Fluorescence Spectra and Chemical Composition of High Oleic Sunflower Oils with Herbs Oil Additives

Galia Gentscheva¹², Aleksandar Pashev¹, Krastena Nikolova³, Stefka Minkova⁴

¹Medical University-Pleven, Department of Chemistry and Biochemistry, Pleven, Bulgaria
²Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
³Medical University-Varna, Department of Physics and Biophysics, Varna, Bulgaria

E-mail: kr.nikolova@abv.bg

Abstract. Fluorescence spectra and chemical composition of cold-pressed sunflower oleic oils with addition of oils from: 1) basil (Ocimum basilicum), 2) rosemary (Rosmarinus officinalis) and 3) oregano (Origanum vulgare) were studied. The aim of the study is to compare chemical composition of cold pressed oil with and without addition of different plant oils. The concentrations of some elements (essential and toxic) in oil were determined by using Inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion. Infrared spectroscopic experiments (ATR and transmittance) and NMR spectrum were used to study the fatty acid profile of the analysed oils.

1. Introduction
Basil, rosemary and oregano have been known and used since ancient times as spices in food to improve the aroma and for medicinal purposes. In recent decades, their essential oils have been of great interest as they are a source of various natural products.

Various synthetic antioxidants are often used to prolong the storage and stability of industrially processed foods. For example, butylated hydroxytoluene (BHT) is widely used as an antioxidant in food, but some laboratory animal studies have questioned its safety [1]. Today, food consumers are increasingly concerned about the negative effects of synthetic additives on their health. That leads to an increasing need to find pure natural products with proven qualities to replace them [2, 3]. Because herbs are rich in antioxidants, vitamins, phenolic compounds, have antimutagenic, antibacterial and other properties, there is growing interest in their use as an alternative to synthetic additives. Oils of different types of herbs have low toxicity, are biodegradable, multifunctional and of low production value. Taking advantage of all these benefits can be a good start for their application in various fields - from agriculture and food production to medicine and pharmacy [4].

Rosmarinus officinalis L., family Lamiaceae, has a huge variety of applications in folk medicine. Infusions of stems and flowers are used to treat headaches and colds, as an analgesic and antispasmodic [5]. The leaves of this plant are considered to have one of the highest antioxidant activities [6]. Its essential oils have, in addition to antioxidant and antimicrobial properties against food pathogens [7, 8].

Basil (Ocimum basilicum L.) family Lamiaceae, one of the most popular herbs in cooking, is used both in flavouring and in food preservation [9]. In folk medicine, the anti-inflammatory, analgesic and
somewhat arousing effect of basil is used. In addition to antimicrobial properties [10], basil essential oil has been shown to have fungicidal activity [11].

*Oregano* (Origanum vulgare) family Lamiaceae, in the form of water infusions is used in folk medicine for respiratory diseases: bronchitis, bronchial asthma, pneumonia and others. The herb has a calming, hemostatic, expectorant, diuretic effect. Stimulates the secretion of the gastrointestinal tract, bile secretion, tones the smooth muscles of the uterus. Like the other two herbs, oregano has antioxidant and antibacterial properties [12, 13].

The aim of the present study is to evaluate the influence of basil, rosemary and oregano oil additives on the optical characteristics and chemical composition of cold-pressed high-oleic sunflower oils in relation to their potential for use in cooking, food industry, cosmetics, pharmacy and others.

2. Materials and methods

2.1. Samples
The samples analyzed in this study were purchased commercially and from one manufacturer
1. Cold-pressed sunflower oleic oils – SO
2. Cold-pressed sunflower oleic oils + basil oil – SO+B
3. Cold-pressed sunflower oleic oils + rosemary oil – SO+R
4. Cold-pressed sunflower oleic oils + oregano oil – SO+O

2.2. Analysis of elements.
Five samples of approximately 0.3 g of each oil were weighted in PTFE vessels. 6 ml of 67% HNO₃ (supra pure) and 2 ml of 30% H₂O₂ (supra pure) were added. Microwave digestion was performed as follows: 15 minutes to reach 200 °C and 15 minutes hold at it. After cooling samples were transferred into 50 ml volumetric flask and diluted to the mark with deionized water. Blank samples were prepared that way as well. Inductively coupled plasma mass spectrometer "X SERIES 2" - Thermo Scientific was used to determine elements concentrations [14].

2.3. Fluorescent analysis
Each sample was put in quartz cuvette and excited by a LED at λ = 245nm, 285 nm, 370 nm, 380 nm, 395 nm, 400nm, 420 by using a fiber optic spectrometer Brolight with sensitivity in the (200-1100) nm range and a resolution of about 8 nm. The spectra were recorded in the 250 to 800 nm region. The validity of the comparison between the different measures was made as each spectrum represents the average of 5 scans recorded after 100 s of excitation. We use these parameters for all the samples in order to measure in the same experimental conditions.

2.4. IR spectrum
The ATR-IR spectra were recorded on a Thermo Fischer Nicolet iS50 instrument featuring an ATR module with a diamond crystal.

2.5. NMR spectrum
The ¹H NMR spectra were recorded on a Thermo Scientific™ picoSpin™ 80 Series II NMR Spectrometer, operating at 82 MHz, with a 2T permanent magnet. The samples were prepared as follows: 0.5 mL of each oil was diluted with 0.5 mL CHCl₃ and the obtained solution was injected in the measuring cell [15].

3. Results and discussion
According to the European regulation for determining maximum levels of contaminants in foodstuffs, maximum lead content is 0.10 mg kg⁻¹ [16]. The four oils are purchased from a local store and are from one manufacturer, which does not guarantee that the cold-pressed sunflower oleic oils are from
the same batch as those of the cold-pressed sunflower oleic oils with an additive, so the results will be commented on in general.

For all oils, the concentrations of some toxic and essential elements given in Table 1 are determined. For the only regulated element lead, the results vary below the norm. The other two toxic elements As and Cd are present in the samples in trace amounts. Another important element for human health is selenium, but in Bulgarian soils it is found in very low concentrations, which is why it can rarely be found in foods from Bulgaria. Only magnesium is in concentrations of the order of 1-2 mg kg\(^{-1}\), all other tested elements are below these values. RSD of the obtained results is 1-7%. In conclusion, we can note that additives do not drastically change the elemental composition of oils.

| Table 1. Concentration of some essential and toxic elements in oils |
|-----------------|-------|-------|-------|-------|-------|
| oil             | Mg, mg kg\(^{-1}\) | Cr, mg kg\(^{-1}\) | Mn, mg kg\(^{-1}\) | Co, mg kg\(^{-1}\) | Ni, mg kg\(^{-1}\) |
| SO              | 0.89  | 0.25  | 0.021 | 0.001 | 0.052 |
| SO+B            | 1.11  | 0.21  | 0.039 | 0.011 | 0.043 |
| SO+R            | 1.63  | 0.18  | 0.021 | 0.002 | 0.033 |
| SO+O            | 1.00  | 0.14  | 0.016 | 0.004 | 0.031 |

(continued)

| oil             | Zn, mg kg\(^{-1}\) | Se, mg kg\(^{-1}\) | As, mg kg\(^{-1}\) | Cd, mg kg\(^{-1}\) | Pb, mg kg\(^{-1}\) |
|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| SO              | <0.0005           | <0.0005           | 0.001             | 0.002             | 0.052             |
| SO+B            | <0.0005           | <0.0005           | <0.0005           | 0.004             | 0.012             |
| SO+R            | <0.0005           | <0.0005           | <0.0005           | 0.002             | 0.016             |
| SO+O            | <0.0005           | <0.0005           | <0.0005           | 0.004             | 0.009             |

According to Sikorska et al. [17], vegetable oils include three major groups of natural fluorophores - tocopherols, chlorophylls and phenols. In our case investigated samples have similar peaks of emission which can be divided in four regions associated to the emission of:

- \(\alpha\)-tocopherols and phenolic components - from 250 nm to 400 nm [18];
- oxidation products [19] – from 400 nm to 500 nm;
- vitamin E (around 525 nm – 560 nm) [20];
- chlorophyll [20].

The most interesting fluorescence spectra are obtained for excitation wavelengths 245 nm and 370 nm. They are presented in figure 1.

**Figure 1.** a) Fluorescence spectra for excitation wavelength 245 nm; b) and 370 nm for cold pressed sunflower oil with addition of herbs

The analysis of the data takes into account the differences in the indicators of the sunflower oil tested with herbal supplements. The addition of rosemary, basil and oregano significantly increases the chlorophyll content. According to Figure 1a) the highest content of \(\alpha\)-tocopherols and phenolic components has the cold pressed sunflower oil with rosemary oil. The probe with oregano oil possess the highest content of oxidation products. The oils with oregano or bacilicum has the maximum of
fluorescence at 682 nm at excitation wavelength 370 nm. The oils with Rosemary oil or bacilicum has the great content of β-caroten and with this is connected content of oxidation products.

The IR spectral analysis in figure 2 confirms the above made conclusions. The functional groups of the major components in the oil samples can be clearly seen, especially those from oleic acid triglyceride derivatives. Notable functional groups include CO (ester) at around 1744 cm\(^{-1}\) and the CH stretch at about 2850 cm\(^{-1}\) and 2910 cm\(^{-1}\). The values are similar to those of free oleic acid, as found in NIST Standard Reference Database 69: NIST Chemistry WebBook.

![IR spectra](image)

**Figure 2.** IR spectra for: a) Cold-pressed sunflower oleic oils – SO; b) Cold-pressed sunflower oleic oils + basil oil – SO+B; c) Cold-pressed sunflower oleic oils + rosemary oil – SO+R; d) Cold-pressed sunflower oleic oils + oregano oil – SO+O.

The oleic acid derivatives were also observed by \(^1\)H NMR spectra recorded on a benchtop NMR instrument (figure 3). The technique used has several advantages – being relatively inexpensive and reliable. The oil samples analyzed, have very similar lipid fingerprints, as seen from the \(^1\)H NMR spectra of figure 3. The major signals, corresponding to different fragments from the oleic acid have been noted, according to the nomenclature for fatty acids. Peak assignments were made using data from the literature [15].
Figure 3. $^1$H NMR spectra for: a) Cold-pressed sunflower oleic oils – SO; b) Cold-pressed sunflower oleic oils + basil oil – SO+B; c) Cold-pressed sunflower oleic oils + rosemary oil – SO+R; d) Cold-pressed sunflower oleic oils + oregano oil – SO+O.

4. Conclusion
The results demonstrate some differences in the characteristics of the tested oils such as changes in the contents of α-tocopherols, phenolic components, β-carotene. Slight increase in Mg content and decrease in Pb. In general, the observed changes affect the quantitative composition, but not the qualitative composition of the oils. High oleic sunflower oil (with and without additives) is a good substitute for refined vegetable oils due to its high content of this useful fat.

Acknowledgments
The financial support of scientific grant № 18001 „Express methodology for examining the relationship between optical properties and antioxidant effects on extracts from medicinal plants and beverages from traditional Bulgarian fruits” (Competitive Session 2018 of the Scientific Research Fund at the Medical University - Varna) is acknowledged.

References
[1] Babich H. 1982 Butylated hydroxytoluene (BHT): A review, Environmental Res. 29 (1) 1-29
[2] Badia V., MSR de Oliveira, G. Polmann, T. Milkievich, AC Galvao, WD Robazza 2020 Effect of the addition of antimicrobial oregano (Origanum vulgare) and rosemary (Rosmarinus officinalis) essential oils on lactic acid bacteria growth in refrigerated vacuum-packed Tuscan sausage, Brazilian J. Microbiol. 51(1) 289-301
[3] Boskovic M., M. Glisic, J. Djordjevic, M. Starcevic, N. Glamoclija, V. Djordjevic, MZ Baltic
2019 Antioxidative Activity of Thyme (Thymus vulgaris) and Oregano (Origanum vulgare) Essential Oils and Their Effect on Oxidative Stability of Minced Pork Packaged Under Vacuum and Modified Atmosphere. J. Food Sci. 84(9) 2467-2474

[4] Kamaneh SAR, M. Qaraaty, M. Tabarrai, M. Mazidi, M. Mojahedi, M. Mazandarani, N. Behnampour, 2020, Effect of oregano oil (Origanum Vulgare L.) on chronic rhinosinusitis: A randomized, double-blind, clinical trial, Indian J. Traditional Knowledge 19(2) 341-349

[5] Al-Sereiti MR, KM Abu-Amer, P. Sen 1999 Pharmacology of rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials, Indian J. Exp. Biol. 3 124–130

[6] Peng Y., J. Yuan, F. Liu, J. Ye 2005 Determination of active components in rosemary by capillary electrophoresis with electrochemical detection, J. Pharm. Biomed. Anal. 39 431–437

[7] Wang W., N. Wu, Y.G. Zu, Y.J. Fu 2008 Antioxidative activity of Rosmarinus officinalis L. essential oil compared to its main components Food Chem. 108 1019–1022

[8] Nieto, G., G. Ros, J. Castillo 2018 Antioxidant and Antimicrobial Properties of Rosemary (Rosmarinus officinalis, L.): A Review Medicines 5 98

[9] Qing X. L. Chion, L. Chang 2016 Chapter 25 - Basil (Ocimum basilicum L.) Oils, Essential Oils in Food Preservation, Flavor and Safety 231-238

[10] Rattanachaikunsopon P., P. Phumkhachorn 2010 Antimicrobial activity of Basil (Ocimum basilicum) oil against Salmonella enteritidis in vitro and in food, Biosci. Biotechnol. Biochem. 74(6) 1200-1204

[11] Oxenham SK., KP Svoboda, DR Walters 2005 Antifungal Activity of the Essential Oil of Basil (Ocimum basilicum), J. Phytopathology 153 174–180

[12] Cervato, G., M. Carabelli, S. Gervasio, A. Cittera, R. Cazzola, B. Cestaro, 2000, Antioxidant properties of oregano (origanum vulgare) leaf extracts, J. Food Biochem. 24(6) 453–465

[13] Masood N., A. Chaudhry, S. Saeed, P. Tariq 2007 Antibacterial effects of oregano (Origanum vulgare) against gram negative bacilli, Pak. J. Bot. 39(2) 609-613

[14] Radusheva P., A. Pashev, G. Uzunova, Kr. Nikolova, G. Gentscheva, M. Perifanova, M. Marudova 2019 Physicochemical characteristics of seed oil of Sambucus ebulus, Coriandrum sativum L. and Silybum marianum L. Bulg. Chem. Commun. 51 Special Issue A 144-149

[15] Gouilleux B., J. Marchand, B. Charrier, GS Remaud, P. Giraudneau 2018 High-throughput authentication of edible oils with benchtop ultrafast 2D NMR Food Chem. 244 153-158

[16] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

[17] Sikorska E., T. Górecki, IV Khmelinskii, M. Sikorski, J. Kozioł 2005 Classification of edible oils using synchronous scanning fluorescence spectroscopy Food Chem. 89(2) 217-225

[18] Dupuy N., Y. Le Dreau, D. Ollivier, J. Artaud, C. Pinatel, J. Kister 2005 Origin of French virgin olive oil registered designation of origins predicted by chemometric analysis of synchronous excitation-emission fluorescence spectra J. Agricultural Food Chem. 53 9361–9368

[19] Sayago A., MT Morales, R. Aparicio, 2004, Detection of Hazelnut Oil in Virgin Olive Oil by a Spectrofluorimetric Method, Eur. Food Res. Technol. 218(5) 480-483

[20] Kyriakidis NB, P. Skarkalis, 2000, Fluorescence Spectra Measurement of Olive Oil and Other Vegetable Oils, J. AOAC Int. 83(6) 1435-1439