Research Article

Tomasz Oniszczyk*, Arkadiusz Matwijczuk*, Alicja Matwijczuk, Sławomir Kocira, Agnieszka Niemczynowicz, Maciej Combrzyński, Agnieszka Wójtowicz, Maciej Kuboń, Andrzej Kusz, Anna Oniszczyk

Impact of storage temperature and time on Moldavian dragonhead oil – spectroscopic and chemometric analysis

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Abstract: Moldavian dragonhead (*Dracocephalum moldavica* L.) is a plant endemic to Asia where it has been used for centuries for the production of essential oils. With the constantly growing demand for vegetable fats it is necessary to find new methods of ensuring their high quality from the moment of production until their eventual purchase by the consumer. Correct assessment of the quality of oleaginous products is therefore one of the intensively explored scientific problems that stimulates the development of innovative methods of analysing such substances. One of the newer and, most importantly,

faster alternatives in the context of analysing the quality of oil and the changes it undergoes during storage is offered by FTIR spectroscopy (Fourier-Transform Infrared spectroscopy). By measuring the relevant FTIR spectra, we were able to observe clear discrepancies in the intensity of bands characteristic of the aging processes taking place in the oleaginous samples due to the oxidation of their fat fractions. The chemometric analysis of FTIR spectra confirmed the high quality of the analysed oils. It is noteworthy that the analysed samples of Moldavian dragonhead oil did not show particularly extensive changes in terms of their FTIR spectra during long-term storage. It confirms their high durability and usability in food production as an excellent product. This is valuable information in order to make progress in the use of Moldavian dragonhead oil in food products.

***Corresponding author: Tomasz Oniszczyk**, Department of Thermal Technology and Food Process Engineering, University of Life Sciences in Lublin, Doświadczalna 44, 20-280 Lublin, Poland, E-mail: tomasz.oniszczyk@up.lublin.pl; **Arkadiusz Matwijczuk**, Department of Physics, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland, E-mail: arkadiusz.matwijczuk@up.lublin.pl; **Alicja Matwijczuk**, Department of Physics, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland; **Sławomir Kocira**, Department of Machinery Exploitation and Production Process Management, University of Life Sciences in Lublin, Głęboka 28, 20-400 Lublin, Poland; **Agnieszka Niemczynowicz**, Department of Analysis and Differential Equations, Faculty of Mathematics and Computer Science, University of Warmia and Mazury, Stłoneczna 54, PL-10-710 Olsztyn, Poland; **Maciej Combrzyński**, **Agnieszka Wójtowicz**, Department of Thermal Technology and Food Process Engineering, University of Life Sciences in Lublin, Doświadczalna 44, 20-280 Lublin, Poland; **Maciej Kuboń**, Department of Agricultural Engineering and Informatics, Faculty of Production Engineering and Energetics, University of Agriculture in Krakow, ul. Balicka 116B 30-149, Kraków, Poland; **Andrzej Kusz**, Department of Technology Fundamentals University of Life Sciences, Doświadczalna 50A, 20-680 Lublin, Poland; **Anna Oniszczyk**, Department of Inorganic Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland

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1 Introduction

In recent years researchers focusing on the problems of healthy nutrition have repeatedly emphasised that the primary factor influencing our health and quality of life is a properly composed diet. The increasing awareness of that fact among consumers has resulted in a growing tendency to look for foods that offer certain health benefits. This group of food products can include functional food produced with health-oriented additives, e.g. in the form of “cold pressed” vegetable oils [1]. Vegetable oils are a valuable addition to the daily diet. They are a source of considerable food energy as well as very important unsaturated fatty acids (USFA), phytosterols, and liposoluble vitamins. The vegetable oil most often found and used in households all over the world is rapeseed oil.

However, many organic food outlets now offer a variety of cold-pressed oils from the seeds of e.g. linseed, hemp, sea buckthorn, sesame, garden cress, blue poppy, or black caraway [2]. Cold-pressed oils can be used in the production of oil extracts, e.g. from black elder flowers, which are used in medicine and cosmetology caraway [2].

One of the particularly valuable oils, due to its chemical composition and content of unsaturated fatty acids, can be obtained from Moldavian dragonhead (*Dracocephalum moldavica* L.) [1]. The Lamiaceae family includes a number of species valued as spices, ornamental plants, or for their health or cosmetology related benefits, e.g. *np. Mentha*, *Salvia*, *Lavandula*, *Thymus*, *Rosmarinus* [3, 4]. The family also includes the dragonhead (*Dracocephalum*), a genus of approximately 70 species most of which are endemic to Central Asia. One member of this group is Moldavian dragonhead [4]. It originates from the Himalayas and southern Siberia but was brought to Europe in the 16th century under the name of *Melissa moldavica* or *M. Turcica* [5]. Due to its visually attractive blue or white, fragrant, melliferous flowers and leaves producing a strong lemon-like scent, it is a welcome addition to most gardens as a decorative, fragrant, and melliferous flower. The plant is valued by beekeepers, mainly due to its good honey output and ease of cultivation. Moldavian Dragonhead is relatively undemanding in terms of soil quality and nutrient availability, although it requires certain care in terms of maintaining correct soil moisture levels. The plant lives for only one year and does not reach excessive size (about 60 cm) and it favours moist and calcium-rich soils. Cultivation in sun-filled areas facilitates higher concentration of the essential oil in the flowers and stalks [4]. Dragonhead flowers collected at the top of the shoot in an apparent ear can, depending on the botanical form, be either blue or white. Similar to other plants from this family, the above ground section of the plant produces essential oils which, combined with large amounts of sugar-rich nectar, attract bees and other pollinating insects [6]. The essential oil has been used to isolate e.g. citral (geranial, neral) geraniol and geranyl acetate [7]. The main components of the herb, other than essential oils, include tannin, phenolic acids and flavonoids. The fruit is a schizocarp containing 4 achenes (seeds). The seeds are small (mass of one thousand seeds (MTN) of 1.8-2.2 g), black, with a shiny tegument and a white V-shaped marking [4]. The oil obtained from dragonhead by way of cold-pressing is rich in valuable unsaturated acids including: α -linolenic (60.0%), linoleic (20%), oleic (8.5%), palmitic (6.5%), and stearic (5.0%) [8-10]. The composition of both the essential oil and the oil pressed

from the seeds renders the plant a valuable resource in the pharmaceutical, cosmetic, and food industries [1, 4, 9].

The physicochemical parameters and content of pro- and anti-oxidative compounds in cold-pressed oils varies significantly from other such substances. The oils are characterised by higher peroxide value (PV) and acid value (AV), as well as lower anisidine value (AnV), which results from the absence of the refining stage. Compared to industrially produced oils, cold-pressed oils are characterised by a darker colour, which results from the content of chlorophyll and carotenoid pigments [5].

The quality of oil is influenced not only by the method of production (refined, cold-pressed), but also the duration, manner, and temperature of storage, exposure to sunlight and oxygen. Improper storage can result in changes in the product's chemical composition and deterioration of its sensory qualities. As a consequence of auto- and photooxidative processes, unsaturated fatty acids undergo oxidation producing fatty acid hydroxides. The use of improper packaging (e.g. clear glass bottles) may lead to photooxidation. The process entails singlet oxygen binding with unsaturated fatty acids, bypassing the stage of free radical reaction [5]. Autooxidation is a free radical process taking place in three stages: initiation, propagation, and termination. At the stage of initiation, a hydrogen atom is detached from the carbon atom located directly at the double bond of the polyunsaturated fatty acid particle, which leads a regrouping of double bonds and emergence of a stable configuration of conjugated bonds. The effects of peroxidation are evidenced by the electromagnetic radiation absorption spectra in the UV range generated by the system of conjugated bonds that are not present in natural fatty acids.

The oxidative changes taking place in oils may be monitored by measuring the following parameters: peroxide value (PV) and anisidine value (AnV) (i.e. parameters that are typically measured in studies of this type [27]). Moreover, the quality of oil is confirmed if its acid value (AV) and iodine value (IV) are within normal ranges, as is its fatty acid profile and composition of the non-tricycloglycerol fraction: concentration of tocopherols, chlorophyll and carotenoid pigments [5], as well as polyphenol and phytosterol compounds.

For this reason, it is very important to also develop new analytical and measurement methods of determining the quality of oil. It is expected that such methods ought to be quick, non-invasive and reliable [23-24].

The evaluation of the quality and usability of oils is a very important criterion, particularly in the context of edible oils and fats. However, traditional methods (which continue to be used by many researchers) of conducting

such evaluation is a time-consuming and laborious process. Recent studies indicate that FTIR spectroscopy combined with chemometric methodology can viably constitute an alternative analytical method of verifying the usability and quality of (edible) oils and fats and offers a simple, fast and environmentally friendly analysis. The technique has been successfully employed for the purposes of quantitative and qualitative measurements of the characteristics of various (edible) oils and fats in the course of numerous scientific studies [11-13]. Chemometric analysis has been applied to monitoring oil mixtures in food products, cosmetics, and pharmaceutical agents. In this context, the use of chemometrics entails relying on chromatographic properties in developing predictive class models capable of quickly processing data on the basis of quality. The current accessibility of IT solutions and multidimensional, chemometric analytical tools allowed this method to become commonly used in quantitative analyses pertaining fats and oils. Chemometrics (PCA) proved itself to be one of the most efficient statistical methods used in the food industry, particularly in the context of food analyses.

The main aim of the study discussed in this article was to apply the methodology of FTIR spectroscopy in combination with advanced chemometrics in order to evaluate the quality of oil obtained from Moldavian dragonhead, relative to the respective time and conditions of its storage. Moreover, the aforementioned methodology was used to conduct a detailed analysis of particular bands and identify the spectroscopic (infrared) markers (relevant bands) potentially reflecting the acceleration or inhibition of aging processes relative to the respective conditions of storage exposing the products to specific external stimuli.

2 Materials and methods

The research material consisted of oil pressed from the seeds of Moldavian dragonhead (*Dracocephalum moldavica* L.). Before pressing, the seeds were stored in bags, in room temperature. Both unheated and thermally processed seeds were used. The heat treatment entailed heating seeds to 70°C, 100 °C, and 130°C, on a metal tray placed in the laboratory drier for a period of 1 hour. The oil pressing process was conducted using a DUO screw press by Farmet (Czech Republic) with the efficiency of 18 to 25 kg/h and engine speed of 1500 rpm. A 10 mm nozzle was used. After pressing, the oil was left for 2 days to allow natural sedimentation, after which it was placed

in 10 cm³ dark-glass bottle impenetrable by sunlight. Oil samples selected for the study were placed in an argon atmosphere and stored at room temperature (20-22°C) or a refrigerator (7°C). The two samples selected for this study were characterised by constant pressing temperature: 80°C, storage in identical dark bottles and the same argon atmosphere. Therefore, the variables were: storage temperature (20-22°C or 7°C) and storage time. The oil obtained from pressing was analysed in terms of its fatty acid profile, acid value (AV), peroxide value (PV), anisidine value (AnV), and iodine value (IV). The general colour (GC) was determined along with the content of carotenoid and chlorophyll pigments, β -carotene, tocopherols, and PC-8. The fatty acid profile was determined with the use of gas chromatography combined with mass spectrometry. The oil samples were used to obtain methyl esters in accordance with PN-EN ISO 12966-2 and their division was conducted using a Trace GC Ultra chromatograph with an ITQ 1100 spectrometer (Thermo Scientific) with the use of a Rtx-2330 column (105 x 0,25 x 0,25 μ m) by Restek. The carrier gas was helium at constant flow rate of 1mL/min., the temperature range was from 60°C to 250°C (5°C/min.) and the injection temperature was 250°C.

Measurements of ATR-FTIR (Fourier-Transform Infrared Spectroscopy) background corrected spectra (25 scans for each sample) were carried out with the use of a HATR Ge trough (45° cut, yielding 10 internal reflections) crystal plate at 20°C, and were recorded with a 670-IR spectrometer (Agilent, USA). The Ge crystal was cleaned with ultra-pure organic solvents (Sigma-Aldrich). The instrument was continuously purged with argon for 40 minutes before and during measurements. Absorption spectra at a resolution of one data point per 1 cm⁻¹ were obtained in the 4000 and 950 cm⁻¹ region. Scans were Fourier-transformed and averaged with Grams/AI 8.0 software (Thermo Fisher Scientific, USA).

The FTIR spectra of the studied oils were analysed chemometrically. PCA (Principal Component Analysis) was based on the correlation matrix for 10 FTIR spectra of the analysed oils (2 samples per experiment, i.e. per each respective time frame). The analysis was conducted using the PCA for spectra application.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

The cold-pressed Moldavian dragonhead oil was characterised by a unique content of fatty acids. Over

90% of acids present in this oil are unsaturated acids, of which over 80% are polyunsaturated acids. The content of palmitic acid (C16:0) was 3.84%, palmitoleic acid (C16:1) – 0.19%, stearic acid (C18:0) – 1.71%, oleic acid (C18:1) – 6.81%, linoleic acid (C18:2) – 19.01%, α -linolenic acid (C18:3 (9,12,15)) – 67.91%, nervonic acid (C24:1) – 0.07%, and other acids approximately 0.47%. Due to its particularly high content of α -linolenic acid (exceeding even that in linseed oil), dragonhead oil offers certain valuable health benefits. It can be used both in the cosmetics industry for the production of high quality creams, as well as in food production and in diet supplements containing ω -3 (α -linolenic) acid.

3.1 FTIR studies

Figure 1 illustrates the ATR-FTIR spectra for the analysed samples of oil obtained from Moldavian dragonhead seeds stored at 7°C (refrigerated). The oil was pressed at the temperature of 80°C (the relevant information is presented in the Materials and Methods section). Figure 2 shows the FTIR spectra for the analysed samples of oil obtained for Moldavian dragonhead seeds stored at room temperature, 20–22°C. The experimental constants were: oil pressing temperature, type of bottle in which the oil was stored (in this case dark glass bottles) and the storage atmosphere (argon gas). The samples were spread on the ZnSe crystal and analysed under N_2 atmosphere. Tables 1 and 2 provide a description of all characteristic bands present in the oil samples selected for the study along with the related vibrations of particular functional groups (with a detailed literature review).

3.2 FTIR spectroscopic analysis of Moldavian dragonhead oil samples stored in various conditions.

All the FTIR spectra recorded for the selected Moldavian dragonhead oil samples revealed very intense and clearly defined bands which correspond to specific vibrations of the respective functional groups contained in the ingredients typically found in this type of food. Most edible vegetable fats and potential oleaginous products are substances composed primarily of various fractions of triglyceride groups, mainly differing in terms of the degree and form of the acyl groups' unsaturation as well as the length of their chains [14]. Numerous publications provide the appropriate associations of the particular spectral bands in oils, both animal and vegetable, and

other fats [15, 16] with specific vibrations in particles or groups thereof. Tables 1 and 2 present in detail the frequencies of characteristic spectra including the most significant broadenings of the respective spectral bands for the four analysed time-frames of oil sample storage, as well as their association with the respective functional groups [16, 17]. It is immediately apparent that the association of the maxima corresponding to stretching vibrations in the FTIR spectra of the analysed samples is considerably easier than assigning the maxima corresponding to deformation vibrations. This is because bands corresponding to vibrations of the latter type often tend to overlap. The presented absorption spectra registered for the oil samples selected for the study reveal vibrations of the methylene group located in the spectral range from 1350 to 1150 cm^{-1} [18]. In this case, they are stretching vibrations originating from the vibrations of the -C-H group bonded with the -CH₂ group (usually at approximately 1350–60 cm^{-1} , in our samples approximately 1370 cm^{-1}) and deformation vibrations in the same group (~ 1160 cm^{-1} , in our case 1159–1166 cm^{-1}). It is noteworthy that the stretching vibrations of the ester bond $\nu(C=O)$ are composed of two merged, asymmetric vibrations, specifically vibrations originating from the C-C(=O)-O and O-C-C groups [19]. The former are usually more intensive [20, 21]. The bands occur in the region from 1300 (as C-C(=O)-O, in our case at approximately 1270 cm^{-1} , as enhancement of the band with the maximum at 1235–9 cm^{-1}) and approximately 1000 cm^{-1} (in our case 1028 to 1036 cm^{-1} for this combination of groups – not shown in Figures 1 and 2). Bands related to saturated esters C-C(=O)-O are present between 1240 and 1160 cm^{-1} (in the case of oils selected for the study approximately 1234–9 cm^{-1}), whereas for unsaturated esters, the vibrations are more often found at lower frequencies [22]. On the other hand, the O-C-O band originating from primary alcohols appears in the region from 1100 to 1020 cm^{-1} (in the analysed case, the band's maximum was located at approximately 1029–31 cm^{-1} , as already mentioned above), whereas in the case of secondary alcohols, the band usually appears with the maximum at approximately 1100 cm^{-1} (in our case approximately 1090–1093 cm^{-1}). Both types of esters described above are present in triglyceride particles. In the literature, the mentioned band (~ approximately 1239–4 cm^{-1}) has been associated exclusively with out-of-plane bending vibrations of the methylene group [22]. Another two bands presented in Tables 1 and 2 (as well as in Figures 1 and 2) are somewhat more difficult to identify: the maximum of the first band is at approximately 1416–18 cm^{-1} and the maximum of the second at approximately 1320 cm^{-1} (most likely a band broadening, see Figures 1

Figure 1: ATR-FTIR spectra for selected Moldavian dragonhead oil samples stored at 7°C (refrigerated): a – immediately after pressing, b – two weeks after pressing, c – four weeks after pressing, d – 8 weeks after pressing.

Figure 2: ATR-FTIR spectra for selected Moldavian dragonhead oil samples stored at room temperature (20–22°C): a – immediately after pressing, b – two weeks after pressing, c – four weeks after pressing, d – 8 weeks after pressing. The spectra are presented within the spectral range from 900 to 3150 cm^{-1} .

Table 1: Positions of the maxima of absorption spectra and assignment to the relevant vibrations recorded for Moldavian dragonhead oil samples stored at 7°C (refrigerated), a – immediately after pressing, b – two weeks after pressing, c – four weeks after pressing and d – 8 weeks after pressing.

FTIR				Type and origin of vibrations
Position of bands [cm ⁻¹]				
a	b	c	d	
3010	3008	3011	3011	$\nu(\text{C-H}_m, \textit{cis-})$
2958	2961	2957	2961	$\nu_{as}(\text{-C-H}_{vst}, \text{-CH}_a)$
2925	2926	2926	2924	and $\nu_s(\text{-C-H}_{vst}, \text{-CH}_a)$ (aliphatic groups in triglycerides)
2853	2854	2854	2859	
1744	1743	1743	1742	$\nu(\text{-C=O}_{vst})$ in esters
1710	1711	1713	1701	$\nu(\text{-C=O}_{vw})$ in acids
1656	1650	1650	1650	$\nu_{vw}(\text{-C=C-}, \textit{cis-})$
-	-	1601	1620	
-	-	1593	1559	
-	-	-	1540	
-	-	-	1518	
1459	1460	1459	1460	$\delta_{vw}(\text{-C-H})$ w CH_2 and in CH_3 groups, deformation (scissoring)
-	-	-	1422	$\nu_{vw}(\text{-C-H}, \textit{cis-})$ deformation (ring)
1373/1394	1377	1374	1367/1391	$\nu_{w, m, vw}(\text{-C-H}, \text{-CH}_3)$ and deformation
1306	1309	1314	1313	$\delta_m(\text{-C-H}, \text{-CH}_3)$
1266	1269	1266	1268	$\nu_m(\text{-C-O})$ or $\delta_m(\text{-CH}_2-)$
1236	1237	1234	1226	$\nu_m(\text{-C-O})$ or $\delta_m(\text{-CH}_2-)$
1161	1161	1164	1164	$\nu_m(\text{-C-O})$
1098	1099	1099	1102	$\nu_{m, vw}(\text{-C-O})$
1064	1067	1067	1067	
1028	-	-	-	
965	989	969	985	$\delta_w(\text{-HC=CH-}, \textit{trans-})$ out-of-plane deformation

ν – stretching vibrations, δ – deformation vibrations, s – symmetric, as – asymmetric, st – strong, w – weak

and 2). The first group of vibrations with their maximum at approximately 1416-18 cm⁻¹ (depending on the time of measurement) may be assigned to the vibrations of the methyl groups in the aliphatic chains of the oils selected for the study [14-16]. The second group of bands (i.e. band broadening) with the maximum at approximately 1320 cm⁻¹ (in all the samples – not shown so as not to obscure the presentation) is observed simultaneously with the bands with the maximum at approximately 980 cm⁻¹ and lower wave numbers. It should be noted that the band at approximately 918 cm⁻¹ which appears in all oil samples, is related to the stretching vibrations of *cis*-substituted olefin groups [14] and can be connected with the vibrations of the vinyl group.

The oil samples selected for the study produced largely similar spectra in the infrared range. However, with sample storage time significant differences in terms of the intensity and frequency of the respective bands (although the shifts were not large) became apparent. In each case we observed the maximum absorbance, which was clearly correlated to the particular storage time.

Other very characteristic vibration regions were also observed with respect to the bands with the maxima at approximately 1745-1 cm⁻¹, which were typical of the stretching vibrations of the carbonyl C=O group [14] in ester groups. Next to the band characteristic of the vibrations of the carbonyl group in esters we observed, on the lower wavenumber side, an enhancement with the maximum at

Table 2: Positions of the maxima of absorption spectra and assignment to the relevant vibrations for Moldavian dragonhead oil samples stored at 23°C (room temperature): a – immediately after pressing, b – two weeks after pressing, c – four weeks after pressing and d – 8 weeks after pressing.

FTIR				Type and origin of vibrations
Position of bands [cm ⁻¹]				
a	b	c	d	
3012	3010	3008	3010	$\nu(\text{=C-H}_m, \text{cis-})$
2960	2962	2961	2959	$\nu_{as}(-\text{C-H}_{vst}, -\text{CH}_a)$
2926	2929	2926	2924	and $\nu_s(-\text{C-H}_{vst}, -\text{CH}_a)$ (aliphatic groups in triglycerides)
2854	2859	2854	2852	
1745	1746	1744	1742	$\nu(\text{-C=O}_{vst})$ in esters
1711	1704	1709	1711	$\nu(\text{-C=O}_{vw})$ in acids
1656	1654	1657	1651	$\nu_{vw}(\text{-C=C-}, \text{cis-})$
-	-	-	1621	
-	-	-	1559	
-	-	-	1541	
-	-	-	1512	
1457	1458	1458	1460	$\delta_{vw}(-\text{C-H})$ w CH_2 and in CH_3 groups, deformation (scissoring)
-	-	-	1422	$\nu_{vw}(-\text{C-H}, \text{cis-})$ deformation (ring)
1373/1393	1375	1372	1370	$\nu_{w, m, vw}(-\text{C-H}, -\text{CH}_3)$ and deformation
1312	1305	1305	1312	$\delta_m(-\text{C-H}, -\text{CH}_3)$
1269	1264	1263	1271	$\nu_m(-\text{C-O})$ or $\delta_m(-\text{CH}_2-)$
1236	1233	1233	1231	$\nu_m(-\text{C-O})$ or $\delta_m(-\text{CH}_2-)$
1162	1162	1162	1159	$\nu_m(-\text{C-O})$
1100	1097	1099	1099	$\nu_{m, vw}(-\text{C-O})$
1067	1066	1069	1069	
1028	1023	-	-	
964	983	969	980	$\delta_w(-\text{HC=CH-}, \text{trans-})$ out-of-plane deformation

ν – stretching vibrations, δ – deformation vibrations, s – symmetric, as – asymmetric, st – strong, w – weak

approximately 1700-15 cm⁻¹, which also corresponds to the vibrations of a carbonyl group, but in this case found in the acidic groups of the analysed samples [13].

The next band, with the maximum at 1655-3 cm⁻¹ corresponds to the stretching vibrations of the -C=C- group (from the *cis*- transformation) [15]. The intensity of those bands increased with longer storage time of the respective samples, which clearly evidences ongoing ageing processes (discussed further in the text). A very characteristic region is also observed for the vibrations with the maximum at 1461-3 cm⁻¹ and originating from the -C-H deformation vibrations in CH₂ and CH₃ groups (bending vibrations). Moreover, the vibrations in the

region from 900 to 650 cm⁻¹ (partially not presented) corresponded in the analysed case to the characteristic deformation vibrations of the -HC=CH- groups (out-of-plane *cis*- conformation) and ring vibrations of the aforementioned groups ($\delta(\text{-(CH}_2)_n\text{-}$ and -HC=CH- (*cis* -)) [21].

Next, proceeding to the range of higher wavenumbers, one should mention the very important =C-H stretching vibrations (*trans*- transformation) with the maximum at approximately 3063-4 cm⁻¹ (not shown due to low intensity of these vibrations) which originate from the vibrations of triglyceride fractions [14]. In turn, with respect to the =C-H stretching vibrations in the *cis*- configuration, the

very characteristic and intense vibrations were observed with the maximum at approximately $3007/12\text{ cm}^{-1}$ (Figures 1 and 2, Tables 1 and 2). Vibrations with the maxima at approximately $2952/8$, $2922/8$, and $2852/7\text{ cm}^{-1}$ originate, respectively from the -C-H stretching vibrations in -CH_3 , CH_2 groups belonging to the aliphatic groups in triglycerides [18, 23].

It should be emphasised that the spectra of Moldavian dragonhead oil samples stored in different conditions revealed clear discrepancies in the shape of bands located in the region from 1780 to 1670 cm^{-1} [14]. Most analysed samples showed a clearly defined, slight enhancement of the band at $1743/6\text{ cm}^{-1}$ (corresponding to the vibrations of the C=O group, as already discussed above) on the lower wavenumber side, with a clear maximum at approximately $1700\text{-}16\text{ cm}^{-1}$ [11], which can be associated with the formation of a hydrogen bond between $\text{C=O}\cdots\text{H-O-H}$ groups (in the analysed samples). Simultaneously to the emergence of the band at $1700\text{-}16\text{ cm}^{-1}$, we observed a clear increase in the intensity of the bands at approximately $1350\text{-}70\text{ cm}^{-1}$ [22, 24] which can also be associated with the stretching vibrations of C-O and C-C groups (as described above). Furthermore, the area between 1100 and 1300 cm^{-1} also corresponds to stretching vibration of C-O groups but indicates minor discrepancies between the analysed oil samples, regardless of the storage time. The bands may display a slight increase in intensity with the decreasing affinity of the particles that generate them toward for the formation of the hydrogen bond between $\text{C=O}\cdots\text{H-O-H}$.

In summary, the spectra obtained using IR spectroscopy revealed considerable differences. Bands wherein the significant differences were observed constitute the spectroscopic markers of the ageing processes taking place in the analysed Moldavian dragonhead oil samples. In particular, observation of peaks in the range 1715 to 1500 cm^{-1} in the samples stored for 8 weeks, revealed the significant changes in terms of the position and intensity of the band characteristic of the carbonyl group, with the maximum at approximately 1744 cm^{-1} [25-26]. The region from 1715 to 1500 cm^{-1} is related mainly to various vibrations originating from the C-C and C=C groups and evidencing the progress of ageing processes (with the oxygenation of fatty acids contained therein). One should also mention the band with the maximum at approximately 1426 cm^{-1} related to the vibrations of C-H groups in acids [23-26].

3.3 Chemometric studies

One of the most effective chemometric methods is Principal Component Analysis (PCA). In essence, PCA

involves converting data into combinations of numerous orthogonal principal components or factors. The data matrix is reduced to two matrices, the score matrix which pertains to information about the objects and the load matrix which pertains to the variables. Each principal component may be understood as a set of variables (e.g. FTIR spectrum) representing a certain primary data feature, while the loads are the values of absorbance or peak heights. The first principal component accounts for the maximum variance of the set, the second accounts for most of the remaining variance, and so on. Additionally, with the PCA method, the noise present in the data set which conveys no information is transferred to the further principal components, which means that the method can also be used for the purposes of data cleansing. The score matrix specifies the contribution of each object to each principal component. The use of PCA for the purposes of grouping objects takes place by verifying the score matrix. The score of the first primary component is usually drawn relative to the object of relative to the score of the second primary component with which each point is identified by the associated object.

PCA is one of the multivariate techniques for data analysis which plays a complementary role with HCA (Hierarchical Cluster analysis) through both data summarization and data reduction. They are statistical approaches that can be used to analyze interrelationships among a large number of variables and explain these variables in the terms of their factors. It means that we can find a way of condensing the information contained a number of original variables (in our case in FTIR spectral data) into a smaller set of variables (principal components) with minimum loss of information. On the other side, including the construction of a hierarchy of agglomerative structure (HCA), in which each object (considered oils samples) is compared by assessing the correspondence of the results. Therefore, the spectroscopic analysis of the analysed oil samples was combined with a chemometric principal component analysis and hierarchical cluster analysis.

In order to confirm visualization of the group and sub-group arrangement of the spectra we use the HCA analysis. Taking into account the similar areas of the groups we can distinguish the difference in the spectral range. Despite this, some questions remain still open. For instance, we are curious how the difference between the variations in the functional group influence the difference in the HCA analysis e.g. what is the band diversity and complication depending on the source of the sample. Therefore, PCA was used further in order to get answer into the above mentioned questions and others.

Figure 3: Plot of eigenvalues for PCA of FTIR spectra.

Figure 4: 3-dimensional loading plot of PCA.

The PCA analysis of the FTIR spectra of the analysed oils within the range $4000\text{--}500\text{ cm}^{-1}$ was conducted. The results are presented in Figures 4, 5, and 6. Based on the Scree Test Criterion (Figure 3) we identify the optimum number of principal components. In the present case, the first three principal components would qualify. The first three principal components accounted for 97.6% of the total variance of the original data set, where PC1 accounted for 79.2%, PC2 for 17%, and PC3 for 1.4% thereof (Figure 8). The first included oil samples d1 and d2 – stored for a period of 8 weeks at 7°C and 22°C respectively. The second included samples a1, c1, and c2 – Subgroup I-A and a2, b1,

Figure 5: 3-dimensional score plot of PCA.

Figure 6: 2-dimensional score plot in the plane of PC1 x PC2.

b2 – Subgroup I-B (Figure 6 and 8). The above grouping of the analysed oils may be due to the physicochemical properties of the respective oil samples' content (also demonstrated by the shift of the relevant infrared bands, Figs 1 and 2, Tables 1 and 2).

Based on the loading plot (Figure 4 and 7a, b) it can be concluded that PC1 is negatively correlated with bands related to bond vibrations $\delta_w(-\text{HC}=\text{CH}-, \text{trans-})$ (range $957\text{--}971\text{ cm}^{-1}$). Its correlation with the remaining vibration

a

b

Figure 7: Loading plot of PC1, PC2, PC3 for the reference spectrum (a) a1 and (b) a2.

Figure 8: Hierarchical Cluster Analysis (HCA) for the FTIR spectra of all oil samples.

bands is positive. Meanwhile, PC2 is negatively correlated with bands within the range of $643\text{--}1291\text{cm}^{-1}$, i.e. $\nu_m(-\text{C}-\text{O})$ or $\delta_m(-\text{CH}_2-)$, $\nu_m(-\text{C}-\text{O})$, $\nu_{m,vw}(-\text{C}-\text{O})$, $\delta_m(-\text{C}-\text{H}, -\text{CH}_3-)$, $\delta_w(-\text{HC}=\text{CH}-, \text{trans-})$ and the band $\nu_s(-\text{C}-\text{H}_{\text{vst}}, -\text{CH}_a)$ (aliphatic groups in triglycerides) (approximately 2854 cm^{-1}).

The use of the cluster analysis technique (HCA) with the Euclidean distance measurement confirmed the hierarchical structure of the samples' distribution in relation to the distribution of their FTIR spectra in the PCA (Figure 6). In this case, distance is converted into measures of similarity, where larger values denote less similarity. If we assume the "cut off" at the level of 0.1, we obtain two branches-two clusters. The first group (cluster) comprises two subgroups. Subgroup A is composed of oil samples a1, c1, and c2 while subgroup B of oil samples a2, b2, and b1. The second group (cluster) comprises oil samples d1 and d2. As easy to see, this analysis confirm the stability of the results obtained by PCA and identify a topology of similarity in considered oils samples based on their FTIR spectras.

4 Conclusions

Cold-pressed oil is a perfect alternative for olive oil as a product for which the respective stages of production can be tightly controlled with the view of preserving all of the oil's health benefits. Maintaining proper pressing and storage conditions can ensure that the resulting oil is of the highest quality. The paper presents the results of a primary analysis (identification of fatty acids present

in the oil) and spectroscopic characteristics combined with a chemometric analysis conducted for two samples of Moldavian dragonhead oil stored under different conditions determined by the storage temperature (room temperature or 7°C).

The analysed oil samples were characterised by a very good fatty acids profile, which confirmed their exceptional health benefits. The spectral analysis of the samples allowed quick and effective characterisation and quality control of the oil in storage, as well as processes affecting the same. Significant differences were observed in terms of bands associated in literature with various fat fractions contained in the oils. The clear discrepancies in IR spectra emerging after 8 weeks within the spectral ranges of $1720\text{--}1500\text{ cm}^{-1}$ and $\sim 1426\text{ cm}^{-1}$ provide markers which could provide evidence of the ageing processes taking place in the samples. Changes related to aging of the sample were related to intensification of bands reflecting the vibrations of C-C, C=C, and C=O groups and as such, they constitute perfect marker bands which can be easily correlated with the given oil's shelf life and the oxidative processes that affect it.

The combination of FTIR spectroscopy and chemometric methodology allowed the identification and classification of marker bands for the ageing processes. PCA and HCA methods were employed to facilitate the analysis. The studied oils were classified under two clusters (groups). The first component differentiated the samples relative to the time and conditions of storage. The samples were clearly distributed in a way to reflect their respective storage conditions. The first component accounted for 79.2% of the total data variance while PC2 for 17% of the same. Samples d1 and d2 were characterised by the highest value of PC1, whereas samples b1 and b2 by the highest value of PC2.

It is our hope that the obtained results may prove useful in identifying spectroscopic markers corresponding to the ageing processes taking place in oil samples. At the same time, the results demonstrate the usability of a reliable, quantitative method of detecting preliminary discrepancies between oil samples and the classification of the same, without the need to resort to the costly, standard chemical methods. It is also clearly observable that the bands gaining in intensity with increasing sample storage time can be grouped by employing the method of chemometric analysis.

Conflict of interest: Authors declare no conflict of interest.

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