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Comparative chemical composition of dill fruits of different origins

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Abstract

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The chemical composition of dill fruits (*Anethum graveolens* L.) originating in Bulgaria, France and Romania was determined. The composition of the lipid fraction and its fatty acid composition are determined. The fruits of France are the richest of essential oil (5.0%).The amount of protein ranges from 19.6 to 20.2% for fruits of different origins. The mineral composition was identified; the highest was calcium content (0.8-0.9%) of macroelements, and – iron (94.3 – 102.2 mg/kg) of microelements.

Keywords: Anethum graveolens L.; fruits; chemical composition

Introduction

Dill (*Anethum graveolens* L.) is an annual herbaceous plant of the Apiaceae family, which is grown in various countries of Central Asia and Europe, in the south of Ukraine and Russia. In Bulgaria it is found throughout the country, but cultivates mainly in the northern part. The dill is widely used spices in the food industry and herb, used in folk medicine, from which the fruits and the over ground part are used (Georgiev & Stoyanova, 2006).

It has been found that fruits have a rich chemical composition which varies according to the origin of the raw material:

According to Altameme et al. (2017), essential oils, fats, proteins (15.68%), carbohydrates (36%), fiber (14.80%), ash (9.8%), furanocoumarin, polyphenol, and mineral are involved in the composition of *A. graveolens*.

Badar et al. (2008) investigated oil originating in Pakistan and determined the following main fatty acids: linolenic (45.13%), oleic (37.05%), capric (8.97%), palmitic (4.66%), and stearic acid (3.86%).

The amount of lipid fractionin the fruits is up to 20%, the major fatty acids are petroselinic (79.91%), linoleic (10.80%), and palmitic (5.79%) acids (Nguyen et al., 2015).

Analyzing of fruit oil from Bulgaria, Zlatanov (1994) found a 2.3% phospholipid content in the lipid fraction. In a further study Zlatanov& Ivanov (1995) reported 17.2% lipid fraction from dill fruits with 0.3% sterol content. The main individual sterols are stigmasterol (28.9-29.0%), β-sitosterol (41.3-41.8%), and Δ^7-stigmasterol (13.9-14.6%). Fruits contain essential oil with general components limonene (about 40%) and (+)-carvone (about 60%) (Altameme et al., 2017; Chahal et al., 2017; Dimov et al., 2017; Jirovetz et al., 2003; Radulescu et al., 2010; Said Al Ahl et al., 2015a; 2015b; Yili et al., 2006).

The fruits contains the following mineral elements: K (66-103%), Na (83-98%), Ca (78-102%), Mg (80-106%), Fe (87-94%), P (91-98%), Zn (68-97%), Cu (75-95%), Cd (78-96%), and Pb (86-104%) (Özcan, 2004; Słupski et al., 2005).

Lipid transfer protein designated as Ag-LTP is isolated from dill, and its properties are studied (Melnikova et al., 2016).

In our country, the dill is grown mainly to produce essential oil of herb and fruits. Bulgarian, French and Romanian seeds are used for sowing.

Due to the wide variety in the chemical composition of dill fruits and opportunities for their more complete use, the purpose of this paper is a comparative analysis of the chemical composition of raw material of different origins.

Materials and Methods

Dill fruits (*Anethum graveolens* L.) were purchased from local markets in Yambol, from Bulgaria, France and Romania.

Before analysis the fruit were finely ground in a laboratory mill to a particle size of 0.05–0.10 cm.

The fruits moisture was determined by drying up to constant weight at 105°*C (Russian Pharmacopoeia, 1990).*

Essential oil

The essential oil was isolated by hydrodistillation for 3 h in a laboratory glass apparatus of the British Pharmacopoeia, modified by Balinova & Diakov (1999). The oil obtained was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis. The essential oil yields are represented on absolute dry weight basis.

Lipid fraction

The lipid fraction is obtained from air-dried fruits at room temperature (22.0±0.5°C) after distillation by extraction with n-hexane in a Soxhlet apparatus for 8 h. (ISO 659:2014).

Fatty acids. The total fatty acid composition of the lipid fraction was determined by gas chromatography (GC) (ISO 12966-1:2014). Fatty acid methyl esters (FAMEs) were prepared by transesterification of the oils with sulfuric acid in methanol (ISO 12966-2:2017). Determination of FAMEs was performed on HP 5890 gas chromatograph equipped with a 75 m × 0.18 mm × 25 μm (film thickness) capillary Supelco column (SP™ – 2560, Fused silica Capillary Column) and a flame ionization detector. The column temperature was programmed from 140°C (hold 5 min), at 4°C/min to 240°C (hold 3 min); the injector and detector temperatures were set at 250°C. Identification was performed by comparison of the retention times with those of a standard mixture of FAME (Supelco, USA 37 comp. FAME mix) subjected to GC under identical experimental conditions.

Tocopherols. Tocopherols were determined directly in the lipids by HPLC analysis on a Merck-Hitachi (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column and fluorescent detector Merck-Hitachi F 1000 (ISO 9936:2016). The operating conditions were mobile phase of n-hexane: dioxane, 96:4 (v/v) and flow rate 1 mL/min, excitation 295 nm, emission 330 nm. 20 μL 1% solution of crude oil in n-hexane were injected. Tocopherols were identified by comparing the retention times with those of authentic individual ones. The tocopherol content was calculated on the base of tocopherol peak areas in the sample vs. tocopherol peak area of standard tocopherol solution.

Sterols. The unsaponifiable lipid fraction was determined by weighting after saponification of the lipids and extraction with n-hexane (ISO 18609:2000) and taking into consideration ISO 12228-1:2014.

Protein content

The protein content is determined in the dried grain according to the method of AOAC 976.06 (1990) with a UDK 152 System (VelpScientiffica, Italy).

Amino acids composition

For the hydrolysis of the protein to free amino acids, 300 mg of dried fruits were put in a glass ampule with 5 mL 6N HCl solution. The ampule was thoroughly sealed and left in a drying chamber at 105°C for 24 h. The ampule content was transferred to a crystallizer and dried in a vacuum chamber at 40-50°C. After evaporation of the water, the residue was fully diluted in 10 mL 20 mM HCl. The solution was filtered through a paper filter, and 20 μL of the collected filtrate were derivatized with AccQ-Fluor kit (WATO52880, Waters Corporation, USA). Initially, 60 μL of AccQ-Fluor borate buffer were added to the filtrate and homogenized. Then, 20 μL of AccQ-Fluor reagent were added, and the sample was homogenized again for 30 s. Before injection, the solution was heated in a water bath at 55°C. The resulting AccQ-Fluor amino acid derivatives were separated by an ELITE LaChrome HPLC (Hitachi) equipped with a DAD and a reverse phase C 18 AccQ-Tag (3.9 mm x 150 mm). The volume of the injected sample was 20 μL, and the elution was made with a gradient system of two mobile phases: A-buffer (WATO52890, Waters) and B – 60% acetonitrile. Amino acids were detected at 254 nm and column temperature 37°C.

Mineral analysis

The mineral composition (except of phosphorus) was determinedby AAC Perkin Elmer sampler 380. The sample is dried in a dry or wet manner and dissolved in acid to obtain a solution with an optimum concentration of the elements. It is atomized in a flame of air-acetylene at a temperature of 2000 – 3000°C. The absorption (optical density) is determined and calculates the concentration using a standard curve.

Phosphorus was determined by the method of Gerike and

Kurmis and measuring the optical density by a wave length of 470 nm (Sandev, 1979).

Statistical analysis

The measurements were performed in triplicate. The results were presented as the mean value of the individual measurements with the corresponding standard deviation (SD), using Excel (Microsoft Inc., USA).

Results and Discussion

The chemical characteristics of dill fruits have been shown in Table 1. The obtained results showed that there were differences in the chemical composition of the samples may be due to the environmental conditions under which the plant has grown. All dill fruits were characterized by higher essential oil, lipid fraction and protein content. The 100% difference is explained by the presence of other substances, such as ash, in the raw material, and other substances (cellulose, carbohydrate, polyphenols, *etc.*), which were not defined in our work. Overall, dill essential oils content in this study was within the range of essential oil content reported previously for dill fruit from other authors. For example, essential oil was previously reported to be 1.75–4.0% (Altameme et al., 2017; Chahal et al., 2017; Said Al Ahl et al., 2015a; Yili et al., 2006). This relatively wide range for dill fruit essential oil content may be due to the different climatic conditions where the plants were growing and to the plant parts processed.

The results for the dill fruit composition of the lipid fraction are in unaccordance with the results reported earlier (Nguyen et al., 2015; Popov & Ilinov, 1986).

The content of the protein is confirmed with the data from literature (Altameme et al., 2017).

Table 1. Chemical composition of fruits from dill

Composition	Bulgaria	France	Romania
Moisture, %	10.6 ± 0.10	10.5 ± 0.10	29.7 ± 0.29
Essential oil, %	3.4 ± 0.03	5.0 ± 0.04	2.6 ± 0.02
Lipid fraction, %	6.1 ± 0.05	5.1 ± 0.04	6.6 ± 0.06
Protein, %	20.2 ± 0.20	19.6 ± 0.19	20.1 ± 0.20

The extracted lipid fraction was observed to be a yellow liquid with specific odor.

The content of their biologically active components is presented in Table 2. The data showed that unsaponifiables and the tocopherols content in the lipid fraction from the Bulgarian fruits were higher.

The sterol content in all samples was similar to that of most plant oils, *i.e.* corn, sunflower, safflower, in which the respective quantities were 0.4-0.9% (Popov & Ilinov, 1986).

Table 2. Lipid fraction composition

Compounds	Bulgaria	France	Romania
Unsaponifiables, %	21.9± 0.18	14.2± 0.09	8.4± 0.01
Sterols, %	0.7± 0.00	0.3± 0.00	0.9± 0.00
Phospholipids, %	5.7± 0.05	4.5± 0.04	4.2± 0.04
Tocopherols, mg/kg	130.0± 1.12	158.0± 1.45	33.0± 0.30

The fatty acid composition of the lipid fraction is presented in Table 3. The data show that 12 fatty acids were determined in the lipid fraction from the samples, constituting 100% of the total oil content. In all samples the main fatty acids were oleic and palmitic. The ratio of unsaturated and saturated fatty acids is presented in Table 3. These results are confirmed with the data by Zlatanov & Ivanov (1995), but are not in agreement with the data reported by Badar et al. (2008).

The tocopherol composition of the lipid fraction has been presented in Table 4. The data shows that the content is different, explained by the origin of the fruits:α- and γ-tocopherol predominate in the fraction of Bulgarian fruits, α-, γ- and δ-tocopherol in the fraction of French fruits, and α-tocopherol in the fraction of Romanian fruits. The quantity of tocopherols in the examined oils was found to be similar than that of other common oils

Table 3. Fatty acid composition of the lipid fraction

Fatty acids, %		Bulgaria	France	Romania
$C_{10:0}$	Capric	-*	0.6 ± 0.00	0.2 ± 0.00
$C_{12:0}$	Lauric	0.3 ± 0.00	1.2 ± 0.01	nd
$C_{14:0}$	Myristic	0.3 ± 0.00	0.2 ± 0.00	0.2 ± 0.00
$C_{14:1}$	Myristoleic	0.1 ± 0.00	nd	0.1 ± 0.00
$C_{15:0}$	Pentadecanoic	0.1 ± 0.00	nd	0.1 ± 0.00
$C_{16:0}$	Palmitic	5.3 ± 0.05	5.8 ± 0.05	5.6 ± 0.05
$C_{16:1}$	Palmitoleic	0.1 ± 0.00	nd	0.1 ± 0.00
$C_{17:0}$	Margaric	0.1 ± 0.00	nd	0.2 ± 0.00
$C_{18:0}$	Stearic	0.7 ± 0.00	nd	0.9 ± 0.00
$C_{18:1}$	Oleic	89.7 ± 0.80	90.7 ± 0.87	91.0 ± 0.88
$C_{18:2}$	Linoleic	3.3 ± 0.03	1.4 ± 0.01	1.6 ± 0.01
$C_{18:3}$	Linolenic	nd	0.1	nd
Saturated fatty acids %		6.8	6.8	1.9
Unsaturated fatty acids,%		93.2	93.2	92.2
Monounsaturated fatty acids, %		89.9	89.9	90.7
Polyunsaturated fatty acids, %		3.3	3.3	1.5

* not determined

(CODEX STAN 210-1999). Tocopherols are a class of organic chemical compounds, many of which have vitamin E activity, where the main dietary sources are olive and sunflower oils, soybean and corn oil (Popov & Ilinov, 1986).

Table 4. Tocopherol and sterol composition of the lipid fraction

Compounds	Bulgaria	France	Romania
Content of tocopherols, % (w/w)			
α-Tocopherol	66.1 ± 0.60	28.0 ± 0.26	100.0 ± 0.98
β-Tocopherol	trace**	nd	nd
γ-Tocopherol	33.9 ± 0.32	37.0 ± 0.36	nd
δ-Tocopherol	nd*	35.0 ± 0.32	nd
Content of sterols, % (w/w)			
Cholesterol	18.3 ± 0.15	12.1 ± 0.11	10.2 ± 0.10
Campesterol	nd*	2.7 ± 0.02	nd
Stigmasterol	29.9 ± 0.21	40.7 ± 0.41	25.8 ± 0.22
β-Sitosterol	47.4 ± 0.44	40.4 ± 0.41	48.4 ± 0.46
Δ^5-Avenasterol	4.4 ± 0.04	4.1 ± 0.04	15.6 ± 0.12

* not determined,** under 0.5%

Sterols were present in the so-called non-saponificated part of the lipid fraction. The individual sterol composition of the lipid fraction has been presented in Table 4. β-Sitosterol, stigmasterol, and cholesterol predominated in the sterol fraction. The data demonstrated that regarding its sterol content and composition, dill oil was similar to the findings for other seed oil (CODEX STAN 210-1999).

Table 5. Amino acid composition of the raw materials

Amino acids	Content, mg/100 g sample		
	Bulgaria	France	Romania
Asp	24.3 ± 0.20	8.9 ± 0.08	35.9 ± 0.30
Ser	18.2 ± 0.17	7.1 ± 0.07	15.8 ± 0.15
Glu	18.0 ± 0.15	26.8 ± 0.25	11.7 ± 0.10
Gly	5.9 ± 0.050	2.9 ± 0.02	6.5 ± 0.06
His	21.2± 0.20	19.7 ± 0.19	18.1 ± 0.17
Arg	11.4 ± 0.10	7.3 ± 0.07	12.9 ± 0.11
Thr	9.8 ± 0.09	5.1 ± 0.05	8.2 ± 0.08
Ala	16.8 ± 0.15	9.0 ± 0.09	15.9 ± 0.15
Pro	12.7 ± 0.12	6.7 ± 0.06	11.9 ± 0.10
Cys	2.4 ± 0.02	0.5± 0.00	4.1 ± 0.04
Tyr	9.0 ± 0.08	4.6 ± 0.04	11.0 ± 0.10
Val	11.2 ± 0.10	5.8 ± 0.05	9.6 ± 0.09
Met	3.2 ± 0.03	1.4 ± 0.01	2.0 ± 0.01
Lys	14.3 ± 0.13	7.4 ± 0.07	12.4 ± 0.11
Ile	12.4 ± 0.11	6.3 ± 0.05	11.4 ± 0.11
Leu	1.9 ± 0.01	1.0 ± 0.00	1.7 ± 0.01
Phe	13.7 ± 0.12	8.0 ± 0.07	13.4 ± 0.13

The amino acid composition of the protein fraction has been presented in Table 5. The data show that fruits from Bulgaria and Romania have a close amino acid composition with the highest content of the following amino acids: aspartic acid, histidine, and serine. In the fruits from France the highest is the content of glutamic acid and histidine. The content of the limiting amino acids leucine and threonine is about twice lower in the fruits from France than in the fruits from Bulgaria and Romania. The differences can be explained by the origin of the studied fruits.

Table 6 presents the mineral composition of the fruits, and the obtained results do not differ significantly from the literature data (Słupski et al., 2005). The data show that, in terms of protein and mineral content, dried dill fruits after distillation are approaching the groups of green fodder, silage, hay, and industrial grain waste (Todorov et al., 2007).

Table 6. Mineralcompositionofthe fruits from dillof different origins

Mineral composition	Bulgaria	France	Romania
Ca, %	0.9 ± 0.00	0.9± 0.00	0.8± 0.00
P, %	0.6 ± 0.00	0.5± 0.00	0.5± 0.00
Mg, mg/kg	3233.9± 31.80	2978.8± 28.70	2700.8± 26.50
Zn, mg/kg	47.7 ± 0.45	52.8 ± 0.51	37.7 ± 0.37
Mn, mg/kg	24.6 ± 0.23	24.9 ± 0.24	20.8 ± 0.19
Fe, mg/kg	102.2± 1.00	94.9 ± 0.93	94.3 ± 0.93
Cu, mg/kg	12.4 ± 0.11	12.4 ± 0.11	8.4 ± 0.08

The differences in chemical composition between the present investigation and the reported data can be explained by the origin of the raw material.

Conclusions

Dill fruits originating in Bulgaria, France and Romania have a comparable chemical composition close to that of the literature. The richest of essential oils are the fruits of France (5.0%); of lipids – the fruits from Romania (6.6%) and of proteins – those from Bulgaria (20.2%).

Based on the results, dill fruits could be established as a potential nonconventional source for the isolation of lipid fraction and protein, with possibilities for application in food, cosmetics, pharmaceutical and other products, and their studying is a potential subject for future research.

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