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GC-MS ANALYSIS OF ESSENTIAL OILS ISOLATED FROM FRUITS OF CHOSEN HOT PEPPER (*CAPSICUM ANNUUM* L.) CULTIVARS

ANALIZA GC-MS OLEJKÓW ETERYCZNYCH WYIZOLOWANYCH Z OWOCÓW WYBRANYCH ODMIAN PAPRYKI OSTREJ (*CAPSICUM ANNUUM* L.)

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Streszczenie. Ostry smak owoców *Capsicum* jest wynikiem obecności grupy związków chemicznych (alkaloidów) zwanych kapsaicynoidami, wśród których kapsaicyna i dihydrokapsaicyna odpowiadają za 90% całkowitej ostrości. Owoce pieprzowca rocznego zawierają również niewielką ilość olejku eterycznego, który nadaje im charakterystyczny zapach i aromat. Określono skład chemiczny olejków eterycznych otrzymanych z owoców trzech odmian papryki ostrej: 'Wulkan' (odmiany polskiej), 'Padron' (odmiany hiszpańskiej) i 'Trakijska Shipka' (odmiany bułgarskiej). Olejki eteryczne wyizolowano z owoców papryki poprzez destylację z parą wodną i analizowano, stosując chromatografię gazową sprzężoną ze spektrometrią mas (GC-MS). Kwas palmitynowy (11,98 i 8,96% odpowiednio w roku 2010 i w roku 2011), pentadekanal (6,46 i 13,70%), furfural (4,02 i 1,28%), 1-nonadecen (3,67 i 5,34%), 2-metoksy-4-winylofenol (3,26 i 1,54%) oraz kwas linolowy (2,23 i 2,16%) stanowiły dominujące składniki olejku odmiany 'Wulkan'. W olejku eterycznym odmiany 'Padron' najwięcej było kwasu palmitynowego (8,45 i 12,52%), furfuralu (6,81 i 3,32%), benzacetaldehydu (4,28 i 1,55%), 2-acetylopirolu (2,31 i 1,48%) oraz 2-metoksy-4-winylofenolu (2,00 i 3,10%). Również kwas palmitynowy (10,00 i 11,17%), pentadekanal (9,04 i 11,81%), 1-nonadecen (6,17 i 10,72%), furfural (6,10 i 2,60%), a także nonadekan (3,99 and 5,19%) oraz 2-metoksy-4-winylofenol (2,82 i 1,77%) dominowały w olejku eterycznym odmiany 'Trakijska Shipka'.

Key words: *Capsicum annuum* L., cultivar, essential oil composition, GC-MS, hot pepper, water--steam distillation.
Słowa kluczowe: *Capsicum annuum* L., destylacja z parą wodną, GC-MS, odmiana, papryka ostra, skład olejku eterycznego.

INTRODUCTION

Hot pepper or chili, which belongs to the plant genus *Capsicum* and to the family *Solanaceae* has been used since ancient times to prepare spicy foods (Govindarajan 1985), as well as for treating asthma, coughs, sore throat, toothache and other ailments (Khan and Abourashed 2010). Nowadays, many different varieties of the genus *Capsicum* are widely cultivated and used all over the world due to the combination of color, taste and nutritional values of its fruits (Kouassi and Koffi-Nevry 2012).

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Fresh pepper fruits are an excellent source of vitamins C, E and B_6, carotenoids, carbohydrates, phenols, flavonoids, pungent principles as well as minerals like potassium, sodium, calcium, iron, copper and manganese (Pruthi 2003; Agrawal and Chakrabarti 2010). They can be eaten raw (salads) or cooked (baked dishes, salsa, pizza), used as spices in dried and powdered form (the fruits with seeds are usually used), or processed into oleoresins (Govindarajan 1986).

Capsicum oleoresins are prepared industrially by organic solvent extraction of the dried pepper fruits and the subsequent removal of the solvent (Jaren-Galan et al. 1999). The obtained concentrates contain a complex mixture of essential oils, waxes, carotenoid pigments and the capsaicinoids. They are commonly used in the food (as natural red colorant for soups, sauces, snacks, meat products, gelatins) and pharmaceutical (plasters, pain-balms, liniments) industries (Govindarajan 1986; Hui 2006).

Capsaicinoids possess antioxidant, antimicrobial, antimutagenic, anti-inflammatory and anti-tumoral properties (Henderson and Hendersor 1992; Cichewicz and Thorpe 1996; Ejechi et al. 1999; Prasad et al. 2006; Suhaj 2006). They are also responsible for the hot flavor and intense burning sensation associated with consumption of hot pepper (Suzuki et al. 1980; Govindarajan 1986; Sukrasno and Yeoman 1993; Prasad et al. 2006; Shaha et al. 2013). Due to the strong irritant activity on skin and eyes they are commonly employed in the production of arthritis creams and in self-defense pepper spray products (Reilly et al. 2001).

Many members of the genus *Capsicum* are of commercial interest, not only for their taste, aroma or capsaicinoids content, but also because of their essential oil and active compounds, such as phenolics, terpenoids and steroids (De 2003; Zimmer et al. 2012).

Pepper fruits have a relatively low volatile oil content, which can vary from 0.1% to 2.6% in dependence on cultivar and the stage of maturity at harvest (Winton and Winton 1939). Paprika powder usually contains less than 0.5% of essential oil. The main chemical classes of compounds identified in *Capsicum* oil are: terpenes and their derivatives, alcohols, aldehydes, ketones, carboxylic acids, esters of carboxylic acids, benzene derivatives, naphthalene skeleton compounds, hydrocarbons, sulphur and nitrogen containing substances, phenolic compounds and carotenoid derivatives (Kocsis et al. 2002; Kocsis et al. 2003).

The essential oil is generally isolated from pepper fruits by steam distillation. However, vacuum distillation-continuous solvent extraction may be applied in the case of present heat-sensitive compounds (Kuzma et al. 2014).

The therapeutic use of the essential oil of *Capsicum* fruits continuously increases day by day. Odoemena and co-workers (1998) showed its antimicrobial activity against *Bacillus subtilis, Salmonella* spp., *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

We previously reported that capsaicinoids, vitamin E, sterols, aliphatic and aromatic hydrocarbons as well as fatty acids dominated in the solvent extracts of selected hot cultivars of *Capsicum* (Wesołowska et al. 2011). Therefore, in the present study, we determined the chemical composition of essential oils isolated by water-steam distillation from the fruits of different cultivars of hot pepper.

MATERIAL AND METHODS

Plant

The experiment was carried out in the years 2010–2011 at the Horticultural Experimental Station, which belongs to the West Pomeranian University of Technology in Szczecin. The research material consisted of fruits of three hot pepper cultivars (*Capsicum annuum* L.): 'Wulkan' (Polish cultivar), 'Padron' (Spanish cultivar) and 'Trakijska Shipka' (Bulgarian cultivar). The pepper seeds used in the experiment came from commercial seed company Thompson and Morgan (cultivar 'Padron'), from Institute of Natural Fibres and Medicinal Plants, Poznan, Poland (cultivar 'Wulkan) and from Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria (cultivar 'Trakijska Shipka').

The seedlings of hot pepper were produced in the greenhouse. Seeds (6 g/m^{-2}) were sown on March 23 into sowing boxes. Three weeks after the sowing, the seedlings of each cultivar were transplanted into plastic pots (8 cm in diameter), filled with peat substrate. At the end of May, the seedlings were planted into the open field at a spacing of 40 x 30 cm, in random blocks pattern, in four replications. The area of one plot was 3.2 m^2 (2.0 m x 1.6 m). The field was prepared according to agronomic recommendations for pepper cultivation. Mineral fertilization was quantified according to the results of the chemical analysis of the soil samples and supplemented to recommended for pepper level of N 150 mg · dm^{-3}, P 60 mg · dm^{-3} and K 60 mg · dm^{-3}. During the growing season, the crop treatment was carried out. It included mainly irrigation, weeding, soil cultivation and plant protection practices. Fruits were collected once on 20th September of both years of the study at the phase of full ripeness.

For the laboratory research 40 fruits in four replications were taken. The fruit samples were chosen randomly. The harvested fruits were dried at 35ºC (electric drier), milled in laboratory grinder and the obtained powder was used for further analysis.

Meteorological conditions during two years of experiment are presented in Table 1.

Table 1. Meteorological data from the period of hot pepper growing in years 2010–2011
Tabela 1. Dane meteorologiczne z lat 2010–2011w okresie uprawy papryki ostrej

Year – Rok	Month – Miesiąc				
	V	VI	VII	VIII	IX
	Mean daily air temperature – Średnia temperatura powietrza [ºC]				
2010	11.1	16.5	21.7	18.5	13.1
2011	14.2	17.8	17.6	18.0	15.1
	Total rainfall – Suma opadów [mm]				
2010	74.9	21.2	62.6	172.4	54.6
2011	37.1	49.8	184.6	51.7	76.5
	Insolation – Usłonecznienie [h]				
2010	115.5	301.0	334.7	151.7	144.7
2011	309.5	300.6	186.0	204.4	180.2

Plants grown in 2011 received more solar energy than those grown in 2010 (Table 1). May, August and September were warmer as compared these months in 2010.

Isolation of the essential oils

The isolation of essential oil was performed according to the method described by Kevresan et al. (2009) with some modifications: Water-steam distillation was carried out by passing steam into a 1-liter round-bottomed flask containing dry plant material (30 g of

powdered plant material was taken for isolation of essential oil) and 400 ml of distilled water for 90 min and collecting the condensate (water and oil) in a Erlenmeyer flask. The condensate was extracted two times with dichloromethane (2 x 50 ml) to completely extract the essential oil (the content of oil in pepper fruits is so small that it does not form a separate layer on the water surface). Sodium sulphate was added to the dichloromethane to remove moisture. Dichloromethane was then removed by rotary evaporation at 40ºC to give yellow oils (0.05 and 0.04% w/w in 2010 and 2011, respectively for 'Wulkan', 0.10 and 0.07% w/w for 'Padron', 0.11 and 0.05% for 'Trakijska Shipka') with characteristic smell. The isolated oils were stored in hermetically sealed glass flask in a refrigerator at 4ºC until GC-MS analysis.

GC-MS analysis of essential oils

Qualitative GC-MS analyses were carried out using an HP 6890 gas chromatograph coupled with HP 5973 mass selective detector operating in electronic ionization mode at 70 eV. Compounds were separated on 30 m long capillary column (HP-5MS), 0.25 mm in diameter and with 0.25 µm thick stationary phase film (5% phenyl-methylpolysiloxane). Helium was used as carrier gas at a flow rate 1.0 ml · min^{-1}. The volume of sample injected was 2µl (20–30 mg of oil dissolved in 1.5 ml of dichloromethane) and split injection was used (split ratio 5 : 1). The injector and the transfer line were kept at 280ºC. The ion source temperature was 230ºC.

The initial temperature of the column was 40ºC for 5 minutes, then increased to 60ºC at a rate of 30ºC per minute, next to 230ºC at a rate of 6ºC per minute (kept constant for 10 min), and then increased to a final temperature of 280ºC at a rate of 30ºC per minute. The oven was held at this temperature for 30 minutes. Solvent delay time was 4 min. The total running time for a sample was about 76 minutes. The mass scan range was from 50 to 550 amu, at 2.94 scans/s.

Most constituents were identified by gas chromatography by comparison of their Kovats retention indices with those reported in NIST 2002, NIST 2005, NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/) databases and literature (Adams 2007). The retention indices were determined in relation to a homologous series of n-alkanes (C_7–C_{40}) under the same operating conditions.

Further identification was made by comparison of their mass spectra with those stored in NBS75K and NIST 2002 mass spectral libraries, using different search engines (PBM, NIST02).

All analyses were performed two times. The relative percentage amounts of the essential oil constituents were evaluated from the total peak area (TIC) using apparatus software.

Statistical analysis

The results of the study presented in Table 3 were subjected to the analysis of variance which was performed with AWAR programme, made by Department of Applied Informatics, Institute of Soil Science and Plant Cultivation in Puławy. The means of two years were separated by the Tukey's test at $p = 0.05$.

RESULTS

The results of the GC-MS analysis of the essential oils obtained by water-steam distillation from fruits of chosen hot pepper cultivars are presented in Table 2. The individual constituents of the analyzed oils are listed in order of their elution from HP-5MS column.

Table 2. Percentage composition of essential oils of chosen cultivars of *Capsicum annuum* L.
Tabela 2. Skład procentowy olejków eterycznych z wybranych odmian *Capsicum annuum* L.

Components – Składniki	RI	2010			2011		
		A	B	C	A	B	C
1	2	3	4	5	6	7	8
Hexanal	802	0.19	0.19	0.23	–	–	0.09
2-Methyl-3-tetrahydrofuranone	809	0.08	0.67	1.44	–	0.12	0.19
2-Methylpyrazine	821	0.15	–	–	–	–	–
Furfural	834	4.02	6.81	6.10	1.28	3.32	2.60
(E)-2-Hexen-1-ol	855	0.15	–	–	–	–	–
2-Furanmethanol	857	0.29	0.82	3.46	–	0.29	0.53
p-Xylene	870	0.06	0.46	0.41	–	0.17	0.13
1-Hexanol	871	0.14	–	0.50	–	–	–
2-Cyclopentene-1,4-dione	886	0.32	1.49	0.73	0.12	0.21	0.24
Methional	907	–	–	0.35	–	0.12	–
2-Acetylfuran	912	0.60	1.39	1.28	0.10	0.90	0.50
Butyrolactone	914	–	–	1.15	–	–	–
3-Hepten-2-one	938	–	0.14	0.19	–	–	–
5-Methyl-2-furanmethanol	956	0.26	0.71	0.71	–	0.59	0.13
Benzaldehyde	961	0.79	1.00	0.40	0.38	0.50	0.22
5-Methyl-2-furfural	965	0.49	1.57	2.53	0.16	–	0.49
Phenol	984	0.71	–	–	0.12	0.26	–
6-Methyl-5-hepten-2-one	986	–	0.33	–	–	0.35	–
3-Octanol	994	–	–	–	–	0.21	–
2-Ethyl-3-methylpyrazine	998	0.20	–	0.16	–	0.21	–
2,3,5-Trimethylpyrazine	1002	–	–	0.32	–	-	–
(E,E)-2,4-Heptadienal	1012	0.36	0.29	–	0.20	0.50	–
2-Ethylhexanol	1031	0.27	0.23	0.18	–	–	–
Phenylmethanol	1035	0.30	–	–	–	–	–
Benzeneacetaldehyde	1045	2.26	4.28	2.00	1.17	1.55	0.24
2-Acetylpyrrole	1063	1.78	2.31	2.13	0.79	1.48	0.56
2-Methylbenzaldehyde	1068	0.11	–	–	–	–	–
4-Methylbenzaldehyde	1070	0.19	–	–	–	–	–
3-Methylphenol	1076	0.32	–	–	–	–	–
Benzyl formate	1079	–	1.30	0.22	–	–	–
2,3,5,6-Tetramethylpyrazine	1087	0.24	–	3.46	–	–	–
2-Phenyl-2-propanol	1091	–	0.22	–	–	–	–
Nonanal	1097	–	–	–	–	0.44	–
Linalool	1101	2.54	1.08	1.14	1.00	0.16	0.89
6-Methyl-3,5-heptadiene-2-one	1107	0.15	–	0.11	0.18	0.30	0.13
2,6-Dimethylcyclohexanol	1110	0.33	–	0.27	–	–	0.25
β-Phenylethanol	1115	0.32	0.44	0.46	–	–	–
α-Ethylcaproic acid	1121	0.29	–	–	–	–	–
Fenchol	1125	–	0.18	–	–	0.13	–
1-Ethyl-2-propylcyclohexane	1143	–	–	–	–	0.13	–
4-Oxoisophorone	1146	0.10	–	–	–	–	–
(E)-2-Nonenal	1161	0.15	0.22	0.20	–	–	0.07
1-Nonanol	1175	–	–	0.76	0.34	0.71	0.25
3,4-Dimethylbenzaldehyde	1178	1.73	0.53	0.51	–	0.23	0.22

Table 2. Percentage composition of essential oils of chosen cultivars of *Capsicum annuum* L. (cont.)
Tabela 2. Skład procentowy olejków eterycznych z wybranych odmian *Capsicum annuum* L. (cd.)

1	2	3	4	5	6	7	8
Terpinen-4-ol	1181	0.22	0.30	–	0.15	–	0.06
2-Acetyltoluene	1190	0.19	–	0.22	–	0.09	–
α-Terpineol	1194	0.57	0.25	0.33	0.20	0.18	0.24
Myrtenol	1197	–	–	–	0.25	0.22	–
Estragol	1201	0.42	0.64	–	0.13	0.18	–
Safranal	1203	0.26	–	0.13	0.12	–	0.13
(E,E)-2,4-Nonadienal	1222	0.28	0.24	–	0.25	0.32	–
β-Cyclocitral	1225	0.24	0.19	0.17	0.15	0.29	0.15
Benzothiazol	1228	0.57	0.52	0.12	0.26	0.23	0.14
Thymol methyl ether	1237	0.78	–	–	–	–	–
Pulegone	1244	0.60	0.71	–	0.39	0.35	–
Geraniol	1258	0.29	–	0.20	–	0.17	–
β-Homocyclocitral	1263	–	–	–	–	–	0.12
1-Decanol	1271	0.34	0.23	–	0.13	0.14	–
2-Phenyl-2-buten-1-al	1277	0.18	0.26	0.18	–	0.20	–
Phellandral	1281	–	–	–	–	–	0.12
(E)-3-Tridecene	1291	–	–	–	0.30	0.62	–
Pelargonic acid	1295	0.51	0.56	–	0.30	0.18	–
Indole	1297	0.59	0.25	–	–	–	0.19
Tridecane	1301	–	–	–	3.54	3.03	-
Carvacrol	1305	2.35	7.82	1.53	–	–	0.66
Undecanal	1309	0.36	0.46	–	–	–	–
2-Methoxy-4-vinylphenol	1318	3.26	2.00	2.82	1.54	3.10	1.77
δ-Elemene	1334	0.65	0.33	–	–	–	–
Piperitenone	1347	1.19	1.75	0.26	1.50	1.46	0.14
2-Methyltridecane	1360	–	–	–	0.22	–	–
Eugenol	1362	0.30	0.27	–	0.17	0.19	–
Capric acid	1368	0.78	–	0.51	–	–	0.30
3-Methyltridecane	1371	–	–	0.28	–	–	0.06
α-Copaene	1379	0.26	0.43	–	0.32	–	–
β-Elemene	1384	0.25	–	0.38	0.81	0.61	0.19
Methyl cinnamate	1388	1.95	1.65	0.43	0.18	0.70	0.14
(E)-β-Damascenone	1390	0.24	0.39	0.16	–	–	0.18
1-Tetradecene	1394	–	–	0.12	–	0.62	0.19
Tetradecane	1398	–	–	0.11	0.13	–	0.24
β-Caryophyllene	1421	0.31	–	0.25	–	–	–
(E)-α-Ionone	1434	0.17	0.17	–	0.13	0.13	0.11
Aromadendrene	1444	0.28	–	0.27	0.32	0.29	0.13
α-Humulene	1447	–	0.18	–	–	1.14	–
(E)-Geranylacetone	1455	1.65	0.94	0.83	1.49	0.13	1.41
2-Methyltetradecane	1459	0.22	–	–	0.34	0.30	0.08
Undecanoic acid	1464	-	0.31	–	–	0.31	–
γ-Muurolene	1473	0.80	0.47	1.36	–	–	0.47
1-Dodecanol	1476	–	0.21	–	–	0.74	–
γ-Himachalene	1483	–	0.18	0.26	0.56	0.12	0.36
Germacrene D	1488	0.30	0.30	–	1.71	2.36	–
(E)-β-Ionone	1492	1.82	1.57	1.58	0.17	0.56	1.56
2-Tridecanone	1495	–	0.49	0.15	0.32	0.62	0.37

Table 2. Percentage composition of essential oils of chosen cultivars of *Capsicum annuum* L. (cont.)
Tabela 2. Skład procentowy olejków eterycznych z wybranych odmian *Capsicum annuum* L. (cd.)

1	2	3	4	5	6	7	8
Pentadecane	1502	0.30	0.42	0.39	–	0.43	0.29
α-Farnesene	1507	–	–	–	1.22	–	0.37
Tridecanal	1513	0.50	0.46	0.72	0.28	0.39	0.86
(Z)-Calamenene	1518	–	–	–	0.42	0.26	–
δ-Cadinene	1523	0.21	0.48	–	–	0.22	0.14
(E)-Cadina-1,4-diene	1527	0.60	–	–	0.38	0.18	–
(E,Z)-Pseudoionone	1533	0.71	0.35	0.21	–	–	–
Dihydroactinidiolode	1541	2.80	2.07	1.26	2.52	2.84	1.57
Elemicin	1559	–	–	–	0.65	0.77	–
2-Methylpentadecane	1563	1.46	0.54	0.91	1.45	2.28	–
Nerolidol	1568	0.90	0.57	0.60	0.27	–	2.33
3-Methylpentadecane	1573	–	–	0.23	1.08	–	0.23
(Z)-3-Hexenyl benzoate	1577	0.46	–	–	0.38	–	-
Spathulenol	1582	–	–	–	–	–	0.19
(E,E)-Pseudoionone	1588	–	–	–	0.52	–	–
Caryophyllene oxide	1592	–	0.22	–	–	–	–
1-Hexadecene	1599	0.50	1.29	0.20	1.31	1.37	0.10
Hexadecane	1602	0.94	0.47	0.22	0.25	–	0.18
Tetradecanal	1614	0.26	–	0.69	0.57	–	0.58
1,10-di-epi-Cubenol	1623	–	0.24	–	0.22	0.25	–
Isopropyl laurate	1628	–	0.26	–	-	–	0.23
Benzophenone	1636	0.29	0.59	0.94	0.32	–	–
γ-Eudesmol	1640	0.20	–	0.17	0.25	–	–
τ-Cadinol	1646	–	–	–	0.25	0.23	–
β-Eudesmol	1650	–	0.29	–	–	0.54	–
α-Muurolol	1656	0.20	–	–	–	–	0.09
7-epi-α-Eudesmol	1659	0.45	0.44	–	0.98	0.39	–
2-Methylhexadecane	1663	0.43	0.47	0.25	–	0.61	0.11
8-Heptadecene	1671	0.24	0.17	0.17	0.23	–	0.13
Cadalene	1673	–	0.35	-	0.50	0.82	–
1-Tetradecanol	1679	–	0.32	0.23	0.24	–	–
α-Bisabolol	1684	–	–	–	–	0.69	–
2-Methylhexadec-1-ene	1687	–	–	–	0.45	–	–
6,6-Dimethyltetradecane	1691	–	–	–	0.59	–	0.11
1-Heptadecene	1697	–	–	0.45	0.90	0.91	0.60
Heptadecane	1700	0.83	0.59	0.57	0.19	0.27	0.25
2-Pentadecanone	1703	–	–	–	0.41	0.42	0.11
5-Ethyl-5-methylpentadecane	1710	–	–	–	-	1.46	0.10
Pentadecanal	1717	6.46	0.50	9.04	13.70	2.54	11.81
(E,E)-Farnesol	1720	0.50	–	–	0.67	–	–
Methyl myristate	1726	0.24	–	0.12	0.13	–	0.29
2,6-Diisopropylnaphthalene	1730	–	–	–	–	1.89	–
5-Phenyldodecane	1734	–	0.88	0.49	–	3.20	0.16
(E,Z)-Farnesol	1742	–	–	0.20	–	–	0.47
(E)-2-Hexylcinnamaldehyde	1749	–	–	–	0.20	–	–
4-Methylheptadecane	1752	–	–	–	0.14	–	–
5-Methylheptadecane	1755	0.26	–	0.40	0.31	–	–
Undecylcyclohexane	1760	–	–	–	0.98	–	–
Myristic acid	1766	1.72	0.46	2.32	0.35	3.27	4.19

Table 2. Percentage composition of essential oils of chosen cultivars of *Capsicum annuum* L. (cont.)
Tabela 2. Skład procentowy olejków eterycznych z wybranych odmian *Capsicum annuum* L. (cd.)

1	2	3	4	5	6	7	8
(E)-α-Atlantone	1775	–	–	–	0.27	–	–
Phenanthrene	1784	–	–	–	0.48	0.21	–
Anthracene	1789	–	0.44	0.34	0.72	–	0.45
1-Octadecene	1794	–	0.63	0.47	–	0.51	0.37
Octadecane	1802	–	–	–	0.33	0.55	–
2-Hexadecanone	1808	0.32	1.17	0.26	0.19	–	0.20
Hexadecanal	1812	–	–	–	0.45	0.24	–
Nootkatone	1818	0.23	0.33	0.21	1.14	0.74	0.16
Isopropyl myristate	1832	1.36	1.08	0.30	0.32	0.23	0.18
Hexahydrofarnesyl acetone	1841	0.72	1.75	0.56	1.64	–	0.23
3,3-Diethylheptadecane	1857	–	–	–	0.79	0.26	–
Pentadecanoic acid	1863	0.43	0.86	0.35	2.19	2.05	0.30
Diisobutyl phthalate	1873	1.07	7.67	0.82	–	–	2.01
1-Hexadecanol	1886	–	–	–	8.50	0.51	–
1-Nonadecene	1895	3.67	0.22	6.17	5.34	0.56	10.72
Nonadecane	1899	2.32	–	3.99	–	–	5.19
5-Ethyl-5-methylheptadecane	1916	–	–	–	1.52	1.40	–
Farnesyl acetone	1920	0.71	0.54	0.23	0.60	0.71	0.90
Methyl palmitate	1927	0.60	–	0.46	–	–	0.43
Z-11-Hexadecenoic acid	1937	–	–	–	–	0.79	-
9-Hexadecenoic acid	1943	0.39	–	0.19	–	–	0.97
Palmitic acid	1968	11.98	8.45	10.00	8.96	12.52	11.17
7,7-Diethylheptadecane	1986	0.42	0.36	0.42	0.25	–	0.26
Ethyl palmitate	1994	–	–	0.70	–	–	0.50
Eicosane	2000	–	–	–	–	–	0.14
Isopropyl palmitate	2018	–	–	–	1.18	0.85	–
Octadecanal	2025	0.56	0.66	0.41	0.34	-	0.48
5-Ethylnonadecane	2032	–	–	–	–	0.42	–
1-Octadecanol	2074	–	–	–	–	–	0.17
Methyl linoleate	2090	–	–	–	0.41	0.39	–
1-Heneicosene	2097	0.55	1.99	0.32	0.22	0.27	0.34
Heneicosane	2103	0.28	0.35	0.19	–	–	0.16
Linoleic acid	2138	2.23	0.78	–	2.16	3.48	–
Oleic acid	2144	2.34	1.30	2.23	1.39	4.26	3.45
Linolenic acid	2147	–	–	2.66	–	–	9.73
Ethyl linoleate	2158	0.90	3.07	0.98	1.94	2.05	0.86
Ethyl linolenate	2172	–	–	0.75	–	–	0.73
1-Nonadecanol	2209	–	0.25	–	–	–	–
Tricosanal	2537	–	–	–	0.46	0.78	–
di-n-Octyl phthalate	2547	0.29	0.98	–	–	1.14	0.20
1-Tricosanol	2558	–	–	–	0.31	–	–
9-Hexacosene	2570	–	–	–	–	0.69	–
1-Heptacosene	2695	–	–	–	0.13	0.12	–
Heptacosane	2702	–	–	–	0.11	0.14	–
Pentacosanal	2739	–	–	–	0.17	–	–
5,5-Diethylpentacosane	2817	–0.20	–	–	0.17	0.36	–
Squalene	2829	–	–	0.11	–	–	0.12
1-Octacosanol	3120		–	–	–	1.05	–
Identified – Zidentyfikowano		96.25	97.75	97.95	97.91	96.17	93.15

A – Polish cultivar 'Wulkan' – odmiana polska 'Wulkan'.
B – Spanish cultivar 'Padron' – odmiana hiszpańska 'Padron'.
C – Bulgarian cultivar 'Trakijska Shipka' – odmiana bułgarska 'Trakijska Shipka'.
RI – retention index – indeks retencji.
– not found – nie wykryto.

One hundred and eleven compounds were identified in the essential oil of 'Wulkan' cultivar. Among them palmitic acid (11.98 and 8.96% in 2010 and 2011, respectively), pentadecanal (6.46 and 13.70%), furfural (4.02 and 1.28%), 1-nonadecene (3.67 and 5.34%), 2-methoxy-4-vinylphenol (3.26 and 1.54%), dihydroactinidiolide (2.80 and 2.52%) and linoleic acid (2.23 and 2.16%) were the major components. The other important compounds were linalool (2.54 and 1.00%), benzeneacetaldehyde (2.26 and 1.17%), (E)-β-ionone (1.82 and 0.17%) and (E)-geranylacetone (1.65 and 1.49%).

One hundred and seven compounds were found in the essential oil of 'Padron' cultivar. Palmitic acid (8.45 and 12.52%) was the main constituent of oil, followed by furfural (6.81 and 3.32%), benzeneacetaldehyde (4.28 and 1.55%), 2-acetylpyrrole (2.31 and 1.48%) and 2-methoxy-4-vinylphenol (2.00 and 3.10%).

The essential oil of 'Trakijska Shipka' cultivar was found to be rich in palmitic acid (10.00 and 11.17%), pentadecanal (9.04 and 11.81%) and 1-nonadecene (6.17 and 10.72%). Other constituents identified in significant amounts were: furfural (6.10 and 2.60%), nonadecane (3.99 and 5.19%), 2-methoxy-4-vinylphenol (2.82 and 1.77%) and (E)-β-ionone (1.58 and 1.56%). A total of 99 compounds were identified in this oil.

Because of high content of furfural, benzeneacetaldehyde, 2-acetylpyrrole, 2-methoxy-4--vinylphenol, (E)-geranylacetone, (E)-β-ionone, dihydroactinidiolide, pentadecanal, 1-nonadecene, nonadecane, palmitic acid, linoleic acid, oleic acid, linolenic acid and ethyl linoleate in the essential oil samples, these major components were analyzed statistically (Table 3).

The statistical analysis of the results presented in Table 3 shows significant differences between the content of the main essential oil constituents of fruits of three hot pepper cultivars.

In the first year of the experiment, among 15 main compounds of hot pepper fruit essential oils, palmitic acid was characterized by the highest concentration – 10.14%. High amounts were also noted for furfural – 5.64%, pentadecanal – 5.33% and 1-nonadecene – 3.53%, while the least for (E)-geranylacetone – 1.14%, linoleic acid – 1.00% and linolenic acid – 0.89%. Comparing the three cultivars, it was found that the fruits of cultivars 'Wulkan' and 'Trakijska Shipka' were characterized by significantly higher mean content of the main 15 essential oil constituents in comparison with cultivar 'Padron'. Moreover, the analysis of the interaction between essential oil constituent and cultivar showed that in case of cultivar 'Wulkan', the main essential oil compound was palmitic acid – 11.98%, for cultivar 'Padron' – palmitic acid (8.45%) and furfural (6.81%), while for 'Trakijska Shipka' – palmitic acid (10.00%) and pentadecanal (9.04%).

In the second year of the experiment, the highest amount, from all of the fifteen main essential oil constituents of hot pepper fruits, was also noted for palmitic acid (10.88%), lower for pentadecanal (9.35%) and then for 1-nonadecene (5.54%), while the least for (E)-geranylacetone (1.01%), benzeneacetaldehyde (0.99%), 2-acetylpyrrole (0.94%) and (E)-β-ionone (0.76%). Moreover, the highest amount of the main essential oil compounds was recorded for the fruits of cultivar 'Trakijska Shipka' in comparison with 'Wulkan' and 'Padron'. The analysis of the interaction for the year 2011, between essential oil constituent and cultivar, showed that in case of cultivar 'Wulkan', pentadecanal was the main essential oil compound – 13.70%, for cultivar 'Padron' – palmitic acid (12.52%), while for 'Trakijska Shipka' – pentadecanal (11.81%), palmitic acid (11.17%), 1-nonadecene (10.72%) and linolenic acid (9.73%).

Table 3. Content of main constituents in the essential oils of three hot pepper (*Capsicum annuum* L.) cultivars in years 2010–2011
Tabela 3. Zawartość głównych składników w olejkach eterycznych trzech odmian papryki ostrej (*Capsicum annuum* L.) w latach 2010–2011

Essential oil constituent (factor I) Składnik olejku eterycznego (czynnik I)	Cultivar (factor II) – Odmiana (czynnik II)											
	2010				2011				2010–2011			
	A	B	C	mean średnia	A	B	C	mean średnia	A	B	C	mean średnia
Furfural	4.02	6.81	6.10	5.64	1.28	3.32	2.60	2.40	2.65	5.07	4.35	4.02
Benzeneacetaldehyde	2.26	4.28	2.00	2.85	1.17	1.55	0.24	0.99	1.72	2.92	1.12	1.92
2-Acetylpyrrole	1.78	2.31	2.13	2.07	0.79	1.48	0.56	0.94	1.29	1.90	1.35	1.51
2-Methoxy-4-vinylphenol	3.26	2.00	2.82	2.69	1.54	3.10	1.77	2.14	2.40	2.55	2.30	2.42
(E)-Geranylacetone	1.65	0.94	0.83	1.14	1.49	0.13	1.41	1.01	1.57	0.54	1.12	1.08
(E)-β-Ionone	1.82	1.57	1.58	1.66	0.17	0.56	1.56	0.76	1.00	1.07	1.57	1.21
Dihydroactinidiolide	2.80	2.07	1.26	2.04	2.52	2.84	1.57	2.31	2.66	2.46	1.42	2.18
Pentadecanal	6.46	0.50	9.04	5.33	13.70	2.54	11.81	9.35	10.08	1.52	10.43	7.34
1-Nonadecene	3.67	0.22	6.17	3.35	5.34	0.56	10.72	5.54	4.51	0.39	8.45	4.45
Nonadecane	2.32	–	3.99	2.10	–	–	5.19	1.73	1.16	–	4.59	1.92
Palmitic acid	11.98	8.45	10.00	10.14	8.96	12.52	11.17	10.88	10.47	10.49	10.59	10.51
Linoleic acid	2.23	0.78	–	1.00	2.16	3.48	–	1.88	2.20	2.13	–	1.44
Oleic acid	2.34	1.30	2.23	1.96	1.39	4.26	3.45	3.03	1.87	2.78	2.84	2.50
Linolenic acid	–	–	2.66	0.89	–	–	9.73	3.24	–	–	6.20	2.07
Ethyl linoleate	0.90	3.07	0.98	1.65	1.94	2.05	0.86	1.62	1.42	2.56	0.92	1.63
Mean – Średnia	3.17	2.29	3.45	2.97	2.83	2.56	4.18	3.19	3.00	2.42	3.81	3.08
$LSD_{\alpha = 0.05}$ for factor I $NIR_{\alpha = 0,05}$ dla czynnika I	2.067				1.459				1.154			
$LSD_{\alpha = 0.05}$ for factor II $NIR_{\alpha = 0,05}$ dla czynnika II	0.590				0.595				0.408			
$LSD_{\alpha = 0.05}$ for interaction I x II $NIR_{\alpha = 0,05}$ dla interakcji I x II	2.283				2.304				1.580			

Explanations see Table 2 – Objaśnienia zob. tab. 2.

Mean results of both years of the experiment proved, that palmitic acid was found to be the main constituent of essential oils of compared in the study fruits of three hot pepper cultivars. High amounts were also noted in case of pentadecanal and 1-nonadecene. The highest average amount of the main essential oil constituents was noted for cultivar 'Trakijska Shipka' (3.81%), while significantly lower for cultivars 'Wulkan' and 'Padron' (on average – 2.71%). More detailed information gave us analysis of interaction between both of the experimental factors. The main compounds of essential oil of the fruits of cultivars 'Wulkan' and 'Trakijska Shipka' were palmitic acid (respectively, 10.47 and 10.59%) and pentadecanal (respectively, 10.08 and 10.43%), and in case of cultivar 'Padron', palmitic acid (10.49%) was found to be the main essential oil constituent.

Generally, aldehydes, terpenoids and hydrocarbons were the main classes of identified compounds. Ketones, alcohols, saturated and unsaturated fatty acids and esters were also detected. However, linolenic acid and ethyl linoleate were found only in 'Trakijska Shipka' oil, while linoleic acid was present only in 'Wulkan' and 'Padron' oils.

DISCUSSION

The aroma profile of *Capsicum* fruits has been previously described by several authors. According to Buttery et al. (1969), 2-methoxy-3-isobutylpyrazine, (E,Z)-2,6-nonadienal, 2,4--decadienal, limonene and methyl salicylate are contributed to the aroma of fresh fruits. Chitwood et al. (1983) associated (E)-3-hexenol, 2-sec-butyl-3-methoxypyrazine and 2-isobuty-3-methoxypyrazine with the green, fruity and floral aromas of pepper fruits, while Haymon and Aurand (1971) showed aliphatic esters as the major aroma compounds of chili peppers.

2-Methoxy-3-isobutylpyrazine and 2-isobutyl-3-methoxypyrazine, compounds which are responsible for characteristic fresh green pepper fruits aroma (Luning et al. 1994), were not detected in this study. However, we found 2-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,3,5--trimethylpyrazine and 2,3,5,6-tetramethylpyrazine in our oils. Moreover, 6-methyl-3,5--heptadien-2-on, 6-methyl-5-hepten-2-one, 2-methylbenzaldehyde and (E)-β-ionone, which we detected in volatile oils of investigated cultivars, are considered as a products of thermal degradation of carotenoids and are characteristic compounds of dried peppers (Cole and Kapur 1957, Van Ruth et al. 1995a, Van Ruth et al. 1995b).

Alkylmethoxypyrazines were also not detected in Hungarian red hot pepper cultivars 'Kalocsai-V-2' and 'Szegedi-178' (Kocsis et al. 2002) and hot samples of Spanish commercial paprika (Mateo et al. 1997). Probably these substances are not characteristic for fragrance of dried red paprika.

Aliphatic aldehydes, alcohols, branched hydrocarbons and terpenoids were the most representative chemical classes of substances identified in the volatile oils of different Calabrian (Italian) *C. annuum* varieties investigated by Ziino et al. (2009). Hexanal, hexanol, (Z)-2-hexenal and (Z)-2-hexenol were the main compounds in all analyzed samples. Similarly to our results, they found 6-methyl-5-hepten-2-one and β-ionone as the products of carotenoid degradation.

In another study, Kevresan et al. (2009) found benzaldehyde, 2-hexenal, heptanal, 2-octenal, nonanal, 2-nonenal, 2,6-nonadienal, safranal, decanal, 2,4-decadienal, tetradecanal (myristyl aldehyde), pentedecanal and 9,17-octadecadienal in the volatile oils of Serbian fresh and dried red pepper (cultivar 'AlevaNK').

Similary, Kocsis et al. (2002 and 2003) found 2,4-heptadienal, benzaldehyde, octanal, 2-octenal, nonanal and 2,6-nonadienal in Hungarian paprika.

Fatty acids and their esters were found as the main volatiles in spice paprika 'AlevaNk' examined under different post-harvest ripening conditions (Kevresan et al. 2013). The palmitic acid (11.55%) was the dominant acid, followed by lauric (6.92%), myristic (5.40%) and linoleic (4.22%). Among the identified esters, methyl linoleate (1.66%) and methyl palmitate (1.31%) were the most abundant. Terpenes and terpenoids were the second main group of compounds identified in Serbian paprika. The main representatives of this class were (E)-β--ionone (1.89%), farnesyl acetone (1.59%), (E)-geranylacetone (1.07%) and linalool (0.79%).

In the essential oils of investigated *Capsicum* cultivars ('Wulkan', 'Padron' and 'Trakijska Shipka') we also detected benzaldehyde, nonanal, safranal, tetradecanal and pentadecanal. Interestingly, furfural (1.28–6.81%), one of the most representative aldehydes in all analyzed oil samples, was not detected in red paprika from Hungary (Kocsis et al. 2002). In Serbian pepper, the concentration of this aldehyde was less than 0.1% (Kevresan et al. 2013). The content of myristic (0.46–4.19%) and linoleic (0.78–3.48%) acids as well as methyl palmitate (0.43–0.60%), methyl linoleate (0.39–0.41%), (E)-β-ionone (0.17–1.82%) and farnesyl acetone (0.23–0.90%) found in our study was lower compared to the results obtained by Kevresan et al. (2013). However, the amount of (E)-geranylacetone in the oil of 'Wulkan' cultivar (1.65 and 1.49% in 2010 and 2011, respectively) was higher than in 'AlevaNk' (Kevresan et al. 2013). Similarly, our cultivars contained more linalool in the essential oil than Serbian pepper (Kevresan et al. 2013). Surprisingly, lauric acid was not detected in our peppers.

CONCLUSIONS

In the present study we have investigated the composition of essential oils obtained from fruits of hot pepper cvs. 'Wulkan' (Polish cultivar), 'Padron' (Spanish cultivar) and 'Trakijska Shipka' (Bulgarian cultivar) using GC-MS.

Among the identified constituents predominant were aldehydes, terpenoids, hydrocarbons, fatty acids and their esters.

In the case of all tested in the experiment cultivars of hot pepper, palmitic acid was found to be the main essential oil constituent. Moreover, for cultivars 'Wulkan' and 'Trakijska Shipka', high amounts were also recorded for another constituent – pentadecanal. Among compared in the experiment hot pepper cultivars, 'Trakijska Shipka' was characterized by the highest amount of the main essential oil constituents.

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Abstract. The burning taste of *Capsicum* fruits is due to the presence of the chemical group of alkaloid compounds called capsaicinoids, among which capsaicin and dihydrocapsaicin are responsible for 90% of the total pungency. The pepper fruits contain also the small amounts of essential oil, which gives them a distinctive flavor and aroma. The main objective of the present study was to determine the chemical composition of essential oils of the fruits of three hot pepper cultivars: 'Wulkan', 'Padron' and 'Trakijska Shipka'. The essential oils were isolated from hot pepper fruits by water-steam distillation and analyzed using gas chromatography-mass spectrometry (GC-MS). Palmitic acid (11.98 and 8.96% in 2010 and 2011, respectively), pentadecanal (6.46 and 13.70%), furfural (4.02 and 1.28%), 1-nonadecene (3.67 and 5.34%), 2-methoxy-4-vinylphenol (3.26 and 1.54%) and linoleic acid (2.23 and 2.16%) were found to be the major constituents of 'Wulkan' cultivar volatile oil. In the essential oil of 'Padron' cultivar, palmitic acid (8.45 and 12.52%), furfural (6.81 and 3.32%), benzeneacetaldehyde (4.28 and 1.55%), 2-acetylpyrrole (2.31 and 1.48%) and 2-methoxy-4-vinylphenol (2.00 and 3.10%) were the most abundant compounds. Similarly, palmitic acid (10.00 and 11.17%), pentadecanal (9.04 and 11.81%), 1-nonadecene (6.17 and 10.72%), furfural (6.10 and 2.60%) as well as nonadecane (3.99 and 5.19%) and 2-methoxy-4-vinylphenol (2.82 and 1.77%) dominated in the essential oil of 'Trakijska Shipka' cultivar.