Physics is an Ever Young Science, Varna, October, 27 – 29, 2017
Физиката – вечно млада наука, Варна, 27 – 29 октомври 2017 г.

THE EFFECT OF EXTRA VIRGIN OLIVE OIL ON THE HUMAN BODY AND QUALITY CONTROL BY USING OPTICAL METHODS

Carsten Tottmann, Valentin Hedderich, Poli Radusheva, Krastena Nikolova
Medical University – Varna (Bulgaria)

Abstract. There are many substances in extra virgin olive oil (EVOO), of which many have a beneficial effect. One of the most interesting substances in EVOO is Oleocanthal. This substance is first recognised in 2005 and can only be found in EVOO. Under further investigations it was proven that *in vitro* Oleocanthal has an anti-inflammatory effect comparable to *Ibuprofen.* This is due to the fact that it is an inhibitor of cyclooxygenase. It is able to form prostanoids, including thromboxane, which takes part in formation of thrombosis, and prostaglandins including prostacyclin, which prevent the formation of the platelet plug during blood clotting and acts as a vasodilator. The quality control of an EVOO is very important, because most often refined oils such as olive-pomace oil, seed oils or synthetic oils are used. In some cases, this may lead to serious health problems after consumption. Such an incident happened 1981 in Spain (Spanish toxic Oil syndrome) where 20000 people were affected from which 330 died from aniline intoxication. Optical techniques are ideal for this purpose because they are simple,

cost-effective, rapid and non-destructive. Fluorescence spectroscopy has different applications: detection of adulteration, geographic region, quantification of fluorescent components, monitoring of photo-oxidation and thermal and assessment of quality changes of olive oil during storage.

Keywords: olive oil; florescence; healthy eating; oxidation

Olive oil is a vastly distributed product and is used in many industries, including foods, cosmetics and pharmaceuticals. Extra virgin olive oil (EVOO) consists of major and minor components. The major components – glycerols, represent more than 98 % of the weight of oil. It is known that fatty acid composition is characterized by a high monounsaturated – to-polyunsaturated fatty acid ratio. Content of minor components is smaller than 2 % of the total oil weigh. They include more than 230 chemical compounds such as aliphatic and triterpenoid alcohols, sterols, hydrocarbons, volatile compounds and antioxidants.

The main antioxidant properties of the EVOO are due to carotenes and phenolic components (Boskou, 1996). EVOO contains different classes of phenolic components such as phenolic acids, phenolic alcohols, flavonoids, lignans. A few authors reported that the phenolic acids are with minor concentration in EVOO (Cortesi et al., 1983; Tsimidou et al., 1996). The other authors are published that compounds such as cafeic, vanilic, syringic, p-coumaric acids are observed in the EVOO (Briante et al., 2003). Rovelli et al. (1997) reported that flavonoids such as luteolin and apigenin were also phenolic components. The vitamins from E group also have antioxidant properties. These are four natural tocopherols (*a*, *β, γ, δ*) and four tocotrienols (αT3, βT3, γT3, δT3) (Cert et al., 2000).

Antioxidants in EVOO based on their mechanism of action can be classified as follow: primary antioxidants, synergistic and secondary antioxidants (Rajalakhmi et al., 1996). It is confirmed from many authors that the antioxidants in olive oil has a different positive influence on the human cells. Manna et al. (1999) examined whether 3,4 – DHPEA can reduce the oxidative damage of human erythrocytes. This investigation suggests that the hydrophilic phenols may have a protective effect on the thrombosis risk. 3,4 – DHPEA may exert a protective activity against cancer by arresting the cell cycle (Fablani et al, 2002). EVOO has a high resistance to oxidative deterioration due to its triacylglycerol composition low in polyunsaturated fatty acids and due to the presence of a group of phenolic antioxidants composed mainly of polyphenols and tocopherols.

Concentration of the phenolic compounds are determined in literature from the following ways: (i) high liquid performed chromatography; (ii) calorimetrically and expressed as the amount of total phenols (Chimi et al.,

1991); (iii) rancimat by using ORAC test (Oxygen Radical Absorbance Capacity) (Papadopoulos & Boskou, 1991).

There are many different kinds of olive oil. These variations depend on the type of used olives and the processing procedure (Veneziani et al., 2017). Besides those there are also refined, pure and olive pomace oils. The difference between them depends on their heat treatment while being processed. EVOO is not treated with heat above 40°C. The chemical compostition is normally that 95 – 99 % of olive oil are triglycerides, glycerin bound to three free fatty acids by esterification (Smyk et al., 2009). The other components are chlorophylls, pheophytins, tocopherols, Vitamin E and oxidative compounds such as oleocanthal and oleuropein (Tena et al., 2012). These substances are the reason for the fluorescence of olive oil. Each of these substances emit light when being excited using different wavelengths: tocopherol and phenols at 300 – 400 nm, Vitamin E at 400 – 600 nm, chlorophyll and pheophytins at 600 – 700 nm.

The positive effect of EVOO on the human body is well-known and one of the most interesting substances is Oleocanthal. This substance is first recognised in 2005 and can only be found in EVOO. Under further investigations it was proven that *in vitro* Oleocanthal has an anti-inflammatory effect comparable to *Ibuprofen.* This is due to the fact that it is an inhibitor of cyclooxygenase (Beauchamp et al., 2005). Cyclooxygenase is able to form prostanoids, including thromboxane, which takes part in formation of thrombosis, and prostaglandins including prostacyclin, which prevent the formation of the platelet plug during blood clotting and acts as a vasodilator (Smith et al., 2000). Oleocanthal therefore acts like a non-steroidal anti-inflammatory drug, which leads to a reduction in inflammatory symptoms. Furthermore, there have been studies which show a cancer killing activity of Oleocanthal. It leads to the destruction of cancer cells by their own enzymes, a process during which healthy cells are not harmed (LeGendre et al., 2015). Besides that, *in vitro* and *in vivo* studies have shown that Oleocanthal potentially enhances β-amyloid clearance in the brain. β-amyloid is a substance whose accumulation is closely related to Alzheimer's disease (Abuznait et al., 2013).

Among Oleocanthal there are many different polyphenols in EVOO, which have a positive effect by decreasing the blood pressure (Ferrara et al., 2000). Furthermore, these polyphenols are anti-oxidants which prohibit the formation of free oxygen radicals, and therefore their mutagenic activity. Additionally, phenols found in EVOO such as hydroxytyrosol, tyrosol and oleuropein show antimicrobial activity against intestinal and respiratory infections, including *Heliobacter pylori*. Through dialdehydic components phenols are able to destroy those cells which cause stomach ulcers or even cancer (Castro et al., 2012).

Moreover, EVOO contains a huge amount of monounsaturated fatty acids (MUFAs) which reduce the risk of heart diseases and strokes (Schwingshackl

& Hoffmann, 2014). According to multiple studies, this is due to the fact that MUFAs tend to lower the low- density lipoprotein (LDL) levels while increasing the high-density lipoprotein (HDL) levels. A higher LDL level increases the accumulation rate of atherosclerosis in the walls of blood vessels, which may lead to plaque ruptures. HDLs, on the other hand, take part in the clearance of fat molecules in the artery walls, thus preventing and regressing atherosclerosis (Gordon et al., 1977).

The quality of EVOO is determine by using spectroscopic techniques, which are ideal for this purpose, because they are cost effective, non-destructive, rapid and simple. But fluorescence spectroscopy has been applied, because its sensitivity is 100-1000 times higher than that of the absorption techniques, enabling to measure concentrations down to parts per billion levels (Sikorska et al., 2004). According Sikorska et al. (2012) it can be used for different applications: (i) discrimination between quality grades; (ii) detection of adulteration; (iii) authentication geographic origin; (iv) quantification of fluorescent components; (v) monitoring of thermal and photo oxidation.

The fluorescent analysis of olive oils takes advantage of the presence of natural fluorescent components, including phenolic compounds, tocopherols and pheophytins, and their oxidation products. Refined oils are characterized by a relatively weak band between 290 – 320 nm, a very broad band spreading to about 500 nm, and a band above 550 nm. All of these bands equally appear in the total fluorescence spectra (Sikorska et al., 2008). Olive oils contain considerable amounts of phenolic compounds, with their concentrations significantly reduced in refined oils. This observation seems to confirm that tocopherols also contribute to the emission observed in the range 295 nm – 360 nm. Most of polyphenols are fluorescent substances, absorbing in the 260 – 310 nm range and emitting in the near-UV range, with their bands centered at 310 – 370 nm (Zandomeneghi & Zandomeneghi, 2005). These phenolic compounds can be detected by fluorescence after separation by HPLC, using excitation/emission wavelengths of 264/354, 310/430 or 280/320 nm (Dupuy et al., 2005). A long-wavelength band is observed in the olive oil spectra, with excitation at about 350 – 420 nm and emission at about 660 – 700 nm, corresponding to the band above 550 nm in total synchronous fluorescence spectra. This band was attributed to pigments of chlorophyll group, based on its excitation and emission characteristics (Zandomeneghi & Zandomeneghi, 2005; Diaz et al., 2003).

Economic aspect is the adulteration of EVOO using lower quality oils. This procedure is due to the high production costs for EVOO and the fact that the user can rarely distinguish the difference. To distinguish pure EVOO from adulterated ones, fluorescence analysis is used. Every oil has a specific spectrum, which is determined previously. It is possible to determine the exact composition of the sample (Xu et al., 2016). The great advantage of this method is the use of 3

dimensional fluorescence spectrum (3DFS) in comparison to the 2-dimensional fluorescence spectrum. The 3DFS has the advantage at the rate of samples and more wavelengths can be compared at the same time to distinguish between different oils.

A similar investigation has been made for olive oils from Bulgarian supermarkets (Nikolova et al., 2014). From the fluorescence spectrum can be concluded: (a) olive oils containing sunflower oil exhibit a fluorescence peak correspondingly at 491.8, 428, and 495 nm with an intensity higher than 3000 arbitrary units; (b) olive oils exhibiting a high contents of chlorophyll show no peaks around 500 to 550 nm; (c) refined olive-pomace oils are obtained from olive pomace after extraction with authorized solvents and a refining process, which includes neutralization, deodorization, and decolorization. All olive oil pomace showed a wide peak between 415 and 550 nm. This means that their content on conjugated hydroperoxides is higher as a result of a greater oxidation (Boggia et al., 2002).

Adulteration of virgin olive involves addition of cheaper oils, including olive oils of lower quality or other plant oils. The most common adulterants found in virgin olive oil are refined olive oil, pomace oil, residue oil, synthetic olive oil–glycerol products, seed oils, and nut oils. Hazelnut oil is chemically similar to virgin olive oil; its presence is difficult to detect at low concentration levels using standard methods. A different approach was tested to detect this type of adulteration using fluorescence (Sayago et al., 2007). The emission spectra of undiluted olive oil mixtures with virgin and refined hazelnut oils with excitation at 350 nm were measured (Sayago et al., 2007). The spectra were subjected to mathematical treatment by calculation of the first derivative.

The addition of cheaper vegetable oils to olive oil causes an increase of the fluorescence in the 450nm to 600 nm spectral range an and a shift in the wavelength of maximum intensity by around 30 nm (Nikolova et al., 2013). Fluorescence emission is stronger for the sunflower and olive oil mixture, rather than for the corn and olive oil mixture. Comparison between fluorescence spectra of vegetable oils and EVOO as well as their first derivatives has been made from Nikolova et al. (2013).

According to international food regulations EVOO shall not contain more than 25% of polar components. By thermooxidation through heating, samples were taken and checked on their fluorescence spectra. The result was that the fluorescence decreased the longer the oil was heated, so the conclusion is that the amount of essential components like phenols and pigments decrease when the oil is heated and their positive effect, such as antioxidation, is lost (Tena et al., 2012).

The studies of thermal deterioration of oils are important because changes during oxidation involve degradation of oil constituents and formation of new products that alter quality attributes and nutritional profile, as the oxidation prod-

ucts are potentially toxic. Thermal deterioration of extra virgin olive oils was studied by (Tena et al., 2012). The sample of virgin olive oil was heated at 190 ^{0}C for 94 h in cycles of 8 h per day. The fluorescence intensity in the spectral region between 290 and 400 nm decreased during the oxidation and a bathochromic shift of the maximum from 350 – 360 nm to around 420 – 440 nm was observed. The observed changes in the spectral profile were explained by the decrease of the tocopherols and phenols and the increase of the oxidation products of vitamin E homologues correlated to hydrolysis products. The intensity of the band between 630 and 750 nm, associated with chlorophylls and pheophytins, decreased exponentially with the thermal oxidation time.

Conclusions

The review of literature data demonstrates that fluorescence measurements conducted directly on olive oil samples can be used for qualitative and quantitative analysis as a valid alternative to conventional, chemical methods of quality assessment. These methods can be used for oil discrimination and for quantitative determination of fluorescent components after an appropriate calibration.

REFERENCES

Abuznait, A.H., Qosa, H., Busnena, B.A., El Sayed, K.A. & Kaddoumi, A. (2013). Olive-oil-derived oleocanthal enhances β-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: in vitro and in vivo studie. *ACS Chem. Neurosci*, *4*, 973 – 982.

Beauchamp, G.K., Keast, R.S.J., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C.-H., Smith, A.B. & Breslin, P.A.S. (2005). Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. *Nature, 437*(7055), 45 – 46.

Boggia, R., Zunin, P., Lanteri, S., Rossi, N. & Evangelisti, F. (2002). Classification and class-modelling of "Riviera Ligure" extra-virgin olive oil using chemical-physical parameters. *J. Agricultural & Food Chem.*, *50*, 2444 – 2449.

Boskou, D. (1996). *Olive oil: chemistry and technology*. Champaign: AOCS Press.

Briante, R., Febbraio, F. & Nucci, R. (2003). Antioxidant properties of low molecular weight phenols present in the Mediterranean diet. *J. Agr. & Food Chem.*, *51*, 6975 – 6981.

Castro, M., Romero, C., De Castro, A., Vargas, J.C., Medina, E.R., Millán, R. & Brenes, M. (2012). Assessment of Helicobacter pylori eradication by virgin olive oil. *Hellicobacter*, *17,* 305 – 311.

Cert, A., Moreda, W. & Pérez-Camino, M.C. (2000). Chromatographic analysis of minor constituents in vegetable oils. *J. Chromatography A, 881,* 131 – 148.

Chimi, H., Cillard, J., Cillard, P. & Rahmani, M. (1991). Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. *J. Amer. Oil Chemists Soc., 68*, 307 – 311.

Cortesi, N., Rovellini, P. & Fedeli, E. (1983). Minor polar components of virgin olive oil: a hypothetical structure by LC-MS. *Riv. Ital. Sostanze Grasse*, *60*, 341 – 351.

Díaz, T.G., Merás, I.D., Correa, C.A., Roldán, B. & Cáceres, M.I.R. (2003). Simultaneous fluorometric determination of chlorophylls A and B and pheophytins A and B in olive oil by partial least-squares calibration. *J. Agricultural & Food Chem.*, *51*, 6934 – 6940.

Dupuy, N., Le Dréau, Y., Ollivier, D., Artaud, J., Pinatel, C. & Kister, J. (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.

Fablani, R., De Bartolomeo, A., Rosignoli, P., Servili, M., Montedoro, G.F. & Morozzi, G. (2002). Cancer chemoprevention by hydroxytyrosol isolated from vergin olive oil through G1 cell cycle arrest and apoptosis. *Eur. J. Cancer Prev., 11*, 351 – 358.

Ferrara, L.A., Raimondi, A.S., d'Episcopo, L., Guida, L., Russo, A.D. & Marotta, T.R. (2000). Olive oil and reduced need for antihypertensive medications. *Arch. Intern.. Med., 160*, 837 – 842.

Gordon, T., Castelli, W.P., Hjortland, M.C., Kannel, W.B. & Dawber, TR. (1977). High density lipoprotein as a protective factor against coronary heart disease: the Framingham study. *Amer. J. Med., 62*, 707 – 714.

LeGendre, O., Breslin, P.A.S. & Foster, D.A. (2015). (-)-Oleocanthal rapidly and selectively induces cancer cell death via lysosomal membrane permeabilization (LMP). *Moll. & Cell Oncol.*, *2*, art. no. 1006077.

Manna, C., Galetti, P., Cucciolla, V., Montedoro, G. & Zappia, V. (1999). Olive oil hydroxytirosol protects human erythrocytes against oxidative damages. *J. Nutr. Biochem., 10*, 159 – 165.

Nikolova, K., Eftimov, T., Antova, G. & Brabant, D. (2013). Detection of corn and sunflower oil adulterations of olive oils using fluorescence spectroscopy. *Trakya Univ. J. Nat. Sci., 14*, 15 – 30.

Nikolova, K., Zlatanov, M., Eftimov,T., Brabant, D., Yosifova ,S., Halil, E., Antova, G. & Angelova, M. (2014). Fluoresence spectra from vegetable oils using violet and blue Ld/Led exitation and an optical fiber spectrometer. I*nt. J. Food Properties*, *17*, 1211 – 1223.

Papadopoulos, G. & Boskou, D. (1991). Antioxidant effect of natural phenols of olive oil. *J. Amer. Oil Chemists Soc., 68,* 669 – 671.

Rajalakhmi, D. & Narasimham, S. (1996). Food antioxidants: sources and methods of evaluation (pp. 65 – 158). In: Madhavi, D.L., Deshpande,

S.S. & Salunkhe, D.K. (Eds.). *Food antioxidants: technological, toxicological and health perspectives.* New York: Marcel Dekker.

Rovelli, P., Cortesi, N. & Fedeli, E. (1997). Analysis of flavonoids from Olea europaea by HPLC – UV and HPLC – electrospray – MS. *Riv. Ital. Sostanze Grasse, 74*, 273 – 279.

Sayago, A., Garcia-Gongález, D.L., Moralez, M.T. & Aparicio, R. (2007). Detection of the presence of refined hazelnut oil in refined olive oil by fluorescence spectroscopy. *J. Agricultural & Food Chem.*, *55*, 2068 – 2071.

Schwingshackl, L. & Hoffmann, G. (2014). Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids Health Dis.*, *13*, 154.

Sikorska, E., Romaniuk, A., Khmelinskii, I.V., Herance, R., Bourdelande, J.L., Sikorski, M. & Kozioł, J. (2004). Characterization of edible oils using total luminescence spectroscopy. *J. Fluorescence*, *14*, 25 – 35.

Sikorska, E., Khmelinskii, I.V., Sikorski, M., Caponio, F., Bilancia, M.T., Pasqualone, A. & Gomes, T. (2008). Fluorescence spectroscopy in monitoring of extra virgin olive oil during storage. *Int. J. Food Sci. & Tech.*, *43*, 52 – 61.

Sikorska, E., Khmelinskii, I.V. & Sikorski, M. (2012). Analysis of olive oils by fluorescence spectroscopy: methods and applications (pp. 63-88). In: Boskou, D. (Ed.). *Olive oil - constituents, quality, health properties and bioconversions.* Rijeka: InTech.

Smith, W.L., DeWitt, D.L. & Garavito, R.M. (2000). Cyclooxygenases: structural, cellular, and molecular biology. *Annu. Rev. Biochem, 69*, 145 – 182.

Smyk, B., Amarowicz, R., Szabelski, M., Gryczynski, I. & Gryczynski, Z. (2009). Steady-state and time-resolved fluorescence studies of stripped Borage oil. *Analitica Chimica Acta, 646*, 85 – 89.

Tena, N., Aparicio, R. & García-González, D.L. (2012). Chemical changes of thermoxidized virgin olive oil determined by excitation–emission fluorescence spectroscopy (EEFS). *Food Res. Int.*, *45*, 103 – 108.

Tsimidou, M., Lytridou, M., Boskou, D., Pappa-Lousi, A., Kotsifaki, F. & Petrakis, C. (1996). On the determination of minor phenolic acids of virgin olive oil by RP-HPLC. *Grasas y Aceites*, *47*, 363 – 370.

Veneziani, G., Esposto, S., Taticchi, A., Urbani, S., Selvaggini, R., Di Maio, I., Sordini, B. & Servili, M. (2017). Cooling treatment of olive paste during the oil processing: impact on the yield and extra virgin olive oil quality. *Food Chem., 221,* 107 – 113.

Xu, J., Liu, X-F. & Wang, Y.-T. (2016). A detection method of vegetable oils in edible blended oil based on three-dimensional fluorescence spectroscopy technique. *Food Chem.*, *212*, 72 – 77.

Zandomeneghi, M. & Zandomeneghi, G. (2005). Comment on cluster analysis applied to the exploratory analysis of commercial Spanish olive oils by means of excitation- emission fluorescence spectroscopy. *J. Agricultural & Food Chem.*, *53*, 5829 – 5830.

✉ **Dr. Krastena Nikolova (corresponding author)**
Department of Physics and Biophysics
Medical University
84, Tsar Oscoboditel Blvd.
9000 Varna, Bulgaria
E-mail: kr.nikolova@abv.bg