 2021. This, in our opinion, will enrich the data for a more versatile application of the fennel fruits, as well as for the content of various biologically active compounds in them.

## 2. Materials and methods

### 2.1. Materials

#### *2.1. Plant material*

The fennel fruits (*Foeniculum vulgare* Mill. var. *dulce* Mill.), family Apiaceae, harvest 2020 and 2021, were used. They were provided by a company, producer of essential oil plants, located in North Eastern Bulgaria near the town of Razgrad (43°32'00" N and 26°32'30" E). The ripe greenish-yellow fennel fruits were air dried and stored in sacks in well-ventilated warehouses (20-25°C), with no sunlight, in cool, and dry conditions.

Before analysis, the fruits were grounded for 30 s to a size of 0.5 mm, using the electric blender Bosch MKM 6003, Stuttgart, Germany.

### 2.2. Methods

#### *2.2.1. Chemical composition of the plants*

Protein content, crude fiber, ash and moisture were determined using methods, described in AOAC. All results in the study are reported at dry weight.

#### *2.2.2. Isolation of mineral elements*

The mineral contents (trace elements, heavy metal and other elements) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion (BSS-EN 17053, BSS-EN 15763).

### *2.2.3. Metabolic profiling by fennel fruits*

0.050 g from each sample (fruits) were mixed with 1.0 mL methanol/distilled water (75:25, v/v) mixture, followed by heating at 70°C for 30 min in a laboratory thermo mixer (Analytik Jena AG, Germany), and then cooled to 25ºC. Next, 500.0 mL chloroform and 200.0 mL distilled water were added, and then the resulted mixture was centrifuged at 13000 rpm for 5 min at 22°C. The lower phase was designed for the analysis of non-polar substances (essential oil, lipid fraction, *etc*. – fraction "A"), whereas the upper phase – for the polar constituents (amino and organic acids, carbohydrates, *etc*. – fraction "B"). The two phases obtained were vacuum-dried in a centrifugal vacuum concentrator (Labconco Centrivap, US) at 40ºC. 1.0 mL 2% $H_2SO_4$ in methanol was added to the dried residue of fraction "A", and the mixture was heated on Thermoshaker TS-100 for 1 h, at 96ºC and 300 rpm. The solution was left to cool and then extracted with *n*-hexane (3x500.0 mL). Combined organic layers were vacuum-dried in the centrifugal vacuum concentrator at 40ºC.

Prior to the gas chromatography-mass spectrometry (GC-MS) analysis, fractions "A" and "B" were derivatized using the procedures: 100.0 µL pyridine and 100.0 µL N, O-Bistrifluoroacetamide (Sigma Aldrich, St. Louis, MO, United States) were added to the dried residue (fraction "A"), then heated on Thermoshaker TS-100, Analytik Jena AG, Germany for 45 min, at 70ºC and 300 rpm, followed by injecting 1.0 µL from the solution into the GC-MS. 300.0 µL solution of methoxyamine hydrochloride (20.0 mg/mL in pyridine) was added to dried residue (fraction "B"), and the mixture was heated for 1 h at 70ºC and 300 rpm. After it was cooled, 100.0 µL BSTFA were added to the mixture then heated for 40 min, at 70ºC and 300 rpm, injecting 1.0 µL from the solution into the GC-MS system.

GC-MS analysis was carried out on Agilent 7890A gas chromatograph (Santa Clara, CA 95051, US) interfaced with an Agilent 5975 C mass selective detector (Santa Clara, CA 95051, US). Separations were performed using a DB-5ms silica-fused capillary column (30 m × 0.25 mm (i.d.)), coated with 0.25 µm film of poly (dimethylsiloxane) as a stationary phase. The carrier gas (helium) flow rate was maintained at 1.0 mL/min. The injector and the transfer line temperature were kept at 250°C. The oven temperature program used was 100°C for 2 min then 15°C/min to 180°C for 1 min then 5°C/min to 300°C for 10 min. The injection volume was 1 µL, and was carried out in a 1:20 split mode. The mass spectrometer was scanned from 50 to 550 m/z. All mass spectra were acquired in electron impact (EI) mode with 70 eV.

A mixture of aliphatic hydrocarbons ($C_8$-$C_{40}$) (Sigma) was injected into the system under the above mentioned temperature program, in order to calculate the retention index RI (as Kovàts index) for each compound. The compounds in the polar fraction were identified as trimethylsilyl (TMS) derivatives with the help of the NIST 08 database (NIST Mass Spectral Database, PC-Version 5.0-2005, National Institute of Standardization and Technology, Gaithersburg, MD, USA), and other plant-specific databases: the Golm Metabolome Database (http://csbdb.mpimp-golm. mpg.de/csbdb/gmd/home/gmd_sm.html accessed on 15 December 2021). In order to calculate the retention index RI of each compound, a mixture of aliphatic hydrocarbons ($C_8$-$C_{40}$) (Sigma-Aldrich, St. Louis, MO, USA) was injected into the system using the above mentioned temperature program. In order to identify the amino acids, a mixture of standard AA (Amino Acid Standard Solution Prod. No A 6407; Sigma-Aldrich, St. Louis, MO, USA) was used as well. The amount of identified metabolites was considered by the percentage peak area that appeared in the total ion chromatogram (TIC) in the GC-MS analysis.

### *2.2.4. Essential oil Isolation by hydrodistillation*

The fruits were hydrodistilled for 4 hours in a British Pharmacopoeia laboratory glassware apparatus, modified by Balinova and Diakov, in 1974. The essential oil was dried over

anhydrous sodium sulfate and stored at 4°C in dark vials. After hydrodistillation, the fruits were air-dried (humidity 7.00±0.06%) for 10 days at 25°C.

***Determination of chemical composition***

The chemical composition of the essential oils was determined as described in subchapter 2.2.3.

***Determination of antimicrobial activity***

Antimicrobial activity of the studied essential oil was tested against the following test microorganisms: Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876; Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NTCC 6017 yeasts: *Saccharomyces cerevisiae* ATCC 2601, *Candida albicans* ATCC 10231 and molds *Aspergillus brasiliensis* ATCC 16404, *Fusarium moniliforme*. Test micro-organisms strains were provided by the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria.

Antimicrobial activity was determined by the diffusion method in agar wells. Growth media were Tryptic soy agar (Merck) for the tested bacteria and Sabouraud-Dextrose-Agar (Merck KGaA, Darmstadt, Germany) for yeasts and molds. Media were inoculated with 24-hour suspension of bacterial species and 48-hour suspension for yeasts and molds with turbidity – 0.5 McFarland standards.

The melted and cooled media at 50°C±2°C were inoculated with 1% of the prepared suspensions of the microorganisms to be tested. 20 mL of inoculated medium was poured into sterile Petri dishes (∅ = 90 mm). 50 µL of essential oil was added drop wise to each well, and then the Petri dishes were placed in thermostatic chambers and incubated at 37°C or 28°C for 24 and 48 h depending on the microbial seasoning.

The diameter of the inhibition zones was measured using digital caliper, and were interpreted as follows: microbial culture up to 15 mm is weakly sensitive; from 15 to 25 mm – sensitive and above 25 mm – very sensitive (Balouiri *et al*., 2016; Hussein *et al*., 2020).

***2.2.5. Lipid fraction by extraction Isolation of lipid fraction***

The lipid fraction was extracted from ground fruits with *n*-hexane using a Soxhlet extractor (ISO 659).

***Fatty acid composition***

The determination fatty acid composition of triacylglycerol's was done using GC method (ISO 12966-1). The triacylglycerols were pre-esterified with methanol in the presence of sulfuric acid in order to obtain fatty acid methyl esters (FAMEs) (ISO 12966-2). Determination of FAMEs was carried out on Agilent 8860 gas chromatograph equipped with a capillary DB-FastFAME column, (30 m x 0.25 mm x 0.25 µm (film thickness)) and a flame ionization detector (FID). The injector and detector temperatures were set at 270°C and 300°C.

The column temperature was from 70°C (1 min), at 6°C/min to 180°C (0 min), and at 5°C/min to 250°C, and the split ratio was 50:1. A standard Supelco, USA mixture (FAME mix 37 components, Supelco, USA) was used for the identification of FAMEs.

***Determination of sterols***

Unsaponifiables were determined according to the ISO 18609 standard. Sterols were isolated from the unsaponifiable matter by thin layer chromatography (TLC) (Ivanov *et al*., 1972) and their total content was determined spectrophotometrically at a 597 nm wavelength. Individual sterol composition was determined on HP 7890 gas chromatograph equipped with DB – 5 (25 m x 0.25 mm) capillary column and FID. Identification was established by comparing retention times with those of a standard mixture of sterols (Across Organics, New Jersey, USA) (ISO 12228-1).

***Determination of tocopherols***

Individual tocopherols were determined by Merck-Hitachi (Merck, Darmstadt, Germany)

high-performance liquid chromatography (HPLC). The column was Nucleosil Si 50-5 (250 mm x 4 mm). Fluorescence detection was used (excitation at 295 nm and emission at 330 nm). The mobile phase used was *n*-hexane: dioxane, 96:4 (v/v) and the flow rate were set at 1 mL/min. (ISO 9936).

***Determination of total phospholipids***

Total phospholipid content was determined spectrophotometrically at 700 nm after mineralization of the lipid fraction with a mixture of perchloric and sulphuric acid (1:1, v/v) (ISO 10540-1).

***2.2.6. Statistics***

All measurements in the study were performed in triplicate ($n = 3$). The results were presented as the mean value with the corresponding standard deviation (SD). ANOVA and Tukey multiple comparison test were used as statistical tools in the assessment of significant differences at $p < 0.05$.

## 3. Results and discussions

### 3.1. Chemical composition of fennel fruits

The chemical composition of the fruits is presented in Table 1. The data show that the fruits of the 2020 harvest have a higher content of protein, essential oil and lipid fraction, which can be explained by the influence of climatic conditions, for example at a higher temperature and drier air, the essential oil evaporates more easily from the plants (Baser and Buchbauer, 2010). For the region of the city of Razgrad, the average monthly temperature in August 2020 was 36.6°C, and the average precipitation is 15 mm, while in 2021 these values were 38.1°C and 10 mm, respectively (Annual Hydrometeorological Bulletin). The amount of essential oil in both samples is higher than reported in the literature, for example 2.5-5% (Kaur and Arora, 2010); 3% (He and Huang, 2011); 1.74% (Diao *et al*., 2014); 3.64-4.14% (Wodnicka *et al*., 2019); from 1.1 to 4.8% (Bahmani *et al*., 2016). The content of the lipid fraction in both samples was lower compared to literature data, for example 19.80% (Ahmad *et al*., 2018); from 12.2 to 22.8% (Ali *et al*., 2016); 20% (He and Huang, 2011); 20.90% (Gulfraz *et al*., 2005). Protein content also differed from literature data 9.5% (Kaur and Arora, 2010); 24.12% (Gulfraz *et al*., 2005). The amount of fiber determined differed as well from literature data: 18.5% (Kaur and Arora, 2010); 9.50% (Gulfraz *et al*., 2005). It was found that the ash value was lower than the data, reported in literature: 7.86% (Unal *et al*., 2013); 8.93-9.78% (Moser *et al*., 2014).

The influence of pedo-climatic conditions, as well as the care given to the cultivation of plants, could lead to the appearance of these differences (Conforti *et al*., 2006; Ehsanipour *et al.,* 2012; Yaldiz and Çamlica, 2019).

The polar compounds are presented in Table 2. From the data it can be seen that the identified components (% of the composition) in the 2020 harvest are 98.69%, and in the 2021 harvest their amount is 98.44%.

**Table 1**. Chemical composition of the fennel fruits

| **Compounds, %** | Harvest 2020 | Harvest 2021 |
|---|---|---|
| Moisture | 13.49±0.11 [a] | 14.70±0.12 [b] |
| Proteins | 15.74±0.14 [b] | 14.30±0.13 [a] |
| Crude fiber | 32.88±3.00 [b] | 31.50±3.00 [a] |
| Ash | 6.49±0.05 [a] | 7.99±0.07 [b] |
| Essential oil | 8.38±0.33 [b] | 7.57±0.09 [a] |
| Lipid fraction | 9.07±0.08 [b] | 5.58±0.04 [a] |

Results: mean value ± standard deviation ($n = 3$). Different letters in the same row indicate significant differences ($p < 0.05$).

**Table 2**. Polar metabolites, trimethylsilyl esters.

| RT [2] | RI [3] | Compounds | Content, % of TIC [1] | |
|---|---|---|---|---|
| | | | Harvest 2020 | Harvest 2021 |
| **Amino acids** | | | | |
| 4.80 | 1088 | Alanine 2TMS | 0.14±0.11 [a] | 0.12±0.10 [a] |
| 5.81 | 1224 | Valine 2TMS | 0.21±0.02 [a] | 0.17±0.01 [a] |
| 6.17 | 1302 | Proline 2TMS | 0.12±0.11 [a] | 0.10±0.01 [a] |
| 7.71 | 1470 | Aspartic acid 3TMS | 0.18±0.01 [a] | 0.15±0.01 [a] |
| **Organic acids** | | | | |
| 5.20 | 1196 | Malonic acid 2TMS | 2.20±2.00 [b] | 1.76±1.50 [a] |
| 6.32 | 1307 | Succinic acid 2TMS | 1.14±0.09 [b] | 0.91±0.09 [a] |
| 6.45 | 1341 | Glyceric acid 2TMS | 0.17±0.01 [a] | 0.14±0.01 [a] |
| 6.58 | 1345 | Fumaric acid 2TMS | 0.19±0.01 [a] | 0.16±0.01 [a] |
| 6.96 | 1397 | Glutaric acid 2TMS | 0.12±0.01 [a] | 0.10±0.01 [a] |
| 7.94 | 1485 | Malic acid 3TMS | 6.96±6.00 [b] | 5.57±5.00 [a] |
| 8.24 | 1498 | Adipic acid 2TMS | 0.80±0.07 [b] | 0.64±0.06 [a] |
| 9.71 | 1636 | *o*-Methoxymandelic acid 2TMS | 0.35±0.03 [b] | 0.28±0.02 [a] |
| 9.97 | 1673 | *p*-Methoxymandelic acid 2TMS | 0.51±0.04 [a] | 0.41±0.04 [a] |
| 10.70 | 1736 | *(E)*-Aconitic acid 3TMS | 5.29±5.00 [a] | 5.41±5.00 [a] |
| 11.38 | 1790 | Shikimic acid 4TMS | 2.86±2.00 [b] | 1.25±1.00 [a] |
| 12.16 | 1804 | Citric acid 4TMS | 2.26±2.00 [b] | 1.78±1.12 [a] |
| **Sugar alcohols** | | | | |
| 5.89 | 1265 | Glycerol 3TMS | 13.03±1.10 [a] | 10.65±0.90 [a] |
| 8.12 | 1489 | Threitol 4TMS | 0.19±0.01 [a] | 0.16±0.01 [a] |
| 8.16 | 1493 | Erythritol 4TMS | 0.55±0.04 [a] | 0.44±0.04 [a] |
| 10.49 | 1690 | Xylitol 5TMS | 9.65±8.00 [b] | 7.72±7.00 [a] |
| 10.57 | 1703 | Arabitol 5TMS | 1.91±1.00 [b] | 1.36±1.00 [a] |
| 15.86 | 2144 | Myo-Inositol | 3.79±3.00 [a] | 4.55±0.40 [b] |
| **Sugar acids** | | | | |
| 14.69 | 2050 | Gluconic acid 5TMS | 2.18±2.00 [a] | 2.60±2.00 [b] |
| 15.01 | 2072 | Glucaric acid 6TMS | 5.21±5.00 [a] | 6.25±0.55 [b] |
| **Saccharides (mono- and di-)** | | | | |
| 12.76 | 1855 | Fructose oxime 5TMS isomer | 1.62±1.10 [a] | 1.95±1.50 [b] |
| 13.03 | 1864 | Fructose oxime 5TMS isomer | 0.67±0.05 [a] | 0.80±0.07 [a] |
| 13.30 | 1881 | Glucose oxime 6TMS isomer | 1.42±1.30 [a] | 1.70±1.50 [b] |
| 13.87 | 1901 | Glucose oxime 6TMS isomer | 3.96±3.00 [a] | 4.71±4.00 [b] |
| 19.11 | 2298 | Glucose 6-phosphate 6TMS | 9.24±9.00 [a] | 11.0 ±1.00 [b] |
| 25.13 | 2611 | Sucrose 8TMS isomer | 19.16±1.80 [a] | 22.91±2.10 [b] |
| 25.96 | 2656 | Sucrose 8TMS isomer | 1.50±1.10 [a] | 1.80±1.70 [b] |
| **Others** | | | | |
| 8.90 | 1575 | *n*-Dodecanol 1TMS | 1.11±0.09 [b] | 0.89±0.08 [a] |

[1] – total ion current; [2]– retention time, min; [3]– retention (Kovat's) index; Results: mean value ± standard deviation ($n$ = 3); Different letters in the same row indicate significant differences ($p < 0.05$).

The distribution of the polar metabolites by groups (expressed as percentage of the identified) is presented in Figure 1. The data show that the saccharides (mono- and di-) predominated in both samples, followed by sugar alcohols, organic acids, sugar acids, others, and amino acids.

From the group of organic acids, the amount of malic acid 3TMS and (E)-aconitic acid 3 TMS was the largest. It is known that aconitic acid has a role as a fundamental metabolite and shikimic acid is polyphenol precursors, as well as it participates in the genesis of aromatic amino-acids like: tyrosine, tryptophan, and phenylalanine (Bontpart *et al*., 2016).

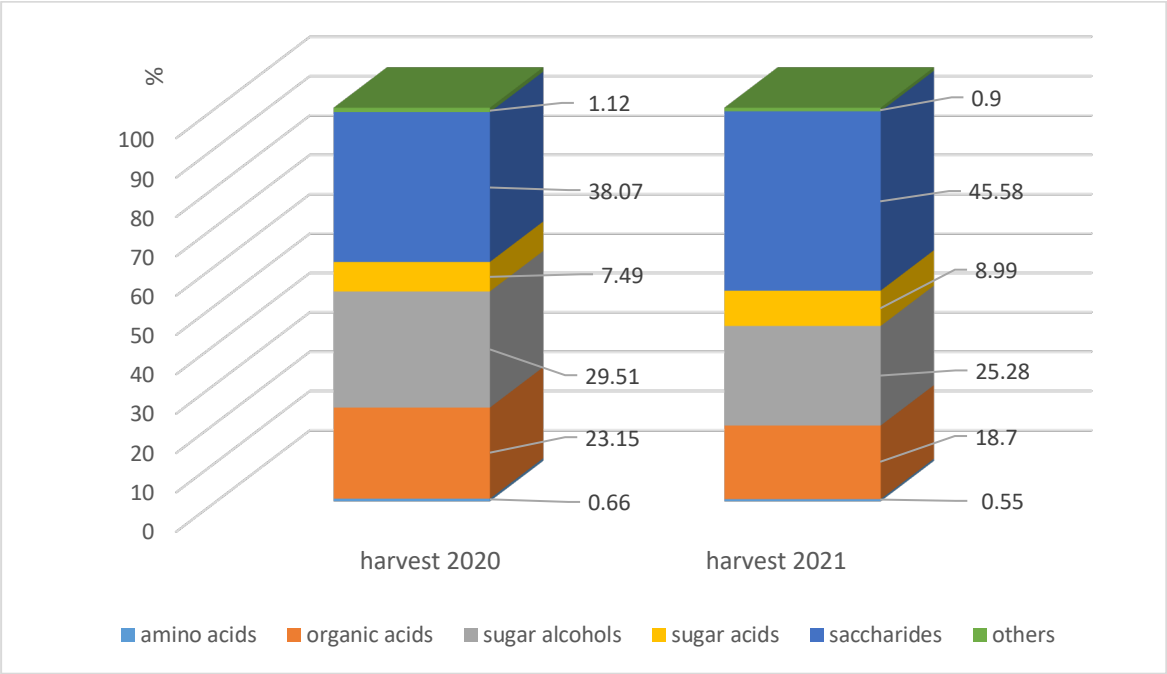



**Figure 1.** Distribution of compounds in polar fraction from fennel fruits.

The sugars that supply energy to the body are precursors for primary and secondary metabolites (Akhatou *et al*., 2016), represented by different group. The data show that in both samples fruits from the group of sugar alcohols predominate xylitol 5TMS and myo-inositol, which are important for health (Chhetri, 2019; Benahmed *et al*., 2020). Sugar acids glucaric acid 6TMS and gluconic acid 5TMS are also biologically important, as the content of the former is twice that of the latter.

From the group of carbohydrates, the content of sucrose is the highest.

The wide variety of polar metabolites defines the fennel fruit as a potential additive in human and animal food.

Mineral elements play a major role in metabolic processes and in the normal functioning of both human and animal bodies. Their main source is fruits and vegetables, and their consumption is a major factor against various diseases and functional disorders. Data in the literature show that the determination of mineral elements is also the subject of research on plant raw materials that are not used for direct consumption, but can be used as an additive in the food of humans and animals.

The mineral composition of fennel fruits is presented in Table 3. The data show significant differences in the amounts of macro elements, while the content of micro and toxic elements was comparable. Generally, the concentrations of the identified macro elements were lower in harvest 2020 compared with harvest 2021, and the greatest differences were with respect to potassium (about 2.5 times), sodium (about 2 times), and magnesium (3 times) contents. The comparative analysis shows that some of the macro elements do not differ from the data in the literature, for example potassium (0.57-40.60 mg/g), sodium (0.07-1.32 mg/g), magnesium (0.57-5.38 mg/g), calcium (1.00-16.78 mg/g), phosphorus (2.13-56.58 mg/g), and sulfur (0.35-52.12 mg/g) (Yaldiz and Gamlica, 2019). However, a lower phosphorus (9 367.80 mg/kg) and calcium (16 452.88 mg/kg) content was found in bitter fennel (Ozcan and Akbulut, 2007). A study by Ullah *et al*., 2012 reported very high values of calcium (70 mg/kg), magnesium (34 mg/kg), and sulfur (21.4 mg/kg). The observed differences in macro elements in our investigation and literature data were probably due to growth conditions, cultural applications

or genetic factors (Guil *et al*., 1998; Ozcan and Akgul, 2007).

Microelements, regardless of their small amounts, participate in various metabolic and regulatory processes in the body of humans and animals. Iron content is highest, followed by magnesium and zinc.

The trace toxic elements, lead, arsenic, cadmium, and mercury, were identified in both harvests.

**Table 3**. Minerals in fennel fruits

| Minerals | Harvest 2020 | Harvest 2021 |
|---|---|---|
| **Macro elements, mg/kg** | | |
| Potassium (K) | 18 328.31±150.00 [a] | 45 003.02±400.00 [c] |
| Calcium (Ca) | 18 904.20±17.00 [a] | 19 426.64±18.00 [b] |
| Phosphorus (P) | 9 771.80±9.50 [a] | 13 201.33±12.00 [h] |
| Sulfur (S) | 5 984.93±5.20 [a] | 7 909.11±7.20 [b] |
| Magnesium (Mg) | 4 661.64±4.10 [a] | 15 871.62±14.00 [b] |
| Sodium (Na) | 523.14±0.50 [a] | 1 213.22±11.00 [b] |
| Aluminum (Al) | 33.37±0.02 [a] | 43.33±0.03 [b] |
| **Micro elements, mg/kg** | | |
| Iron (Fe) | 65.66±0.05 [a] | 70.54±0.06 [b] |
| Manganese (Mn) | 51.67±0.04 [b] | 40.31±0.03 [a] |
| Zinc (Zn) | 32.29±0.02 [a] | 30.23±0.02 [a] |
| Copper (Cu) | 14.16±0.01 [a] | 13.80±0.01 [a] |
| Nickel (Ni) | 1.53±0.01 [a] | 2.12±0.0 [b] |
| Cobalt (Co) | 0.11±0.0 [a] | 0.20±0.0 [a] |
| Selenium (Se) | 0.14±0.0 [a] | 0.14±0.0 [a] |
| **Toxic elements, mg/kg** | | |
| Chromium (Cr) | 0.27±0.0 [a] | 0.35±0.0 [b] |
| Arsenic (As) | trace[1] | trace |
| Lead (Pb) | trace | trace |
| Cadmium (Cd) | trace | trace |
| Mercury (Hg) | trace | trace |

[1] up to 0.01 mg/kg; Results: mean value ± standard deviation ($n$ = 3); Different letters in the same row indicate significant differences ($p < 0.05$).

Data for micro- and toxic elements do not differ from data in the literature (Yaldiz and Gamlica, 2019).The established differences in the amount of mineral elements reflect the influence of environmental factors on their presence and accumulation during the vegetation of plants.

The obtained results show that with its balanced composition of mineral elements, the fruits of the fennel can be used as a supplement to the food of humans and animals.

### 3.2. Chemical composition and antimicrobial activity of essential oil

All the essential oils were easily mobile liquids, with a pale-yellow color and a characteristic odor of anethole.

The chemical composition of the essential oils is presented in the Table 4.

From the data it can be seen that the identified components (% of the composition) in the essential oils obtained by hydrodistillation are 98.45% (harvest 2020) and 98.79% (harvest 2021). In this variant the two essential oils have a similar composition. In the 2020 harvest, the main components (above 2%)

were: anethole (73.72%), fenchone (17.09%), and estragole (2.67%). The essential oil obtained from the fruits of the 2021 harvest had the following main components (over 2%): anethole (76.15%), fenchone (13.70%), *α*-pinene (2.27%), and estragole (2.05%). Comparison of chemical composition data shows that both samples were high in anethole and low in fenchone. The increased amount of anethole in the essential oil obtained from the fruits of the 2021 harvest and the lower amount of fenchone can be explained by the climatic conditions when the plant was grown. In terms of content of essential components, the essential oils correspond to the data from the literature for oils of sweet fennel. In the variant extraction/derivatization the identified components (% of the composition) in both essential oils are 98.92%. The essential oils do not differ in chemical composition. Their main components (above 2%) were: anethole (74.03-75.20%), fenchone (14.38-15.25%), estragole (2.15-2.80%), and *α*-pinene (1.88-2.39%).

The quantity of the main components in all investigated essential oils differ from the data in the literature: anethole (82%), limonene (6.55%), estragole (3.53%) (Renjie *et al*., 2010); anethole (46.2%), limonene (27.78%), fenchone (12.78%), estragole (6.34%), and *α*-pinene (3.26%) (Shahat *et al*., 2012); anethole (68.53%), estragole (10.42%), and limonene (6.24%) (Diao *et al*., 2014); *trans*-anethole (82.9-87.4%), estragole (4.47-5.87), and limonene (3.64-4.97%) (Najdoska-Bogdanov *et al*., 2015); anethole (1.2-88.4%), estragole (0.2-59.1%), fenchone (1.1-14.7%), and limonene (5.3-15.7%) (Bahmani *et al*., 2016); anethole (69.95%) and fenchone (18.14%) (Wodnicka *et al*. 2019).

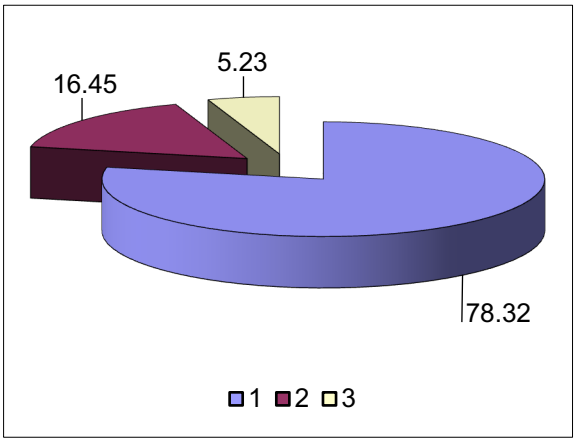
The distribution of the components by functional groups (expressed as percentage of the identified) is presented in Figure 2.

**Table 4.** Chemical composition of essential oils of fennel fruits (% of TIC[1])

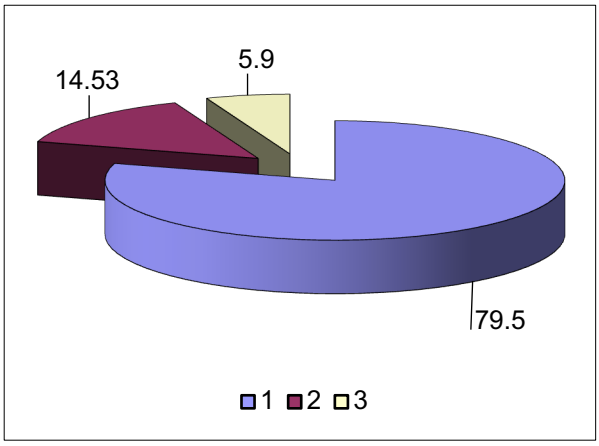
| RT[2] | RI[3] | Compounds | By hydrodistillation | | By extraction/ derivatization | |
|---|---|---|---|---|---|---|
| | | | Harvest 2020 | Harvest 2021 | Harvest 2020 | Harvest 2021 |
| **Monoterpene hydrocarbons** | | | | | | |
| 10.09 | 933 | *α*-Pinene | 1.79±0.16 [a] | 2.27±0.21 [b] | 1.88±0.17 [a] | 2.39±2.22 [b] |
| 10.59 | 947 | Camphene | 0.21±0.02 [a] | 0.24±0.02 [a] | 0.22±0.02 [a] | 0.25±0.02 [a] |
| 11.36 | 968 | Sabinene | 0.08±0.0 [a] | 0.11±0.01 [a] | 0.08±0.0 [a] | 0.11±0.0 [a] |
| 11.51 | 979 | *β*-Pinene | 0.10±0.0 [a] | 0.13±0.01 [a] | 0.10±0.0 [a] | 0.14±0.01 [a] |
| 11.94 | 988 | Myrcene | 0.49±0.04 [b] | 0.36±0.03 [a] | 0.51±0.05 [b] | 0.38±0.03 [a] |
| 12.46 | 1001 | *α*-Phellandrene | 0.25±0.02 [a] | 0.32±0.03 [b] | 0.27±0.02 [a] | 0.34±0.03 [b] |
| 13.11 | 1024 | Limonene | 1.64±0.15 [a] | 1.73±0.16 [b] | 1.72±0.15 [b] | 1.82±0.06 [c] |
| 13.44 | 1033 | *(Z)-β*-Ocimene | 0.07±0.0 [a] | 0.08±0.0 [a] | 0.07±0.0 [a] | 0.09±0.0 [a] |
| 14.04 | 1054 | *γ*-Terpinene | 0.43±0.04 [a] | 0.52±0.04 [b] | 0.45±0.03 [a] | 0.54±0.04 [b] |
| 14.93 | 1073 | Terpinolene | 0.09±0.0 [a] | 0.07±0.0 [a] | - [4] | - |
| **Oxygenated monoterpenes** | | | | | | |
| 13.22 | 1027 | Eucalyptol | 0.10±0.0 [a] | 0.13±0.01 [a] | 0.11±0.01 [a] | 0.14±0.01 [a] |
| 14.52 | 1062 | *(Z)*-Sabinene hydrate | 0.11±0.01 [a] | 0.08±0.0 [a] | 0.12±0.01 [a] | 0.09±0.0 [a] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 15.36 | 1085 | Fenchone | 15.47±1.40 [c] | 13.70±1.20 [a] | 15.25±1.40 [c] | 14.38±1.30 [b] |
| 16.10 | 1114 | endo-Fenchol | 0.09±0.0 [a] | 0.10±0.00 [a] | 0.10±0.0 [a] | 0.11±0.0 [a] |
| 16.30 | 1139 | *(Z)*-Pinene hydrate | 0.08±0.0 [a] | 0.06±0.0 [a] | 0.09±0.0 [a] | 0.07±0.0 [a] |
| 16.84 | 1165 | Camphor | 0.34±0.03 [a] | 0.28±0.02 [a] | 0.35±0.03 [a] | 0.29±0.02 [a] |
| **Phenyl propanoids** | | | | | | |
| 12.95 | 1020 | *p*-Cymene | 0.10±0.01 [a] | 0.08±0.0 [a] | 0.12±0.01 [a] | 0.08±0.0 [a] |
| 18.37 | 1195 | Estragole | 2.67±0.21 [b] | 2.05±0.21 [a] | 2.80±0.22 [b] | 2.15±0.20 [a] |
| 20.03 | 1237 | *o*-Anisaldehyde | 0.15±0.01 [a] | 0.11±0.01 [a] | 0.16±0.01 [a] | 0.12±0.0 [a] |
| 20.43 | 1248 | *p*-Anisaldehyde | 0.46±0.04 [b] | 0.22±0.02 [a] | 0.49±0.04 [b] | 0.23±0.02 [a] |
| 21.15 | 1290 | Anethole | 73.72±7.20 [a] | 76.15±7.50 [b] | 74.03±7.00 [a] | 75.20±7.20 [b] |

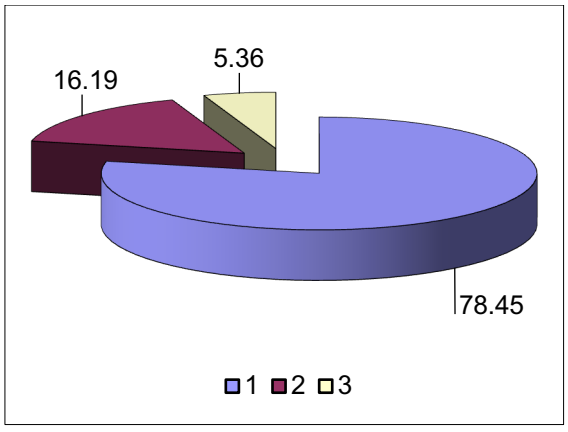
[1] – total ion current; [2] – retention time, min; [3]– retention (Kovat's) index; [4] – not identified; Results: mean value ± standard deviation ($n = 3$); Different letters in the same row indicate significant differences ($p < 0.05$).



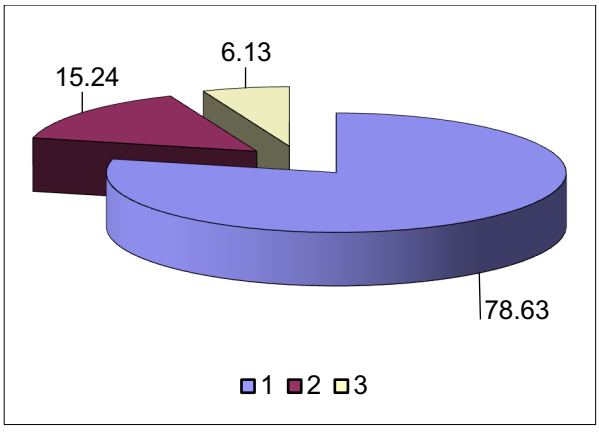

By hydrodistillation, Harvest 2021




By hydrodistillation, Harvest 2021




By extraction/derivation, Harvest 2020




By extraction/derivation, Harvest 2020

**Figure 2.** Distribution of compounds in essential oils from fennel fruits:
1 – phenyl propanoids; 2 – oxygenated monoterpenes; 3 – monoterpene hydrocarbons.

The data show that in variant hydrodistillation phenyl propanoids were the dominant group in both essential oils. The comparative analysis shows that the amount of phenylpropanoids and monoterpene hydrocarbons was higher in the 2021 harvest, and the monoterpene oxygen derivatives was higher in the 2020 harvest.

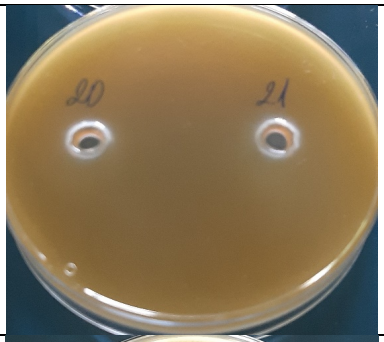
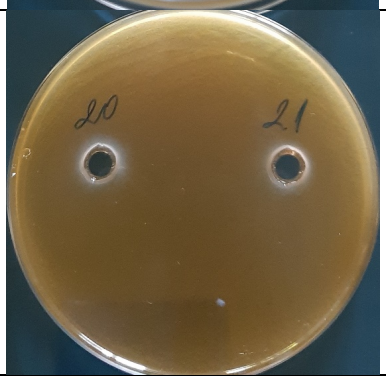
In variant extraction/derivatization the distribution of components in both essential oils by group of compounds does not differ: phenyl propanoids (from 78.32% to 79.50%), followed by oxygenated monoterpenes (from 14.53% to 16.45%), and monoterpene hydrocarbons (from 5.23% to 6.13%). These groups of compounds in all essential oils differ from the literature: monoterpene hydrocarbons (6.20%), oxygenated monoterpenes (11.40%), and phenyl propanoids (74.37%) (Ahmad *et al*., 2018).

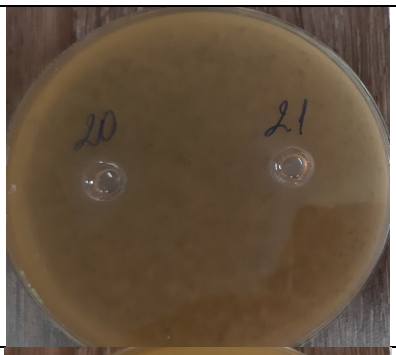
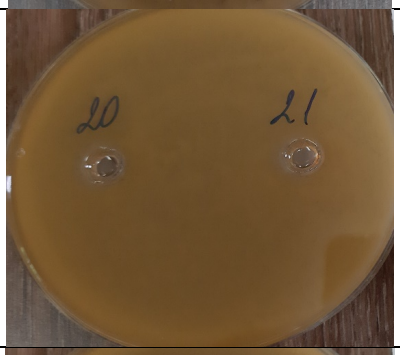
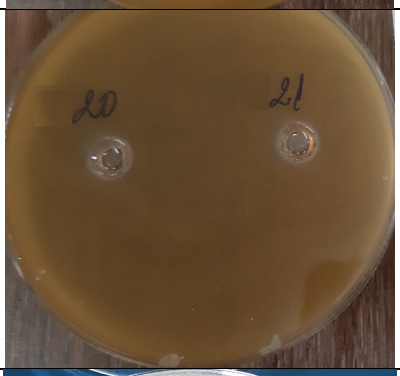
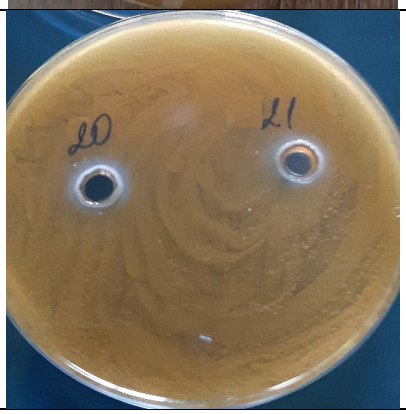
The differences in the aromatic compounds of the investigated essential oils and those from the literature can be both explained by the climatic conditions in different countries.

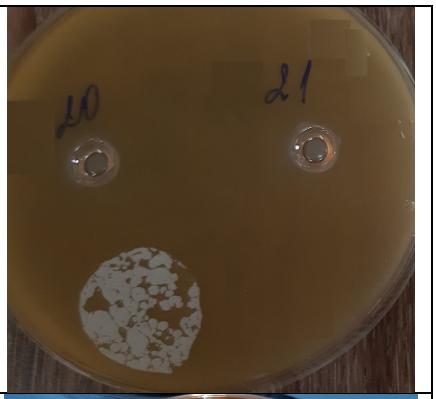
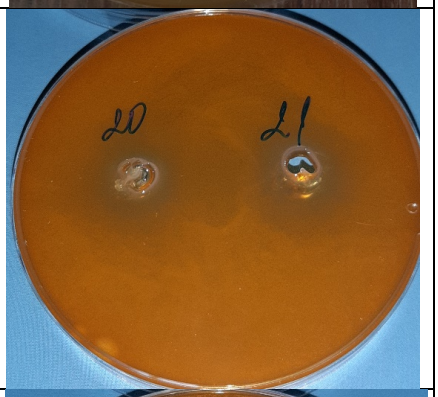
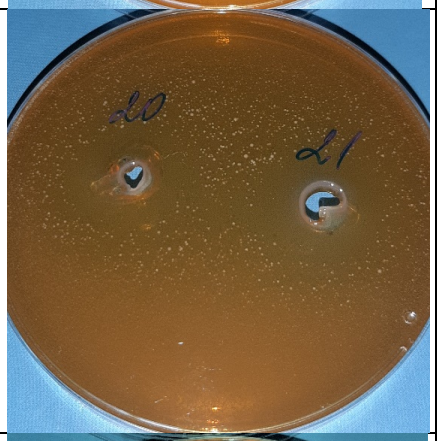
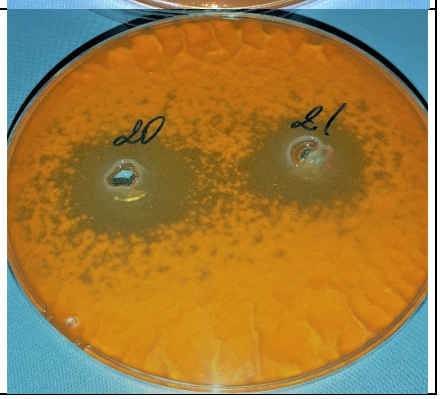
Anethole is characterized by various biological properties – antimicrobial, antioxidant, anti-inflammatory, *etc*. (Ghelardini *et al*., 2001; Freire *et al*., 2005; Tognolini *et al*., 2007; Huang *et al*., 2010; Ponte *et al*., 2012; Giustarini *et al*., 2014; Kim *et al*., 2017). However, anethole taken orally in large quantities has an effect on the central nervous system, which is why essential oils containing it have limited use in the food industry and cosmetics (Poon and Freeman, 2006; Aschenbeck and Hylwa, 2017; Horst *et al*., 2017; Sarkic and Stappen, 2018).

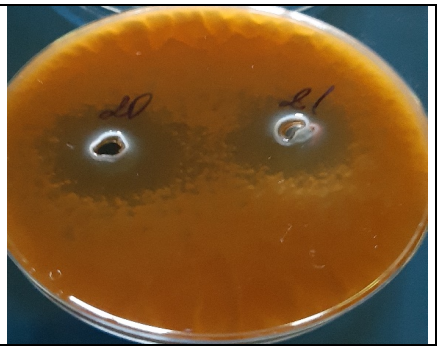
The results for the antimicrobial activity of the essential oils of the fennel fruits are presented in Table 5.

**Table 5.** Antimicrobial activity of essential oils from fennel fruits

| **Tested microorganisms** | **Inhibition zone (mm)** | | |
|---|---|---|---|
| | **Harvest 2020** | **Harvest 2021** | |
| *Staphylococcus aureus* ATCC 6538 | 8.0±0.0 [a] | 8.0±0.0 [a] |  |
| *Staphylococcus epidermidis* ATCC 12228 | 8.0±0.0 [a] | 8.0±0.0 [a] |  |

| | | | |
|---|---|---|---|
| *Bacillus subtilis* ATCC 6633 | 8.0±0.0 [a] | 8.0±0.0 [a] |  |
| *Bacillus cereus* ATCC 10876 | 8.0±0.0 [a] | 8.0±0.0 [a] |  |
| *Escherichia coli* ATCC 8739 | 8.0±0.0 [a] | 8.0±0.0 [a] |  |
| *Pseudomonas aeruginosa* ATCC 9027 | 16.3±0.3 [b] | 14.9±0.4 [a] |  |

| | | | |
|---|---|---|---|
| *Salmonella abony* NCTC 6017 | 8.0±0.0 [a] | 8.0±0.0 [a] |  |
| *Candida albicans* ATCC 10231 | 13.6±0.2 [a] | 13.7±0.5 [a] |  |
| *Saccharomyces cerevisiae* ATCC 9763 | 22.7±0.7 [a] | 21.2±0.3 [a] |  |
| *Aspergillus brasiliensis* ATCC 16404 | 19.0±0.5 [b] | 17.8±0.6 [a] |  |

| | | | |
|---|---|---|---|
| *Fusarium moniliforme* | 17.4±0.4 [a] | 18.3±0.2 [b] |  |

Results: mean value ± standard deviation ($n$ = 3); Different letters in the same row indicate significant differences ($p < 0.05$).

It can be seen that they have an almost identical profile of antimicrobial activity – good against yeasts and molds and weak to none antibacterial activity.

The strongest activity of the oils was observed against the yeast *S. cerevisiae* and the molds *A. brasiliensis* and *F. moniliforme*. The yeast *C. albicans* is weakly sensitive to the tested essential oils. These results are also confirmed by the research of other authors (Javed *et al*., 2012; Atanasova-Pančevska *et al*., 2021).

Among the bacteria tested, only the Gram-negative bacterium *Pseudomonas aeruginosa* was sensitive to the oils. The remaining bacteria are not affected by the two oils.

Research by other authors confirmed the low antibacterial activity of the essential oil of the fennel against *S. aureus*, *L. monocytogenes, B. cereus,* and *Salmonella* sp. (Miguel *et al*., 2010) and against pathogenic and bacteria that cause spoilage of food products (Borotova *et al.*, 2021).

Other studies have found high inhibitory activity of the essential oil against the bacteria *A. baumannii*, *E. coli*, *P. aeruginosa*, *S epidermidis*, and *S. aureus* (Diao *et al*., 2014; Barrahi *et al*., 2020).

The established differences in the antimicrobial activity of the oils are due to differences in their chemical composition, due to the geographical origin of the fennel fruits, differences in the methods of collection, drying and preparation of the essential oil (Bozin *et al*., 2006).

### 3.3. The chemical composition of lipid fractions

The lipid fractions are a viscous mass with a green to dark green color and a specific odor.

In Table 6 it is presented the fatty acid composition of the lipid fraction.

In the variant of extracting the fruits after their distillation the sum of petroselinic + oleic acids predominated in both oils (84.1 and 79.5%, respectively), followed by saturated palmitic (6.5 and 9.5%), the unsaturated linoleic (4.0 and 4.2%) acid, and stearic acid (1.6 and 2.1%). The other fatty quantities are negligible (from 0.1 to 0.7%). The main fatty acid in both samples was C $_{18:1}$. In this case, it is a mixture of isomers that cannot be separated under the conditions of the analysis. In this variant a tendency to increase the content of palmitic acid and decrease of linoleic acid was observed. This was probably due to the possible transformation of these acids under the influence of temperature, resulting in oxidation of the methylene group near the carboxyl. Free radicals were produced, which in subsequent oxidative degradation are cleaved off, resulting in acids with a shorter chain (Gonstone, 1967).

In the variant extraction/derivatization very few fatty acids were identified. The main fatty acid was petroselinic acid (72.7-74.2%), followed by oleic acid (3.4-3.8%), and palmitic acid (2.95-3.2%).

The values of the identified fatty acids differ from the data in the literature: petroselinic acid (74.80%), linoleic acid (12.74%), and palmitic acid (5.34%) (Ahmad *et al*., 2018); linoleic acid (37.01%), linolenic

acid (35.54%), and palmitic acid (17.7%) (Morales *et al*., 2012); petroselinic acid (75.18%), linoleic acid (11.18%), oleic acid (6.15%), and palmitic acid (4.76%) (Cosge *et al*., 2008); ($C_{18:1}$) methyl esters (80.9-83.0%), linoleic acid (10.8-11.9%), and palmitic acid (4.27-5.15%) (Najdoska-Bogdanov *et al*., 2015). The established differences in the amount and fatty acid composition of the studied oils, and those from the literature can be explained both by the varietal characteristic of the fennel and by the climatic conditions of the respective years during which the studied fruits ripened.

The distribution of saturated and unsaturated fatty acids shows that in the extraction version after distillation, unsaturated fatty acids predominated in both oils (89.6 and 85.7%), and the monounsaturated ones were in a greater amount (85.3 and 81.1%) than the poly-unsaturated ones (4.3 and 4.6%). Saturated fatty acids were found to be 10.4% (2020 harvest) and 14.3% (2021 harvest) respectively.

**Table 6.** Fatty acid composition of lipid fraction from fennel fruits (variant extraction after distillation).

| **Fatty acids, %** | | **By extraction after distillation** | | **By extraction/ derivatization** | |
|---|---|---|---|---|---|
| | | **Harvest 2020** | **Harvest 2021** | **Harvest 2020** | **Harvest 2021** |
| **Saturated fatty acids** | | | | | |
| $C_{8:0}$ | Caprylic | 0.3±0.0[a] | 0.3±0.1[a] | - | - |
| $C_{10:0}$ | Capric | -[1] | 0.1±0.0 [a] | - | - |
| $C_{12:0}$ | Lauric | 0.1±0.0 [a] | 0.1±0.0 [a] | - | - |
| $C_{14:0}$ | Myristic | 0.3±0.1 [a] | 0.4±0.0 [a] | - | - |
| $C_{15:0}$ | Pentadecanoic | 0.1±0.0 [a] | 0.2±0.0 [a] | - | - |
| $C_{16:0}$ | Palmitic | 6.5±0.5 [b] | 9.5±0.7 [c] | 2.95±0.2 [a] | 3.2±0.2 [a] |
| $C_{17:0}$ | Margaric | 0.3±0.0 [a] | 0.2±0.0 [a] | - | - |
| $C_{18:0}$ | Stearic | 1.6±0.2 [b] | 2.1±0.1 [c] | 0.3±0.1 [a] | 0.4±0.1 [a] |
| $C_{20:0}$ | Arachidic | 0.7±0.1 [b] | 0.7±0.2 [b] | 0.1±0.0 [a] | 0.1±0.0 [a] |
| $C_{22:0}$ | Behenic | 0.5±0.1 [a] | 0.7±0.2 [a] | - | - |
| **Monounsaturated fatty acids** | | | | | |
| $C_{14:1}$ | Myristoleic | - | 0.1±0.0 [a] | - | - |
| $C_{15:1}$ | Pentadecenoic | 0.2±0.0 [a] | 0.2±0.0 [a] | - | - |
| $C_{16:1}$ | Palmitoleic | 0.2±0.0 [a] | 0.3±0.1 [a] | - | - |
| $C_{17:1}$ | Heptadecenoic | 0.4±0.1 [a] | 0.5±0.1 [a] | - | - |
| $C_{18:1}$ (n-9) | Oleic | - | - | 3.8±0.2 [a] | 3.4±0.2 [a] |
| $C_{18:1}$ (n-12) | Petroselinic | - | - | 72.7±7.0 [a] | 74.2±7.0 [a] |
| $C_{18:1}$ | (Petroselinic + Oleic) | 84.1±8.0 [b] | 79.5±7.8 [b] | - | - |
| $C_{20:1}$ | Gondoic | 0.3±0.0 [a] | 0.3±0.0 [a] | - | - |
| $C_{22:1}$ | Erucic | 0.1±0.0 [a] | 0.2±0.0 [a] | - | - |
| **Polyunsaturated fatty acids** | | | | | |
| $C_{18:2}$ (n-6) | Linoleic | 4.0±0.2 [b] | 4.2±0.3 [b] | 1.6±0.2 [a] | 1.5±0.2 [a] |
| $C_{18:2}$ | *trans*-Linoleic | 0.1±0.0 [a] | 0.1±0.0 [a] | - | - |
| $C_{18:3}$ (n-6) | $\gamma$-Linolenic | 0.1±0.0 [a] | 0.1±0.0 [a] | 0.2±0.1 [a] | 0.2±0.1 [a] |
| $C_{18:3}$ (n-3) | $\alpha$-Linolenic | 0.1±0.0 [a] | 0.1±0.0 [a] | - | - |
| $C_{20:3}$ (n-3) | Eicosatrienoic | - | 0.1±0.0 [a] | - | - |

[1] – not identified; Results: mean value ± standard deviation ($n$ = 3); Different letters in the same row indicate significant differences ($p < 0.05$).

In the variant extraction/derivatization the quantity of saturated fatty acids was (2 times) as low compared to the variant extraction after hydrodistillation. The amounts of the monounsaturated and polyunsaturated fatty acids were comparable. Petroselinic acid, which is the main fatty acid in the lipid fraction, is an important raw material for oleochemical processes and can be easily processed into lauric and adipinic acid. This acid also plays a significant role in the food, chemical, and cosmetics industries (Uitterhaegen *et al*., 2016).

In Bulgaria there is a tradition of growing the fennel fruits. Due to the high content of petroselinic acid, the fruits can be a raw material for its preparation, replacing the fruits of coriander.

Unsaponifiable matter in both lipid fractions is presented in Table 7 and their values were found to be comparable. They contain terpenic (sterols, tocopherols, tocotrienols, carotenoids, *etc.*) and aliphatic (fatty alcohols, saturated and unsaturated hydrocarbons) compounds (Fontanel, 2013). The data show that their content is higher than that in other lipid fractions from maize (2.8%), rape seed (2.0%), grape seed oil (2.0%), and sunflower (1.5%) (Codex Stan 210).

Total sterols in both lipid fractions were 1.17-1.34% and phospholipids were found to be 0.92 and 1.33%. The total amount of sterols in the oil is higher compared to that of other vegetable oils, for example, from sunflower, soybean, cotton, safrole, *etc*. (0.24-0.64 %) (Codex Stan 210). Since numerous studies demonstrate that phytosterols from the lipid fraction lower total and LDL cholesterol levels, therefore the sterols from the lipid fractions from fennel fruits could constitute a sanogenic ingredient in the prevention and treatment of hypercholesterolemia.

Tocopherols are a class of organic compounds that have vitamin E activity, contained in the lipid fraction of various oils - olive, sunflower, soybean, *etc*. Total tocopherols in the fractions were relatively low, but their content in the lipid fraction from the fruits harvest 2020 (197 mg/kg) was higher than in the fraction from fruits harvest 2021 (160 mg/kg). However, their content in both lipid fractions is lower than that of soy (600-3370 mg/kg), corn germ (330-3720 mg/kg) and rapeseed (430-2680 mg/kg) (Codex Stan 210). The biological role of phospholipids in the construction of cell membranes is known, as well as their antioxidant properties (Tlili *et al*., 2011; Küllenberg *et al.*, 2012). Their quantity is higher in the 2021 harvest. Sterol and tocopherol profiles of the examined lipid fractions are given in Table 7. The data show significant differences in individual sterols. The main ones in the 2020 harvest were *β*-sitosterol (61.1%) and stigmasterol (24.6%), and in the 2021 harvest – *β*-sitosterol (39.7%), stigmasterol (41.2%), and $\Delta^5$-avenasterol (13.8%). In the content of *β*-sitosterol, the main sterol is comparable to that of other lipid fractions such as olive, soybean, and sunflower oil (Codex Stan 210). For tocopherols, the values of the individual components are comparable. The main tocopherol in both lipid fractions is *γ*-tocotrienol, followed by α-tocopherol. In composition, the lipid fraction differs from other commonly used, such as sunflower (where *α*-tocopherol predominated), soybean (*γ*- and *δ*-tocopherols were the main components) and corn oils (where *γ*-tocopherol predominated but there were also traces of *α*- and *δ*-tocopherols), and was rather similar to those of sesame oil (where *γ*-tocopherol was also the major one) (Codex Stan 210).

The biological components in the lipid fraction define the fennel fruit as a suitable supplement to the food of humans and animals.

**Table 7.** Biological component, sterol and tocopherols in lipid fraction from fennel fruits.

| Compounds | Harvest 2020 | Harvest 2021 |
|---|---|---|
| **Biologically components** | | |
| Unsaponifable matter, % | 25.7±0.2 [a] | 27.4±0.2 [a] |
| Sterols, % | 1.17±0.12 | 1.34±0.08 [b] |
| Phospholipids, % | 0.92±0.10 [a] | 1.33±0.07 [b] |
| Tocopherols, mg/kg | 197.0±14.0 [d] | 160.0±8.0 [d] |
| **Sterols** | | |
| Cholesterol | 0.4±0.1 [a] | 0.5±0.1 [a] |
| Brassicasterol | 0.1±0.0 [a] | -[1] |
| Campesterol | 2.3±0.1 [a] | 4.1±0.1 [b] |
| Stigmasterol | 24.6±0.3 [a] | 41.2±0.2 [b] |
| $\Delta^7$-Campesterol | 2.1±0.2 [b] | 0.7±0.1 [a] |
| $\beta$-Sitosterol | 61.1±0.4 [b] | 39.7±0.3 [a] |
| $\Delta^5$-Avenasterol | 9.4±0.2 [a] | 13.8±0.2 [b] |
| **Tocopherols** | | |
| $\alpha$-Tocopherol | 41.6±0.2 [b] | 38.2±0.1 [a] |
| $\gamma$-Tocotrienol | 58.4±0.2 [a] | 61.8±0.4 [b] |

[1]- not identified; Results: mean value ± standard deviation ($n$ = 3); Different letters in the same row indicate significant differences ($p < 0.05$).

## 4. Conclusions

As the fennel fruits are widely used in the food industry, in this research we focus at determining the phytochemical composition and biological activity of sweet fennel fruits, cultivated during two harvest years 2020 and 2021 in North Eastern Bulgaria. The content of protein, crude fiber, essential oil, polar and lipid fractions, and mineral elements was determined in the fennel fruits. The polar metabolites are present in saccharides (mono- and di-), sugar alcohols, organic acids, sugar acids, others, and amino acids. Fennel fruits have high content of macro elements potassium, sodium, and magnesium, and low content of toxic elements. The main components of both essential oils are anethole and fenchone. The essential oils have antimicrobial activity, more pronounced against molds and yeasts. The main sitosterols in both lipid fractions are $\beta$-sitosterol and stigmasterol, and major tocopherol is $\gamma$-tocotrienol. Based on the results we achieved, we can conclude that because of its biological components in the lipid fraction and with its balanced composition of mineral elements, the fennel fruits can be used as a suitable supplement to the food of humans and animals.

## 5. References

Ahmad, B., Talou, T., Saad, Z., Hijazi, A., Cerny, M., Kanaan, H., Chokr, A., Merah, O. (2018). Fennel oil and by-products seed characterization and their potential applications. *Industrial Crops and Products,* 111, 92-98.

Akhatou, I., González-Domínguez, R., Recamales, Á. (2016). Investigation of the effect of genotype and agronomic conditions on metabolomic profiles of selected strawberry cultivars with different sensitivity to environmental stress. *Plant Physiology and. Biochemistry,* 101.

Ali, M., Sameeh, A., Abd El-Razik, M. (2016). Gamma-irradiation affects volatile oil constituents, fatty acid composition and antimicrobial activity of fennel (*Foeniculum vulgare*) seeds extract. *Research Journal of Pharmaceutical, Biological and Chemical Science,* 7, 524-531

Anka, Z., Gimba, S., Nanda, A., Salisu. L. (2020). Phytochemistry and pharmacological activities of *Foeniculum vulgare*. *Journal of Pharmacy and Biological Sciences*, 10(1), 1-10.

Annual Hydrometeorological Bulletin (2020, 2021). National Institute of Meteorology and Hydrology, Sofia, Bulgaria.

AOAC (2016). Association of Official Analytical Chemist. *Official methods of analysis*, 20th edn. Washington, DC.

Aschenbeck, K., Hylwa, S. (2017). Brushing your way to allergic contact dermatitis: Anethole allergy. *Dermatitis*, 28(3), 219-220.

Atanasova-Pančevska, N., Kungulovski, D., Najdoska-Bogdanov, M., Bogdanov, J., Stefova, M. (2021). Comparative study of essential oils from fennel fruits and anise fruits: chemical composition and in vitro antimicrobial activity. *Macedonian Journal of Chemistry and Chemical Engineering,* 40( 2), 241-252.

Bahmani, K., Izadi, A., Al-fekaiki, D., Sticklen, M. (2016). Phytochemical diversity of fennel landraces from various growth types and origins. *Agronomy Research*, 14(5), 1530-1547.

Balinova, A., Diakov, G. (1974). On improved apparatus for microdistillation of rose flowers. *Plant Science,* 11, 77-85.

Balouiri, M., Sadiki, M., Koraichi Ibnsouda, S. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis,* 6, 71-79.

Barrahi, M., Esmail, A., Elhartiti, H., C., Nabileh, B., Raja, A., Noraldin, R., Abodolghader, Z., Mohamad, O. (2020). Chemical composition and evaluation of antibacterial activity of fennel (*Foeniculum vulgare* Mill) seed essential oil against some pathogenic bacterial strains. *Caspian Journal of Environmental Sciences,* 18 (4), 295-307.

Baser, K., Buchbaue, G. (2010). Hand Book of Essential Oils: Science, Technology, and Applications. Taylor and Francis Group, LLC CRC Press in an imprint of Taylor and Francis Group, an Informa business.

Benahmed, A., Gasmi, A., Arshad, M., Shanaida, M., Lysiuk, R., Peana, M., Pshyk-Titko, I., Adamiv, S., Shanaida, Y., Bjørklund, G. (2020). Health benefits of xylitol. *Applied Microbiology and Biotechnology,* 104(17), 7225-7237.

Bontpart, T., Marlin, T., Vialet, S., Guiraud, J.-L., Pinasseau, L., Meudec, E., Sommerer, N., Cheynier, V., Terrier, N. (2016). Two shikimate dehydrogenases, VvSDH3 and VvSDH4, are involved in gallic acid biosynthesis in grapevine. *Journal of Expimental Botany,* 67, 3537-3550.

Borotova, P., Galovičova, L., Valkova, V., Ďuranova, H., Vuković, N., Vukić, M., Babošova, M., Kačaniova, M. (2021). Biological activity of essential oil from *Foeniculum vulgare*. *Acta Horticulturae et Regiotecturae,* 24(2), 148-152.

Bozin, B., Mimica-Dukic, N., Simin, N., Anackov, G. (2006). Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of Agricultural and Food Chemistry*, 54(5), 1822-1828.

Bulgarian State Standard-EN 17053:2018. Animal feeding stuffs methods of sampling and analysis – Determination of trace elements, heavy metals and other elements in feed by ICP-MS (multi-method), Sofia, Bulgaria.

Bulgarian State Standard-EN 15763:009. Foodstuffs – Determination of trace elements – Determination of arsenic, cadmium, mercury and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion.

Chhetri, D. (2019). Myo-Inositol and its derivatives: Their emerging role in the treatment of human diseases. *Frontiers in Pharmacology.* 11.

Codex Stan 210. Codex standard for named vegetable oils. Revisions 2001, 2003, 2009. Amendment 2005, 2011.

Conforti, F., Statti, G., Uzunov, D., Menichini, F. (2006). Comparative chemical composition and antioxidant activities of wild and cultivated *Laurus nobilis* L. leaves and *Foeniculum vulgare* subsp. *piperitum* (Ucria) coutinho seeds. *Biological and Pharmaceutical Bulletin*, 29(10), 2056-2064.

Coşge, B., Kiralan, M., Gürbüz, B. (2008) Characteristics of fatty acids and essential oil from sweet fennel (*Foeniculum vulgare* Mill. var. *dulce*) and bitter fennel fruits (*F. vulgare* Mill. var. *vulgare)* growing in Turkey. *Natural Product Research,* 22, 1011-1016.

Dadalioglu, I., Evrendilek, G. (2004). Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas L.*), and fennel *(Foeniculum vulgare*) on common foodborne pathogens. *Journal of Agriculture and Food Chemistry,* 52, 8255-8260.

Damianova, S., Stoyanova, A. (2007). Antimicrobial activity of aromatic products. 14. Extracts from fruits of sweet fennel (*Foeniculum vulgare* Mill. var. *dulce* Mill.) and coriander (*Coriandrum* sativum L.). *Journal of Essential Oil Bearing Plants,* 10(5), 440-445.

Diao, W.-R., Hu, Q.-P., Zhang, H., Xu, J.-G. (2014). Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (*Foeniculum vulgare* Mill.). *Food Control*, 35, 109-116.

Díaz-Maroto, M., Pérez-Coello, M., Esteban, J., Sanz, J. (2006). Comparison of the volatile composition of wild fennel samples (*Foeniculum vulgare* Mill.) from Central Spain. *Journal of Agricultural and Food Chemistry*, 54, 6814-6818.

Dikova, B. (2014). Inhibiting effect of lavender and fennel oils on *Tomato spotted Wilt virus*. *Journal of Balkan Ecology*, 17(4), 369-376.

Dikova, B., Dobreva, A., Djurmanski, A. (2017). Essential oils of lavender and fennel for inhibiting *Tomato spotted Wilt virus* in pepper plants. *Acta Microbiologica Bulgarica*, 33(1), 36-43.

Ehsanipour, A., Razmjoo, J., Zeinali, H. (2012). Effect of nitrogen rates on yield and quality of fennel (*Foeniculum vulgare* Mill.) accessions. *Industrial Crops and Products,* 35, 121-125.

Fontanel, D. (2013). Unsaponifiable Matter in Plant Seed Oils. Springer, London, UK.

Freire, R., Morais, S., Catunda-Junior, F., Pinheiro, D. (2005). Synthesis and antioxidant, anti-inflammatory and gastroprotector activities of anethole and related compounds, *Bioorganic and Medicinal Chemistry*, 13(13), 4353-4358.

Ghelardini, C., Galeotti, N., Mazzanti, G. (2001). Local anaesthetic activity of monoterpenes and phenylpropanes of essential oils. *Planta Medica*, 67, 564-566.

Gonstone, F. (1967). An introduction the chemistry and biochemistry of fatty acids and their glycerides, Charman & Hall Ltd., London.

Guil, J., Martinez, J., Isasa, M. (1998). Mineral nutrient compasition of edible wild plants. *Journal of Food Composition and Analysis,* 11, 322-328.

Giustarini, D., Fanti, P., Sparatore, A., Matteucci, E., Rossi, R. (2014). Anethole dithiolethione lowers the homocysteine and raises the glutathione levels in solid tissues and plasma of rats: a novel non-vitamin homocysteine-lowering agent. *Biochemical Pharmacology*, 89(2), 246-254.

Gulfraz, M., Arshad, M., Uzma, N., Kanwal, Shabir, K. (2005). Composition in various bioactive compounds of leaves and seeds of *Foeniculum vulgare* Mill. *Ethnobotanical Leaflets*, 9, 6-14.

He, W., Huang, B. (2011). A review of chemistry and bioactivities of a medicinal spice: *Foeniculum vulgare*, *Journal of Medicinal Plants Research*, 5(16), 3595-3600.

Horst, N., Leysen, J., Mellaerts, T., Lambert, J., Aerts, O. (2017). Allergic contact cheilitis from anethole-containing toothpastes: a practical solution. *Journal of the European Academy of Dermatology and Venerology*, 31(8), 374-375.

Huang, Y., Zhao, J., Zhou, L., Wang, J., Gong, Y., Chen, X., Guo, Z., Wang, Q., Jiang, W. (2010). Antifungal activity of the essential oil of *Illicium verum* fruit and its main component *trans*-Anethole. *Molecules*, 15(11), 7558-7569.

Hussein, K., Molnár, T., Pinter, R., Toth, A., Ayari, E., Friedrich, L., Dalmadi, I., Kiskó, G. (2020). *In vitro* antimicrobial activity of plant active components against *Pseudomonas lundensis* and *Listeria monocytogenes*. *Progress in Agricultural Engineering Sciences,* 16(S2), 163-172.

ISO 659:2014. *Oilseeds. Determination of oil content* (Reference method).

ISO 10540-1:2014. Animal and vegetable fats and oils. Determination of phosphorus content. Part 1: Colorimetric method.

ISO 12966-1:2014. *Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters – Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters*.

ISO 12966-2:2017. *Animal and vegetable fat and oils. Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids*.

ISO 18609:2000. *Animal and vegetable fats and oils. Determination of unsaponifiable matter. Method using hexane extraction*.

ISO 12228-1:2014. *Animal and vegetable fats and oils. Determination of individual and total sterols contents. Gas chromatographic method*.

ISO 9936:2016. *Animal and vegetable fats and oils. Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography*.

Ivanov, S., Bitcheva, P., Konova, B. (1972). Méthode de détermination chromatographyque et colorimétrique des phytosterols dans les huiles végétales et les concentres steroliques. *Revue Francaise des Corps Gras*, 19, 177-180.

Jamwal, N., Kumar, S., Rana, A. (2013). Phytochemical and pharmacological review on *Foeniculum vulgare*. *Pharma Science Monitor*, 4(3), 327-341.

Javed, S., Mushtaq, S., Khokhar, I., Ahmad, R., Haider, M. (2012). Comparative antimicrobial activity of clove and fennel essential oils against food borne pathogenic fungi and food spoilage bacteria. *African Journal of Biotechnology*, 11(94), 16065-16070.

Kaur, G., Arora, D. (2010). Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbeliferae – Current status. *Journal of Medicinal Plants Research,* 4(2), 87-94.

Kim, K., Lee, H., Seol, G. (2017). Anti-inflammatory effects of *trans*-anethole in a mouse model of chronic obstructive pulmonary disease. *Biomedicine and Pharmacotherapy*, 91, 925-930.

Küllenberg, D., Taylor, L., Schneider, M., Massing, U. (2012). Health effects of dietary phospholipids. *Lipids Health and Disease,* 11, 3.

Miguel, M., Cruz, C., Faleiro, L., Simões, M., Figueiredo, A., Barroso, J., Pedro, L. (2010). *Foeniculum vulgare* essential oils: chemical composition, antioxidant and antimicrobial activities. *Natural Product Communications,* 5, 319-328.

Mohsenzadeh, M. (2007). Evaluation of antibacterial activity of selected Iranian essential oils against *Staphylococcus aureus* and *Escherichia coli* in nutrient broth medium. *Pakistan Journal of Biological Sciences,* 10, 3693-3697.

Morales, P., Ferreira, I., Carvalho, A., Sanchez-Mata, M., Camara, M., Tardio, J. (2012). Fatty acids profiles of some Spanish wild vegetables. *Food Science and Technology International*, 18(3), 281-290.

Moser, B., Zheljazkov, V., Bakota E., Evangelista, R., Gawde, A., Cantrell, C., Winkler-Moser, Hristov, A., Astatkiee, T.,

Jeliazkova, E. (2014). Method for obtaining three products with different properties from fennel (*Foeniculum vulgare*) seed. *Industrial Crops and Products*, 60, 335-342.

Najdoska-Bogdanov, M., Bogdanov, J., Stefova, M. (2015). Simultaneous determination of essential oil components and fatty acids in fennel using gas chromatography with a polar capillary column. *Natural Product Communications,* 10(9), 1619-1626.

Ozcan, M., Akbulut, M. (2007). Estimation of minerals, nitrate and nitrite contents of 9 medicinal and aromatic plants used as spices, condiments and herbal tea. *Food Chemistry,* 106, 852-858.

Ponte, E., Sousa, P., Rocha, M., Soares, P., Coelho-de-Souza, A., Leal-Cardoso, J., Assreuy, A. (2012). Comparative study of the anti-edematogenic effects of anethole and estragole. *Pharmacology Report*, 64, 984-990.

Poon, T., Freeman, S. (2006). Cheilitis caused by contact allergy to anethole in spearmint flavoured toothpaste. *Australasian Journal of Dermatology*, 47(4), 300-301.

Rather, M., Dar, B., Sofi, S., Bhat, B., Qurishi, M. (2012). Foeniculum vulgare: a comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. *Arabian Journal of Chemistry*, 9, S1574-S1583.

Renjie, L., Zhenhong, L., Shidi, S. (2010). GC-MS analysis of fennel essential oil and its effect on microbiology growth in rats' intestine. *African Journal of Microbiology Research,* 4, 1319-1323.

Sarkic, A., Stappen, I. (2018). Essential oils and their single compounds in cosmetics. A Critical Review. *Cosmetics*, 5(11), 1-21.

Shahat, A., Hammouda, F., Shams, K., Saleh, M. (2012). Comparative chemical analysis of the essential oil of wild and cultivated fennel (*Foeniculum vulgare* Mill). *Journal of Essential Oil Bearing Plants*, 15(2), 314-319.

Tlili, N., Trabelsi, H., Renaud, J., Khaldi, A., Mayer, P., Saida, T. (2011). Triacylglycerols and phospholipids composition of caper seeds (*Capparis spinosa*). *Journal of the American Oil Chemists Society,* 88, 1787-1793.

Tognolini, M., Ballabeni, V., Bertoni, S., Bruni, R., Impicciatore, M., Barocelli, E. (2007). Protective effect of *Foeniculum vulgare* essential oil and anethole in an experimental model of thrombosis. *Pharmacology Research*, 56(3), 254-260.

Uitterhaegen, E., Sampaio, K., Delbeke, E., De Greyt, W., Cerny, M., Evon, P., Merah, O., Talou, T., Stevens, S. (2016). Characterization of French coriander oil as source of petroselinic acid. *Molecules*, 21, 1202.

Ullah, R., Khader, J., Hussain, I., Mohamed, N., Elsalam, A., Talha, M., Khan, N. (2012). Investigation of macro and micro-nutrients in selected medicinal plants. *African Journal of Pharmacy and Pharmacology,* 25, 1829-1832.

Unal, H., Izli, N., Kacar, O., Goksu, E. (2013). Physical and nutritional properties of fennel seed. *Journal of Food, Agriculture & Environment*, 11(3&4), 6-11.

Wodnicka, A., Huzar, E., Dzięcioł, M., Krawczyk, M. (2019). Comparison of the composition and fungicidal activity of essential oils from fennel fruits cultivated in Poland and Egypt. *Polish Journal of Chemical Technology*, 21(2), 38-42.

Yaldiz, G., Çamlica, M. (2019). Variation in the fruit phytochemical and mineral composition, and phenolic content and antioxidant activity of the fruit extracts of different fennel (*Foeniculum vulgare* L.) genotypes. *Industrial Crops and Products*, 142(1), 1-14.

Zheljazkov, V., Horgan, T., Astatkie, T., Schlegel, V. (2013). Distillation time modifies essential oil yield, composition, and antioxidant capacity of fennel (*Foeniculum vulgare* Mill.). *Journal of Oleo Science,* 62(9), 665-672.

**Acknowledgements**

The authors acknowledge the support by the Science Fund of the University of Ruse "A. Kanchev", Branch-Razgrad, project No FRz-01/22.