Spectroscopic Examination and Chemometric Analysis of Essential Oils Obtained from Peppermint Herb (Mentha piperita L.) and Caraway Fruit (Carum carvi L.) Subjected to Pulsed Electric Fields

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Urszula Sadowska [1,*], Arkadiusz Matwijczuk [2], Agnieszka Niemczynowicz [3], Tomasz Dróżdż [1] and Andrzej Żabiński [1]

[1] Institute of Machinery Exploitation, Ergonomics and Production Processes, Faculty of Production and Power Engineering, University of Agriculture in Kraków, Łupaszki 6, 30-198 Kraków, Poland
[2] Department of Physics, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland
[3] Department of Analysis and Differential Equations, Faculty of Mathematics and Computer Science, University of Warmia and Mazury, Słoneczna 54, PL-10-710 Olsztyn, Poland
* Correspondence: urszula.sadowska@ur.krakow.pl; Tel.: +48-504-318-098

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Abstract: In the food industry, the pulsed electric field (PEF) technique is used to support the process of extraction of various substances. The aim of this study was to analyze the effect of a number of PEF pulses applied to peppermint and caraway on both the content of essential oils (EO) and their spectroscopic properties. The examined herb species were placed in a special vessel in the working chamber of the device between two electrodes providing high voltage electric pulses. The pulses were delivered 0, 150, 250, and 350 times per a studied sample of each herb. Essential oils were then obtained by way of hydrodistillation. The infrared spectra for all samples were measured using an FTIR spectrometer in the spectral range of 3700–730 cm^{-1}. The applied electric field of a predetermined number of pulses had no significant effect on the amount of distilled essential oil from caraway fruit, while in the case of peppermint, it caused a slight decrease in relation to the raw material not subjected to PEF exposure. It was found that the analysis of infrared spectra made it possible to compare the quality of the obtained oils with each other and to pre-determine their compositions.

Keywords: peppermint herb; caraway fruit; essential oils; spectroscopic studies; pulsed electric field; chemometric analysis and spectroscopic studies

1. Introduction

Peppermint (*Mentha pipperita* L.) and caraway (*Carum carvi* L.) are two of the most important herb species grown in Poland [1,2]. Their healing properties are primarily determined by essential oils (EO) [3]. Essential oils from various plant species are widely used in the pharmaceutical, cosmetic, and food industries [4]. In addition to their traditional uses, they may have a potential for additional applications due to their proven inhibitory effects on the development of pathogenic microorganisms [5–9] or their insecticidal activity [10]. Unfortunately, the content of EO in herbal plants is low. Thus, it is important to extract the highest possible amounts of these valuable ingredients. In the case of peppermint pharmaceuticals, raw material comes in the form of leaves, while in the case of caraway, in the form of schizocarps [3]. In pharmaceutics, only raw materials with the required operational parameters and that meet strictly defined standards can be used, including inter alia, the minimum, normatively required amount of the examined active compounds. In accordance with

requirements [3], the minimum oil content in peppermint leaves must be 12 mL·kg^{-1} for uncrushed substance, while in the case of caraway, it must not be less than 30 mL·kg^{-1}. The determination of essential oil content in plant substances in the context of pharmaceutical applications is carried out by means of steam distillation, in accordance with a methodology strictly defined for each respective species [3].

At times, the process of substance extraction is preceded by various preliminary procedures. One of the more common and effective entails exposure to a pulsed electric field (PEF). Bazhal et al. [11] showed that PEF application leads to increased efficiency of apple juice pressing processes and changes the physical and optical properties (such as absorbance and transmittance) of the obtained products. Similar results were obtained by Praporscic et al. [12], who used PEF to increase the efficiency of apple and carrot juice pressing. They proved that a pulsed electric field not only increases the pressing capacity, but also positively affects the quality characteristics of the products obtained. Bouzrara and Vorobiev [13] showed that the use of pulsed electric field before sugar beet juice pressing increases juice yield by 18–28%. Inspired by these studies showing beneficial effects of PEF on other food products, we conducted research on the use of PEF treatment to support the secretion of essential oils from herbal plants in the hydrodistillation process.

The aim of the study was to analyze the effect of the number of PEF pulses applied to peppermint and caraway on both the content of essential oils and their spectroscopic properties, which allowed the authors to identify the composition of essential oils from selected plants. The FTIR infrared spectroscopy and chemometric analysis results obtained for selected samples of essential oils from the two plant species were used in the performance of the aforementioned goal.

2. Materials and Methods

The research material consisted of dried peppermint herb (*Mentha piperita* L.) with a moisture content of 11%, from conventional cultivation, Poland (N 50°30′17″, E 20°21′38″) and Kończewicki caraway fruit (*Carum carvi* L.) with a moisture content of 9%, both of which were bought at the local seed store.

The study on PEF application was carried out on a prototype research stand. Its basic elements included a high voltage pulse generator, a working chamber (Figure 1), and a computerized control system. The examined herb species were placed in a special vessel in the working chamber of the device between two electrodes providing high voltage electric pulses. The pulses were of rectangular shape and were applied 0, 150, 250, or 350 times per sample of each herb. The duration of a single pulse was ≈25 μs and the time interval between two pulses was 10 s, i.e., the process lasted at least 25 min per sample at the lowest level of intensity (150 pulses).
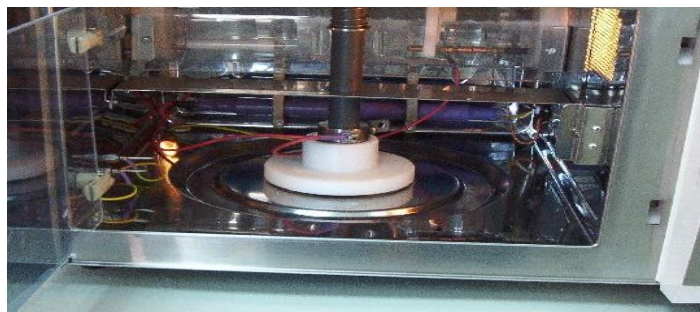
Figure 1. Working chamber of pulsed electric field (PEF) research stand.

The intensity of the electric field was 30 kV·cm^{-1}. The volume of the vessel in the pulse chamber was 20 mL. One vessel contained 4 g of dried peppermint leaves moistened with 9 mL of distilled water, the other, 10 g of caraway schizocarps with 9 mL of distilled water. In each run of the experiment, tests for peppermint were carried out in 15 replicates (15 × 4 g), so as to obtain 60 g of raw material. This mass

was necessary to perform the analysis of the content of essential oils in three replicates, in accordance with pharmaceutical requirements [3]. In the case of caraway seeds, three replicates (3×10 g) were performed because that was enough to obtain the oil content required [3]. After application of PEF was completed, the temperature of the tested material was measured. The temperature was measured using a K-250PC probe co-operating with a PC720M digital gauge made by the company SANWA.

Due to time constraints, it was impossible to directly analyze the content of oils (a long process), therefore, the obtained material was transferred to an airtight container and placed at 3–4 °C for 24 h. After this time, the content of essential oils was analyzed. The study was carried out in triplicate for each species and experimental condition.

The content of essential oils in the herbal species from individual experimental combinations was determined by 2 h hydrodistillation, in a Clevenger apparatus. A single test sample for the peppermint had a weight of 20 g, and in the case of caraway, 10 g. Hydrodistillation for each species was carried out using 200 mL H_2O, in accordance with the pharmaceutical requirements for EO [3]. The tests were performed in three replicates for each species and combination of runs (0, 150, 250, and 300 pulses). The material was tested uncrushed. After distillation, the essential oil was brought to the scaled part of the receiver and its volume was determined. After the reading, the oil was poured into an airtight container and stored at 3–4 °C until spectroscopic analysis. The determinations were carried out in triplicate for each condition.

Infrared absorption spectra were recorded with a 670-IR (Varian) spectrometer. The attenuated total reflection (ATR) configuration was used with 20 internal reflections of the ZnSe crystal plate (45° cut). Typically, 16 scans were collected, Fourier-transformed, and averaged for each measurement. Absorption spectra at a resolution of one data point per 1 cm^{-1} were obtained in the region between 4000 and 600 cm^{-1}. The instrument was continuously purged with N_2 for 40 min. before and during measurements. The ZnSe crystal plate was cleaned with ultra-pure organic solvents from Sigma-Aldrich (Poznań, Poland). The spectral analysis was performed with Grams/AI software from ThermoGalactic Industries (Waltham, MA, USA). All experiments were carried out at 23 °C.

Statistical analyses were carried out using the STATISTICA 13.1 software (Stat Soft, Inc., Kraków, Polska). A one-way analysis of variance (ANOVA) was performed separately for each species. The factor of the experiment was the number of pulses (0, 150, 250, 350). The arithmetic mean as well as the standard deviations (SD) were calculated. When comparing variables between groups, the ANOVA with post-hoc analysis was used to show specific differences between groups. The significance of differences between the mean values was evaluated using Fisher's test for $p = 0.05$.

Principal component analysis of the FTIR spectra were performed using OriginPro software (OriginLab, Northampton, MA, USA) using the Principal Component Analysis for Spectroscopy app.

3. Results and Discussion

The results of the chromatographic analysis of the essential oil isolated from the mint herb are presented in Table 1. Fifty-six ingredients were identified, and monoterpenes, menton, and menthol were the dominant compounds. According to many authors, these compounds are the essential components of this plant's oil [5,14–16]. However, in terms of their relative percentages, quite significant differences can sometimes be noticed. Their causes result primarily from the genetic diversity of plants, but they can also have a technological character [17]. Mentone and menthol accounted for more than 60% of the total content in our research. Similar results of the percentages of these compounds were reported by Schmidt et al. [18]. The other essential components of the oil were distinguished by an increased proportion of menthofuran and menthyl acetale (Table 1), which is also consistent with the results obtained by Schmidt et al. [18].

Table 1. Chemical composition of peppermint oil.

Peak Name	Time (min)	Kovats Retention Index		The Volatile Compounds %
		Exp.	Lit.	
2-(*E*)-Hexenal	4.304	830	827	tr.
1-Hexanol	4.346	861	854	tr.
3-Heptanol	5.242	889	884	0.02
α-Pinene	6.279	938	932	0.29
Sabinene	7.450	981	969	0.21
β-Pinene	7.575	984	974	0.12
Myrcene	7.967	998	988	0.19
3-Octanol	8.079	1001	989	0.40
Limonene	9.333	1032	1024	tr.
Eucalyptol	9.433	1035	1026	3.13
Ocimene, (*Z*)-β	9.625	1040	1032	0.24
Ocimene, (*E*)-β	10.029	1050	1044	0.04
trans-Sabinene hydrate	10.790	1069	1065	0.03
Linalool	12.104	1100	1095	0.19
2-Methylbutyl-3-methyl butyrate	12.450	1109	1111	0.07
3-Octyl acetate	13.121	1124	1123	0.06
trans-Sabinol	13.817	1140	1137	0.07
cis-p-Mentha-2.8-dien-1-ol	13.925	1142	1140	tr.
neo-Isopulegol	14.142	1147	1144	0.21
Menthone	14.629	1152	1148	38.19
Menthofurane	15.008	1164	1159	11.44
Menthol	15.479	1172	1167	28.35
Isomenthol	15.813	1185	1179	0.50
neo-Isomenthol	16.033	1190	1183	0.19
Myrtenol	16.367	1198	1194	0.04
cis-Piperitol	16.492	1202	1195	tr.
trans-Piperitol	16.808	1210	1207	tr.
Isopipetitenol	17.171	1220	1228	0.10
Isovaleric acid *cis*-3-hexenyl ester	18.100	1237	1238	0.10
Pulegone	18.250	1240	1240	0.24
Piperitone	18.925	1254	1249	1.42
neo-Menthyl acetate	19.879	1276	1271	0.18
Perilla alcohol	20.513	1289	1294	0.08
Menthyl acetate	20.775	1297	1304	8.39
p-Menth-1-en-9-ol	20.875	1298	1294	tr.
iso – Menthyl acetate	21.379	1310	1304	0.20
Dihydrocarveol acetate[τ]	21.571	1314	1310	tr.
α-Copaene	24.413	1374	1378	tr.
β-Bourbonene	24.817	1386	1387	0.32
β-Elemene	25.121	1392	1389	0.26
Caryophyllene(E)-β	26.337	1420	1417	1.52
β-Copaene	26.737	1432	1430	tr.
Himachalene	27.413	1443	1449	0.04
unknown sesquiterpene	27.479	1447	-	0.12
Humulene	27.783	1456	1452	0.16
E-β-Farnesene	27.862	1458	1454	0.08
cis-Muurola-4(15).5-diene[τ]	28.183	1466	1465	tr.
Germacrene D	28.979	1482	1484	1.81
Bicyclogermacrene	29.612	1500	1499	0.10
γ-Cadinene	29.975	1508	1512	0.14
δ-Cadinene	30.696	1525	1522	0.08
Germacrene D-4-ol	32.846	1576	1574	tr.
Sapathulenol	32.925	1580	1577	tr.
Caryophyllene oxide	33.167	1585	1582	0.18
Viridiflorol	33.517	1594	1592	0.24
epi-Cubenol [τ]	34.429	1625	1627	0.04
neo-Intermedol [τ]	35.971	1653	1658	0.16

[τ] -Tentatively identified, tr.—amount less than 0.01%. Prepared based on Sadowska et al. [19].

Studies conducted by Seidler-Łożykowską et al. [20,21] indicated little differentiation in the content of the main ingredients of carvone and limonene in oil obtained from the fruit of Kończewicki caraway. This supported the choice of this particular cultivar for the study, particularly coupled with the fact that it is the only cultivar of that species currently registered in the COBORU Register of Agricultural Plant Varieties. It is a variety of plant recommended for cultivation in Poland. The current study pertained to oil extracted from the fruit of Kończewicki caraway.

The chromatographic analysis of oil obtained from the caraway fruit (*Carum carvi* L.), Kończewicki cultivar, revealed the dominance of two compounds: carvone and limonene, constituting, respectively, 65.60 and 30.27% of the content (Table 2). The contents of other analyzed compounds fluctuated between 0.01 (α-thujone) and 0.16% (Carveol). When analyzing the content of essential oils in caraway fruit obtained from various cultivations and over a number of years, Bosko et al. [22] also observed a negative correlation between the overall oil content and the share of its respective constituents. Specifically, with increasing oil content, the concentration of limonene decreased in favor of carvone. Seidler-Łożykowska et al. [20] also demonstrated, in their studies on various caraway cultivars, a mutual, negative correlation between the two compounds; a higher carvone content in the oil always correlated with a lower content of limonene.

Table 2. Chemical composition of oil from Kończewicki caraway fruit.

Peak Name	Time (min)	% Composition
Carvone	45.17	65.60
Limonene	15.69	30.27
Dihydro-carvone	38,04	0.04
Dihydro-carveol	45.50	0.04
Carveol	49.91	0.16
α-thujone	25.25	0.01
α-pinene	9.16	0.05
β-pinene	11.83	0.02

Prepared based on Seidler-Łożykowska et al. [20,21].

The results of tests on the total content of chemically active compounds in peppermint and caraway are presented in Table 3. A decrease in the content of oils was observed in the case of peppermint after pulsed electric field application, irrespective of the number of pulses used, compared to the material not exposed to PEF. There were no statistical differences between the combinations where a different number of pulses were used. Heating of the substrate subjected to the pulsed electric field was noticed in relation to the herb not subjected to these procedures (Table 3). The highest temperature was recorded for peppermint herb after the application of 250 pulses, slightly lower values were obtained in the case of 350 pulses. However, given the values of the standard deviation for these combinations, the differences should be considered minimal.

Table 3. The average content of essential oils and temperature of the examined material after pulsed electric field application.

Species	Number of Pulses	Essential Oils (mL·100g^{-1}) $\pm$ SD	Temperature ($^\circ$C) $\pm$ SD
Peppermint	0	2.25 $\pm$ 0.017 a *	25.00 $\pm$ 1.00 d
	150	2.03 $\pm$ 0.006 b	34.06 $\pm$ 3.26 c
	250	2.02 $\pm$ 0.006 b	44.80 $\pm$ 1.93 a
	350	1.95 $\pm$ 0.01 b	40.40 $\pm$ 2.06 b
Carway	0	1.67 $\pm$ 0.006 a	25.00 $\pm$ 1.00 c
	150	1.73 $\pm$ 0.025 a	38.00 $\pm$ 1.00 b
	250	1.73 $\pm$ 0.006 a	41.00 $\pm$ 1.00 a
	350	1.77 $\pm$ 0.006 a	42.33 $\pm$ 0.58 a

*—averages with the same letter in the same column for the tested species did not differ statistically significantly according to Fisher's test at the significance level $p = 0.05$.

It was found that caraway fruit, regardless of the experimental combination, was characterized by a similar amount of essential oil. Similarly to peppermint, heating of caraway fruit subjected to PEF was noted. The fruit reached temperatures in the range of 38–42.3 °C.

In the available literature, there is no data on the impact of pulsed electric fields on the content of essential oils in peppermint herb and caraway fruits. A reduced amount of essential oils isolated from peppermint leaves subjected to PEF application was observed, irrespective of the number of pulses used, in relation to the material not subjected to these treatments. It seems that this may be due to the increasing temperature of the tested material during PEF application. Heating of peppermint above 34 °C may already promote a significant loss of essential oils. Similar observations in the case of sage dried within a comparable temperature range were made by Sellami et al. [23], and by Sadowska et al. [24] for sage and thyme. The results published by these authors indicate 0.3% to 0.26% loss of oil during convective drying at 45 °C compared to natural drying at a temperature of about 22 °C [23], and increase of said loss at drying temperatures between 35 and 40 °C by 0.23 mL·100 g^{-1} for sage, and 0.16 mL·100 g^{-1} for thyme. According to Vega-Mercado et al. [25] and Ngadi et al. [26], short pulses of electrical energy cause the perforation of cell membranes. Thus, loss of oils can occur during the PEF application itself. In the case of peppermint, the oils are located in glandular hairs located on the leaf surface [27]. It should be noted that the material under investigation was already dried, and, according to the observations previously made by Diaz-Maroto [28] using a scanning electron microscope on the leaves of *Mentha spicata* L., glandular hairs are already deformed under the influence of drying. Additional damage to cell membranes probably occurs during this treatment, and with simultaneous heating of the material, this leads to the loss of accumulated oils.

The basis for pharmaceutical qualification of caraway is the minimum content of essential oils [3]. In the case of caraway, this should be not less than 30 mL·kg^{-1}. It should be noted that the tested raw material did not meet the required standards in any experimental combination. The literature on the subject indicates a large span in the content of essential oils in caraway fruits, in the range of 1–7% [2]. The amount of essential oils in herbal plants is determined genetically, but is also modified by environmental or agrotechnical conditions [29]. The obtained results of this research were within the given range.

The PEF application to caraway schizocarps did not cause essential oil loss, as was the case with peppermint. On the contrary, even a small increase in the amount of isolated oils was noticed, however, this dependence was not confirmed statistically. Thus, the results obtained are more promising than in the case of peppermint. Morphological diversity of the location of essential oils in these two species should be noted. In caraway fruits, differently than in peppermint, they are located in oil canals. Studies on the ultrasonic treatment of caraway fruit before essential oil hydrodistillation process, carried out by Assami et al. [30], indicated faster release of the volatile fraction, but, similarly to the results presented in this paper, no major differences in their amount were found.

Figure 2 shows ATR-FTIR spectra for selected essential oil samples obtained for testing from peppermint and caraway fruit after pulsed electric field exposure at the respective doses (mentioned above). Figure 2A presents spectra of peppermint oils for field exposure dosed at 0, 150, 250 and 350 PEF (continuous lines: black, gray, blue, and red, respectively). The spectra obtained for caraway samples are presented in the same order in Figure 2B. The samples were applied onto the ZnSe crystal and tested under N$_2$ (as explained in the Materials and Methods section).
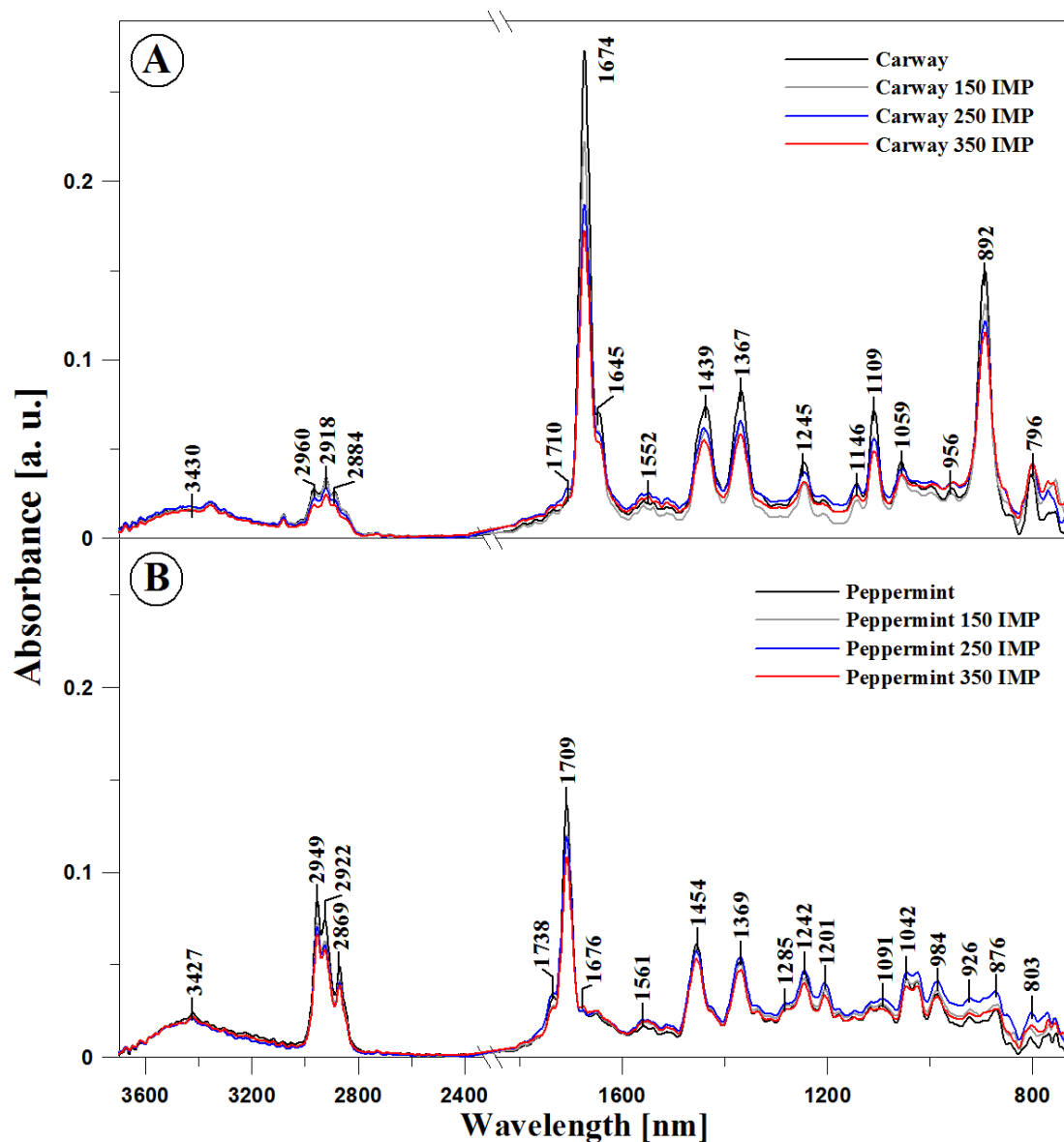
Figure 2. ATR-FTIR spectra of essential oils samples obtained from peppermint (**A**) and caraway fruit (**B**) after an application of pulsed electric field at different doses.

Table 4 presents all characteristic spectra observed in selected samples for essential oils of both species and for individual doses of pulsed electric field stimulation, as well as specific functional groups assigned to particular vibration spectra (with a detailed review of the literature).

Examination of peppermint and caraway essential oil samples after the application of pulsed electric field was performed with the use of FTIR infrared spectroscopy. It should be noted that all infrared spectra (ATR-FTIR) for essential oil samples selected for the study display very intense and distinct bands, which can be attributed to specific vibrations of appropriate functional groups, corresponding in turn to the components contained therein. Essential oils are mixtures of various compounds, for example, carvone (about 50%), limonene, pinene, cymol, and terpenic alcohols in the case of caraway oil. In the case of peppermint essential oils, the main components with a clear contribution to the FTIR spectra include: menthol and menthone (various isomers), piperitone, methyl acetate, and germacrene D.

Table 4. The location of the maxima of absorption bands FTIR [31,32] with arrangement of appropriate vibrations for selected for sampling: a—Caraway, b—Caraway 150 IMP, c—Caraway 250 IMP, d—Caraway 350 IMP, e—Peppermint, f—Peppermint 150 IMP, g—Peppermint 250 IMP, h—Peppermint 350 IMP made in terms of spectra 3700–730 cm^{-1}.

FTIR								Type and Origin of Vibrations
Position of Bands (cm^{-1})								
a	b	c	d	e	f	g	h	
3434	3427	3431	3423	3416	3428	3420	3432	−C=O$_w$ (overtone) and ν(=C–H$_{vw}$, *trans*-) or ν(–OH)
2973	2968	2972	2965	2950	2950	2950	2954	ν$_{as}$(–C–H$_m$, –CH$_3$, –CH$_2$)
2924	2923	2923	2920	2923	2926	2926	2918	ν$_{as}$(–C–H$_{vst}$, –CH$_2$) and ν$_s$(–C–H$_{vst}$, –CH$_a$) (alifatyczne grupy w trigricerydach)
2886	2889	2886	2886	2875	2817	2871	2871	
2845	2848	2852	2844	-	-	-	-	
-	-	-	-	1737	1737	1735	1733	ν(–C=O$_{vst}$) in ester
1705	1707	1707	1707	1708	1708	1706	1706	ν(–C=O$_{vw}$) in acid
1674	1674	1676	1676	1675	1675	1675	1675	ν$_{vw}$(–C=C–, *cis*-)
1649	1645	1639	1645	1648	1648	1650	1648	ν$_{vw}$(–C=C–, *cis*-) and δ(–OH)
1557	1559	1551	1551	1559	1557	1562	1562/1510	δ$_{vw}$(–C–H) in CH$_2$ and CH$_3$ group, bending (scissoring) or ν$_{vw}$(–C–H, *cis*-) bending (rocking)
1438	1440	1440	1440	1458	1454	1454/1421	1454/1415	
1372	1368/1329	1368/1320	1368/1324	1372/1337	1368/1281	1372/1331	1372/1335	ν$_{w, m, vw}$(–C–H, –CH$_3$), banding
1246	1246	1246	1246	1285/1244	1246	1281/1246	1281/1246	ν$_m$(–C–O) or δ$_m$(–CH$_2$-)
1205	1213	1203	1217	1203	1207	1205	1203	
1141	1143	1143	1143	1116	1116	1178	1112	ν$_{st}$(–C–O) or δ$_{st}$(–CH$_2$–)
1108	1109	1109	1108	1094	1091	1149	1094	ν$_m$(–C–O)
1055	1059	1059	1057	1044/1023	1048/1028	1091/1044	1046/1026	ν$_{m,vw}$(–C–O)
999	999	958	954	991/923	986/923	1030	982	δ$_w$(–HC=CH–, *trans*-) bending out of plane
958	960	891	891	873	869	986/925	925	
893	893	798	802	805	-	871	867	δ$_{vw}$(–HC=CH–, *cis*-) bending out of plane
798	820	-	-	-	805	801	799	δ(–(CH$_2$)$_n$– and –HC=CH– (*cis*-) bending (rocking)
761	-	767	769	770	756	770/758	768/756	

ν—stretching vibrations, δ—deformation vibrations, s—symmetric, as—asymmetric, st—strong, vst—very strong, w—weak.

In many studies, the authors have made appropriate adjustments to specific bands in the spectra of essential oils of plant origin of various species [33–36] regarding specific vibrations in molecules or their moieties. However, many bands are difficult to properly assign to a specific functional group, which may correspond to the content of a given amount of different substances (the reason is the fact that the substances present in particular oils often have similar chemical compositions). Table 4 presents in detail the frequencies of the characteristic spectra, along with the major extensions of the respective bands in the spectra of essential oil samples selected for the study, their assignment to the appropriate functional groups (with appropriate review and comparison with literature data [33]. The bottom indices also show the intensity of observed bands in typical spectra in the IR region. It should be noted that the assignment of spectra in the present case, corresponding to the tensile vibrations in the infrared spectra of such samples, is usually easier than the assignment of bands corresponding to deformation vibrations (this is often caused by the overlap of the bands responsible for the type of vibrations [33–35].

Vibrations of the methylene group, located in the spectral range from 1375 to 1150 cm^{-1} can be observed in the presented FTIR spectra of two of the species' essential oil samples selected for testing [33,34]. These are, in this case, the stretching vibrations derived from the vibrations of the group –C–H associated with the group –CH$_3$ and the deformation vibrations in this group (~1140–1180 cm^{-1} in our case). These bands seemed to be much richer in the case of oils derived from caraway than from peppermint, which was caused by a much greater number of these groups in the ingredients (listed above) in oils from caraway. It is also worth noting that tensile vibrations of the ester bond ν (C–O) consist of two connected asymmetric vibrations, namely vibrations of the C–C (=O)–O and O–C–C groups [35]. The first of these vibrations is usually more intense [36,37]. These bands occur in the region between 1300 and 1200 cm^{-1} (like C–C(=O)–O, in our case, about 1285 cm^{-1} (in the case of samples from caraway, practically invisible in peppermint oils), as a sub-band with a maximum at 1236–1238 cm^{-1}), and about 1050–1090 cm^{-1} (in our case, 1050 to 1100 for these groups for both types of oils). Bands associated with saturated esters C–C(=O)–O are usually observed between 1240 and 1160 cm^{-1} (in the case of oil varieties selected for testing, ~1245 cm^{-1} for peppermint, and ~1242 cm^{-1} for caraway), while for unsaturated esters, the vibrations are more often formed at lower frequencies [33]. However, on the other hand, the O–C–O band originating from primary alcohols appears in the zone between 1090 and 1020 cm^{-1} (in the present case, the maximum of this band fell around 1059 for peppermint and 1042 cm^{-1} for caraway), while for secondary alcohols, this band usually appears with a maximum of approximately 1100 cm^{-1} (in our case, about 1109 for peppermint and 1091 cm^{-1} for caraway). Both types of vibration described above were present because of the small amount of methyl acetate in peppermint oils, in the case of caraway oils, they may belong to the vibrations of C–H groups. However, some authors often attribute the above-mentioned band (at about 1240 cm^{-1}) only to the bending vibrations of the methylene group beyond the plane [36–38]. Subsequently, the next two bands presented in Table 4 (as well as in Figure 1) may cause slight difficulties in their proper recognition: one band with a maximum at about 1439 cm^{-1} and the second with a maximum at about 1367 cm^{-1} (for peppermint), and 1454 and 1369 cm^{-1} (for caraway). The first of these vibrations, with a maximum of about 1440–1460 cm^{-1} (depending on the sample, Figure 2), can be assigned to vibrations of methyl groups in aliphatic groups of oils selected for testing [33,38]. The second group of bands, with a maximum of about 1360–1370 cm^{-1} (for all samples), was observed simultaneously with bands with a maximum of about 960–990 cm^{-1} (for both types of oils), much more intensely for caraway than peppermint samples. It can be noted that the bands below 920 cm^{-1} (depending on what samples we are dealing with) that appear in all oil samples are related to tensile vibrations of cis-substituted olefinic groups [33], and may be related to vibrations of the vinyl group. These vibrations in peppermint oils come mainly from a high proportion of menthol and menthone, while in the case of caraway oils, mainly from carvone and limonene, as well as other substances containing the abovementioned groups.

The samples of oils selected for testing (in a given type) had quite similar spectra in the infrared area. However, depending on the type of the electric field factor used, they differed in terms of intensity.

No band shift was observed (in a given type of oil), which also confirms that the applied electric field did not modify the composition of the tested oils to a negative degree. With an increase in the number of pulses of the electric field in both cases, a decrease in the intensity of all observed bands can be noted, which was much larger for samples of peppermint than caraway oil. The decrease in intensity also indicates a slightly negative effect of the applied electric field factor in the case of peppermint oils on essential oil secretion. In the case of spectra obtained for caraway, the intensity decreased and confirmed the previously observed fact of the small field effect on the amount of essential oils obtained from a given plant.

Other very characteristic areas of vibration included the bands with a maximum at about 1745–1640 cm^{-1}, characteristic of the stretching vibrations of the carbonyl group C=O [33] in menthol esters for peppermint oils (as well as small amounts of piperitone and methyl acetate). In the case of caraway essential oils, the main source of vibrations of the carbonyl group came from the carvone constituting its main component (in this case, a much smaller contribution may come from other substances). In the case of these bands, significant differences were observed between peppermint and caraway samples. In the case of samples of peppermint essential oils, we observed a very intense band with a maximum at approximately 1674 cm^{-1}, with sub-bands at 1710 and 1645 cm^{-1}. In turn, in the case of caraway oils, we observed a very intense band at 1709 cm^{-1}, as well as sub-bands at 1738 and 1676 cm^{-1}. Bands with a maximum at approximately 1710–1715 cm^{-1}, also corresponding to the carbonyl group vibrations, are characteristic for acid groups of oil samples selected for testing [33,39]. Differences in the intensity of these groups (significant ones) indicate different factions of substances such as menthone (in the case of peppermint) or carvone (in the case of caraway) in selected oil samples. The subsequent bands with a maximum at 1552–1561 cm^{-1} (depending on the type of oil) are the vibrations that originate from the stretching vibrations of the –C=C– group (from the cis- transformation) [33,40]. A very characteristic area also included vibrations with a maximum at 1439 cm^{-1} (in peppermint) or 1454 cm^{-1} (in caraway), derived from deformation vibrations of the groups –C–H in the groups CH$_2$ and CH$_3$ (bending vibrations). In addition, it is also worth mentioning the vibrations in the area from 900 to 650 cm^{-1}, which in our case represented characteristic deformation vibrations belonging to –HC=CH– groups (cis- conformation, beyond the plane) and swinging vibrations of the above mentioned groups (δ(–(CH$_2$)n- and –HC=CH– (cis-)) [33].

Next, moving to vibrations in the range of larger wave numbers, very significant stretching vibrations =C–H (trans- transformation) with a maximum at about 3400 cm^{-1} (Table 3) derived from vibrations of the menthol and menthone fractions (for peppermint) and carvone and limonene in the case of caraway oils should be mentioned [33,34,41,42]. The vibrations from this area also indicated a small amount of water present in both types of oils (or stretching vibrations of the –OH groups). The vibrations from this area can also came mainly from menthol (in peppermint). The vibrations with a maximum at about 2965 to 2850 cm^{-1} were derived successively from the C–H stretching vibrations in the –CH$_3$, CH$_2$ groups (symmetrical or asymmetrical) belonging to the aliphatic groups for the compounds mentioned above (such as menthol, menthone, or carvone and limonene for caraway) [33,42].

For the majority of the examined samples, a slight enhancement of the band at 1738 cm^{-1} (responsible for the vibrations of the C=O group, as already described above) (for peppermint about 1710 cm^{-1}) on the side of the lower wave numbers was clearly visible (more clearly for caraway) [43], which can also be attributed to hydrogen bond formation between C=O . . . H–O–H groups (in the samples tested). The appearance of the band at 1710–1712 or 1738 cm^{-1} was accompanied by intense bands at about 1350–1370 and below 800 cm^{-1} [34,40,42], which can also be attributed to stretching vibrations in C–O and C–C groups (described above). These bands, along with the variable amount of water in a given sample and the possibility of hydrogen bond formation between C=O . . . H–O–H, may show a slight increase in their intensity.

Chemometric analysis including principal component analysis (PCA) was used in order to determine the similarity between the respective samples' FTIR spectra. PCA is a method that extracts

spectral information and uses the maximum variance principle. Furthermore, the data reduction methods which provide decompositions of an FTIR spectrum in terms of principal component loading use the new low-dimensional variables instead of the original high-dimensional variables to achieve data dimension reduction (Figure 3) [44,45].
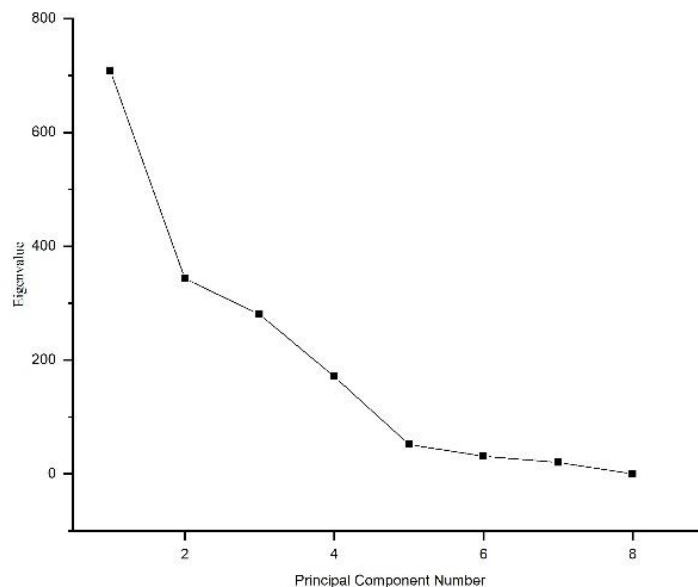

Figure 3. Scree plot for principal components (PCs).

Figure 4 shows the PCA score plots in PC1 × PC2 space, obtained from the correlation matrix of absorbancies in the region of 4000–600 cm^{-1}.
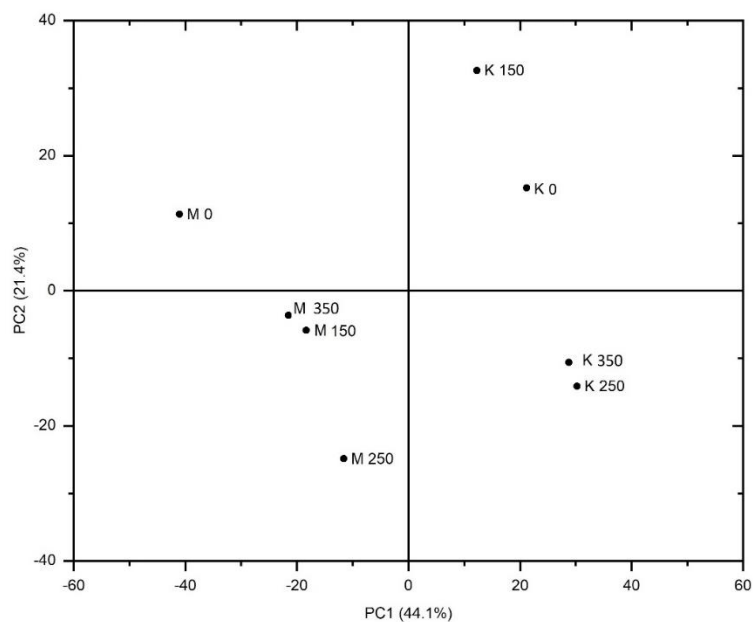

**Figure 4.** The score plot of PC1 and PC2 for all samples.

The oils' FTIR spectra were rather similar and only small variations could be seen between the different structures, illustrating that although the technique is very efficient at identifying band regions, the mean spectra give little insight into the basis of differentiation at a molecular structure level. PCA can provide further insight into the source of the spectral variability, meaning the differentiation of the different band regions.

PCA results showed that six factors were needed in order to explain almost all variability (98.76%, see Table 5). Based on the percentage of variance criterion, four factors qualified. In contrast, using the Scree plot (Figure 3) provided us with one more factor. In order to simplify interpretation of oil FTIR spectra, this analysis is aimed at understanding the information contained in the first two PCs.

Table 5. Values of Eigenvalue and percentage of variance with cumulative corresponding to each principal component.

Principal Component Number	Eigenvalue	Percentage of Explained Variance (%)	Cumulative Percentage of Explained Variance (%)
1	708.81	44.14	44.14
2	343.05	21.36	65.50
3	280.25	17.45	82.95
4	171.05	10.65	93.60
5	51.65	3.22	96.81
6	31.25	1.95	98.76
7	19.94	1.24	100
8	5.29×10^{-28}	3.29×10^{-29}	100

Factor loadings for the PC1 and PC2 are shown in Figure 5. The loading with reference spectra (Figure 5a,b, spectra of K0 and M0) shows that PC1 and PC2 had negative values, which can be attributed to C–H stretching and –C–O stretching vibrations from around 1400–1100 nm, and 3600–3400 nm for a –C=O stretching weak overtone and =C–H or –OH stretching vibrations.
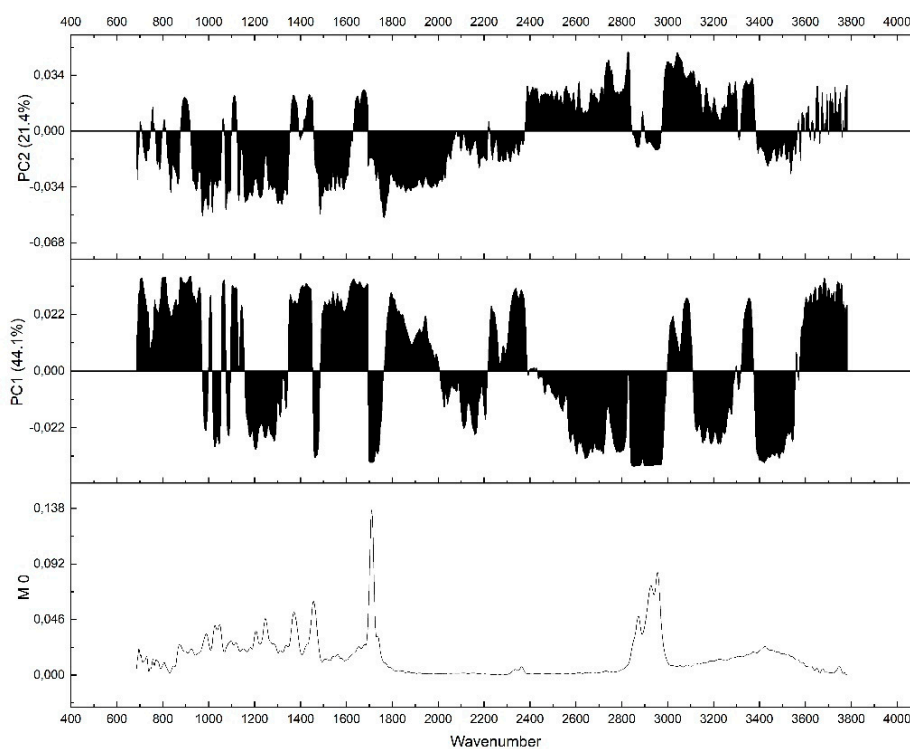
(a)

Figure 5. *Cont.*
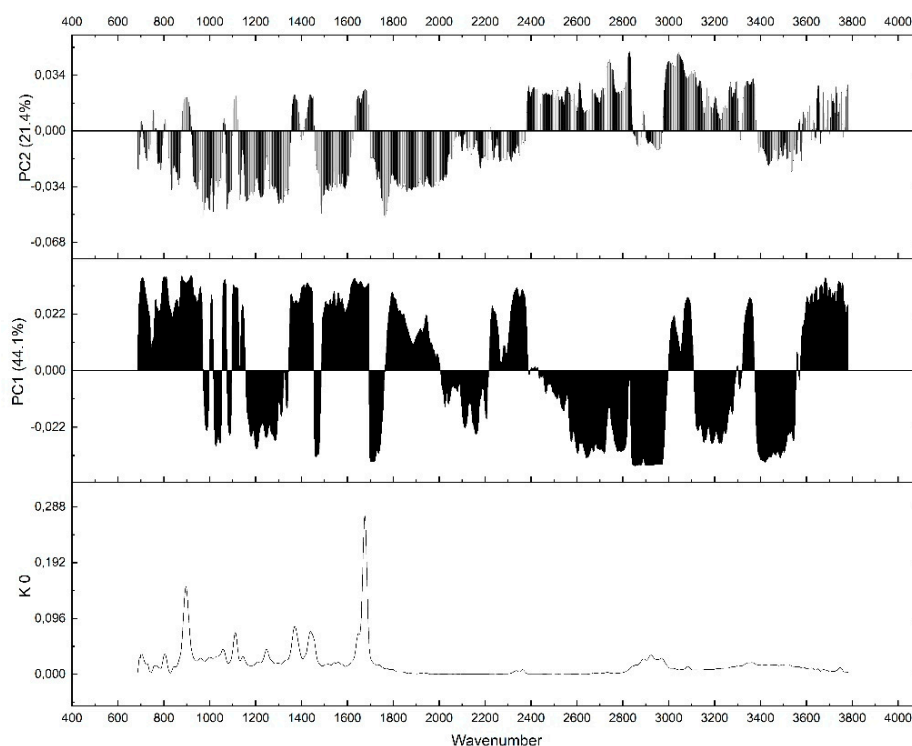
(**b**)

Figure 5. Loading plots of PC1 and PC2 for references spectra K0 (**a**) and M0 (**b**).

The PC1 respective negative and positive loadings contributed substantially to the differentiation of absorption around 1500–1400 nm and 1700–1600 nm, due to weak stretching deformation vibrations –C–H in CH_2 and CH_3 groups, bending (scissoring) or weak stretching vibrations (–C–H, cis-), bending (rocking), and very strong stretching vibrations –C=C–, cis-, –C=O attributed to esters or acids.

It is noteworthy that a correlation existed between the values of the loading and the positions of the spectra in the PCA plot (Figure 5a,b). Thus, there was a correspondence between the composition of the analyzed oil samples and the profile of the loading of PC1, which is a representation of the composition of each oil sample.

The score plots (Figure 4) reveal that among the studied samples, K0–K350 were separated on the positive side in PC1, but M0–M350 on the negative side of PC1. The groups were well defined and discriminated on the scores plot, indicating that the PCs are a clear representation of inter-group variance.

4. Conclusions

1. The PEF application does not lead to an increase in the yield of essential oils in dried peppermint herb and caraway schizocarps.

2. FTIR infrared spectroscopy is a method characterized by ease of use and non-invasiveness, and can be successfully used in the assessment of the quality of biological samples, even in small quantities.

3. The analysis of spectra (specific areas of wave numbers) in infrared FTIR gives a quick comparison between the quality of obtained oils by applying the diversified doses of the pulsed electric field. Moreover, changes in the appropriate bands can signal information about the composition (quality) of a specific variety of the obtained essential oils.

4. The changes in the obtained FTIR spectra were significantly emphasized in the chemometric analysis.

5. The greatest differences between the oils could be observed in the vibrations of bands associated with carbonyl groups, which indicates differences in terms of menthone (in the case of peppermint) and carvone fractions (in the case of caraway oils) contained in the oil. In addition, large changes were observed for groups characteristic of stretching vibrations of –CH$_2$ and –CH$_3$ groups, also present in the abovementioned substances.

6. The use of FTIR, combined with chemometrics to analyze the essential oil compounds, effectively classified and identified the samples. It could also suggest reasons for their varying chemical compositions. Moreover, the results obtained in this study can provide a comprehensive evaluation of sample oil quality and an optimization evaluation method for medicinal herb quality control.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "conceptualization, U.S. and A.M.; methodology, A.N. and U.S., A.M., T.D.; software, A.N., A.M.; validation, U.S., T.D. and A.Ż.; formal analysis, U.S.; investigation, U.S., A.M.; resources, U.S.; data curation, U.S., A.N., A.M.; writing—original draft preparation, U.S., A.M., A.N.; writing—review and editing, U.S., A.M., A.N., T.D., A.Ż.; visualization, U.S.; supervision, U.S, A.M.; project administration, U.S.; funding acquisition, T.Ż.".

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mikołajczyk-Grzelak, N. Produkcja roślin zielarskich w Polsce. *Roczniki Naukowe Stowarzyszenia Ekonomistów Rolnictwa i Agrobiznesu* **2008**, *10*, 270–273. (In Polish)
2. Seidler-Łożykowska, K.; Król, D.; Bocianowski, J. Content of essential oil and its composition in fruit originated from caraway (*Carum carvi* L.) collection. *Rośl. Oleiste—Oilseed Crops* **2010**, *31*, 145–158. (In Polish)
3. *European Pharmacopoeia 7*; Council of Europe Publishing: Strasbourg, France, 2011.
4. Bhat, S.V.; Nagasampagi, B.A.; Sivakumar, M. *Chemistry of Natural Products*; Narosa Publishing House: New Delhi, India, 2006; p. 115.
5. Iscan, G.; Kirimer, N.; Kürkcüoglu, M.; Baser, H.C.; Demirci, F. Antimicrobial screening of Mentha piperita essential oils. *J. Agric. Food Chem.* **2002**, *50*, 3943–3946. [CrossRef] [PubMed]
6. Edris, A.E.; Farrag, E.S. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. *Nahrung* **2003**, *47*, 117–121. [CrossRef] [PubMed]
7. Gupta, C.; Garg, A.P.; Uniyal, R.C.; Kumari, A. Antimicrobial activity of some herbal oils against common food-borne pathogens. *Afr. J. Microbiol. Res.* **2008**, *2*, 258–261.
8. Soković, M.; Glamočlija, J.; Marin, P.D.; Brkić, D.; van Griensven, L.J. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules* **2010**, *15*, 7532–7546. [CrossRef]
9. Skrinjar, M.M.; Mandic, A.I.; Misan, A.C.; Sakac, M.B.; Šaric, L.C.; Zec, M.M. Effect of mint (Mentha piperita L.) and caraway (*Carum carvi* L.) on the growth of some toxigenic Aspergillus species and aflatoxin B1 production. *Proc. Nat. Sci. Matica Srpska Novi Sad* **2009**, *116*, 131–139. [CrossRef]
10. Kumar, P.; Mishra, S.; Malik, A.; Satya, S. Insecticidal properties of Mentha species: A review. *Ind. Crop. Prod.* **2011**, *34*, 802–817. [CrossRef]
11. Bazhal, M.I.; Lebovka, N.I.; Vorobiev, E. Pulsed electric field treatment of apple tissue during compression for juice extraction. *J. Food Eng.* **2001**, *50*, 129–139. [CrossRef]
12. Praporscic, I.; Shynkaryk, M.V.; Lebovka, N.I.; Vorobiev, E. Analysis of juice colour and dry matter content during pulsed electric field enhanced expression of soft plant tissues. *J. Food Eng.* **2007**, *79*, 662–670. [CrossRef]
13. Bouzrara, H.; Vorobiev, E. Beet juice extraction by pressing and pulsed electric field. *Int. Sugar J.* **2000**, *102*, 194–200.
14. Soković, M.D.; Vukojević, J.; Molecules, M. Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. *Molecules* **2009**, *14*, 238–249. [CrossRef] [PubMed]

15. Derwich, E.; Benziane, Z.; Taouil, R.; Senhaji, O.; Touzani, M. Aromatic Plants of Morocco: GC/MS Analysis of the Essential Oils of Leaves of Mentha piperita. *Adv. Environ. Biol.* **2010**, *4*, 80–85.

16. Stanojević, L.; Marjanović-Balaban, Ž.D.; Kalaba, V.; Stanojević, J.; Cvetkovic, D.; Cakic, M.; Gojkovic, V. Chemical Composition and Antibacterial Activity of the Essential Oil of *Menthae piperitae* L. *Qual. Life (Banja Luka)* **2018**, *16*, 1–2.

17. Stanojevic, L.J.; Marjanovic-Balaban, Ž.; Kalaba, V.; Stanojevic, J.; Cvetkovic, D. Chemical Composition, Antioxidant and Antimicrobial Activity of Chamomile Flowers Essential Oil (*Matricaria chamomilla* L.). *J. Essent. Oil-Bear. Plants* **2016**, *19*, 2017–2028. [CrossRef]

18. Schmidt, E.; Bail, S.; Buchbauer, G.; Stoilova, I.; Atanasova, T.; Stoyanova, A.; Krastanov, A.; Jirovetz, L. Chemical Composition, Olfactory Evaluation and Antioxidant Effects of Essential Oil from Mentha x piperita. *Nat. Prod. Res.* **2009**, *4*, 1107–1112. [CrossRef]

19. Sadowska, U.; Żabiński, A.; Szumny, A.; Dziadek, K. An effect of peppermint herb *(Mentha piperita* L.) pressing on physico-chemical parameters of the resulting product. *Ind. Crop. Prod.* **2016**, *94*, 909–919. [CrossRef]

20. Seidler-Łożykowska, K.; Król, D.; Bocianowski, J. Zawartość olejku eterycznego i jego skład w owocach pochodzących z kolekcji kminku zwyczajnego (*Carum carvi* L.). *Rośl. Oleiste—Oilseed Crops* **2010**, *31*, 147–160.

21. Seidler-Łożykowska, K.; Barańska, M.; Barański, R.; Krol, D. Raman Analysis of Caraway (*Carum carvi* L.) Single Fruits. Evaluation of Essential Oil Content and Its Composition. *J. Agric. Food Chem.* **2010**, *58*, 5271–5275. [CrossRef]

22. Bosko, R.; Vagnerova, L.; Pluhackova, H.; Sofrova, J.; Smirous, P. The variability of caraway (*Carum carvi* L.) essential oils. *MendelNet* **2016**, *23*, 30–34.

23. Sellami, I.H.; Rebey, I.B.; Sriti, J.; Rahali, F.Z.; Limam, F.; Marzouk, B. Drying sage (*Salvia officinalis* L.) plants and its effects on content, chemical composition, and radical scavenging activity of the essential oil. *Food Bioprocess. Technol.* **2012**, *5*, 2978–2989. [CrossRef]

24. Sadowska, U.; Kopeć, A.; Kourimska, L.; Zarubova, L.; Kloucek, P. The effect of drying methods on the concentration of compounds in sage and thyme. *J. Food Process. Preserv.* **2017**, *41*, 1–11. [CrossRef]

25. Vega-Mercado, H.; Pothakamury, U.R.; Chang, F.J.; Barbosa-Cànovas, G.V.; Swanson, B.G. Inactivation of Escherichia coli by combining pH, ionic strength and pulsed electric fields hurdles. *Food Res. Int.* **1996**, *29*, 117–121. [CrossRef]

26. Ngadi, M.O.; Bazhal, M.I.; Raghaven, G.S.V. Engineering aspects of pulsed electroplasmolysys of vegetable tissues. *Agric. Eng. Int.* **2003**, *5*, 1–10.

27. Shanker, S.; Ajaykumar, P.V.; Sangwan, N.S.; Kumar, S.; Sangwan, R.S. Essential oil gland number and ultrastructure Turing Mentha arvensis leafonkogeny. *Biol. Plant.* **1999**, *42*, 379–387. [CrossRef]

28. Díaz-Maroto, M.C.; Pérez-Coello, M.S.; González, M.A.V.; Cabezudo, M.D. Influence of drying on the flavor quality of spearmint (*Mentha spicata* L.). *J. Agric. Food Chem.* **2003**, *51*, 1265–1269. [CrossRef] [PubMed]

29. Raut, J.S.; Karuppayil, S.M. A status review on the medicinal properties of essential oils. *Ind. Crop. Prod.* **2014**, *62*, 250–264. [CrossRef]

30. Assami, K.; Pingret, D.; Chemat, S.; Meklat, B.Y.; Chemat, F. Ultrasound induced intensification and selective extraction of essential oil from *Carum carvi* L. seeds. *Chem. Eng. Process.* **2012**, *62*, 99–105. [CrossRef]

31. Gandolfi, M.; Taddei, P.; Pondrelli, A.; Zamparini, F.; Prati, C.; Spagnuolo, G. Demineralization, Collagen Modification and Remineralization Degree of Human Dentin after EDTA and Citric Acid Treatments. *Materials* **2019**, *12*, 25. [CrossRef]

32. Alosmanov, R.; Imanova, J.; Wolski, K.; Ziemmermann, R.; Fiejdasz, S.; Przewoźnik, J.; Goc, K.; Kapusta, C.; Zapotoczny, S.; Szuwarzyński, M. Fabrication of Functional Carbon/Magnetic Nanocomposites as A Promising Model of Utilization of Used Crosslinked Polymers. *Materials* **2018**, *11*, 2595. [CrossRef]

33. Guillen, M.D.; Cabo, N. Infrared spectroscopy in the study of edible oils and fats. *J. Sci. Food Agric.* **1997**, *75*, 1–11. [CrossRef]

34. Guillén, M.D.; Cabo, N. Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chem.* **2002**, *77*, 503–510. [CrossRef]

35. Rohman, A.; Che Man, Y.B. Monitoring of virgin coconut oil (VCO) adulteration with palm oil using Fourier transform infrared spectroscopy. *J. Food Lipids* **2009**, *16*, 618–628. [CrossRef]

36. Tapp, H.S.; Defernez, M.; Kemsley, E.K. FTIR spectroscopy and multivariate analysis can distinguish the geographic origin of extra virgin olive oils. *J. Agric. Food Chem.* **2003**, *51*, 6110–6115. [CrossRef] [PubMed]

37. Scholze, B.; Meier, D. Characterization of the water-insoluble fraction from pyrolysis oil (pyrolytic lignin). Part I. PY–GC/MS, FTIR, and functional groups. *J. Anal. Appl. Pyrolysis* **2001**, *60*, 41–54. [CrossRef]

38. Gurdeniz, G.; Ozen, B. Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data. *Food Chem.* **2009**, *116*, 519–525. [CrossRef]

39. García, M.J.L. Introduction. In *Characterization and Authentication of Olive and Other Vegetable Oils*; Springer: Berlin/Heidelberg, Germany, 2012; pp. 1–43.

40. Yang, D.; Ying, Y. Applications of Raman spectroscopy in agricultural products and food analysis: A review. *Appl. Spectrosc. Rev.* **2011**, *46*, 539–560. [CrossRef]

41. Cozzolino, D.; Smyth, H.E.; Gishen, M. Feasibility study on the use of visible and near-infrared spectroscopy together with chemometrics to discriminate between commercial white wines of different varietal origins. *J. Agric. Food Chem.* **2003**, *51*, 7703–7708. [CrossRef] [PubMed]

42. Vlachos, N.; Skopelitis, Y.; Psaroudaki, M.; Konstantinidou, V.; Chatzilazarou, A.; Tegou, E. Applications of Fourier transform-infrared spectroscopy to edible oils. *Anal. Chim. Acta* **2006**, *573*, 459–465. [CrossRef] [PubMed]

43. Bendini, A.; Cerretani, L.; Di Virgilio, F.; Belloni, P.; Bonoli-Carbognin, M.; Lercker, G. Preliminary evaluation of the application of the FTIR spectroscopy to control the geographic origin and quality of virgin olive oils. *J. Food Qual.* **2007**, *30*, 424–437. [CrossRef]

44. Anderson, T.W. *An Introduction to Multivariate Statistical Analysis*; Wiley: New York, NY, USA, 2003.

45. Bonnier, F.; Byrne, H.J. Understanding the molecular information contained in principal component analysis of vibrational spectra of biological systems. *Analyst* **2012**, *137*, 322–332. [CrossRef] [PubMed]