

## THERMAL AGING OF EDIBLE OILS: SPECTROPHOTOMETRIC STUDY

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### ABSTRACT

The aim of the present study was to determine the spectrophotometric and thermal aging properties of various edible oils (olive, peanut, rapeseed, soybean and sunflower oils) which are commonly available in the Czech market. The samples were measured by UV/VIS absorption spectrometry and fluorescence spectroscopy. Detected substances of UV/VIS spectra were compared to expected oil composition; the highest absorbance values were detected in a wavelength range 300-550 nm which can be related to the presence of unsaturated fatty acids. The mixtures of oils were characterized by fluorescence spectroscopy; the individual oils were successfully distinguished according to their excitation-emission profiles. This method was also used to detect the samples of adulterated oils, i.e., the adulteration of high-quality oils with soybean oil. From a physicochemical point of view, the influence of temperature on the compounds of extra virgin olive oil was examined by thermal stress simulation. This thermal aging analysis demonstrated that the amount of oxidation products in olive oil increased during the heating whereas the chlorophyll content decreased. The results showed the ability of the techniques used, UV/VIS absorption spectrometry and fluorescence spectroscopy, to characterize the quality and composition of oils, and to distinguish individual oils in blends. UV/VIS spectrometry was also successfully employed for the evaluation of olive oil qualitative parameters according to the standard quality parameters by the "International Olive Council" (EEC 702/2007).

**Keywords:** fluorescence spectroscopy; UV/Vis; thermal aging; oils; quality

### INTRODUCTION

The investigation of physicochemical properties of oils, their composition, the effect of heat on their stability and quality and other technological aspects are the main topic of many research articles published at the present time (Burg et al., 2017; Munasinghe and Wanspala, 2015; Chen et al., 2015; Timilsena et al., 2017). Particularly, the oxidation stability of edible oils and the possibilities of its enhancement are of growing interest nowadays (Comunian et al., 2017; Hernández Sánchez et al., 2016; Zhang et al., 2017).

Edible oils have their own characteristics that differ from each other. They have specific aroma, taste, color and nutritional properties. These properties may change during oil storage and thermal treatment, due to the changes in oil composition. Changes in oil components can be influenced in some ways, e.g., by proper storage, suitable packaging material, and light access. In general, oils must be kept in a dark place protected from sunlight to prevent oxidation and degradation (Gutiérrez and Fernández, 2002).

During only a few months of storage, changes can occur not only on lipids and fatty acids but also on minor components of stored oil. There are significant losses of chlorophyll, carotenoids and total oil phenol content

throughout the whole period (Morelló et al., 2004; Thanh et al., 2006).

Autooxidation is the main cause of oil deterioration, and it is of fundamental importance in the processing of oils in the food industry (Behlau and Widmann, 2003). This undesirable process depends on a number of factors such as the initial composition of the oil, its chemical structure, the presence of minor substances, the content of antioxidants (minerals, tocopherols, carotenoids, chlorophylls) and storage conditions. Some oils are prone to autooxidation because of a high content of polyunsaturated fatty acids. For that reason, antioxidants such as tocopherol are added to the oils to control oxidation during their processing (Zuta et al., 2007; Gunstone, 2013; Arora et al., 2010).

On the other hand, triesters can hydrolyze and produce glycerides and free fatty acids, and non-saturated chains can react with oxygen to produce oxidative products responsible for lipids deterioration. These two phenomena are the cause of two major forms of changes in food fats, i.e., acidification and oxidation (Burg et al., 2017).

During thermal treatment of oils (e.g., frying), many chemical reactions give rise to a wide range of substances. The frying oils can contain more than 400 different heat-induced reaction products, the majority of which become absorbed into the fried food. Unfortunately, many of these

compounds can be harmful to human health (Sebastian et al., 2014). Triacylglycerols (TAGs) as the major constituent of oils are hydrolyzed under these conditions, and pyrolysis occurs when the so-called smoke point is exceeded. Above this limit, free fatty acids (FA) are cleaved from TAGs and acrolein is formed (Pamies and Vilanova, 2014; Osório and de Lourdes Cardeal, 2011; Suh et al., 2017; Bastos et al., 2017).

Free FA increase thermal oxidation of oils, and their unsaturation rather than chain length lead to significant effects upon thermooxidative degeneration of oils. The oxidation rate of a frying oil increases as the content of unsaturated fatty acids of the oil increases. The content of linolenic acid is critical to the frying performance, the stability of oil, and the flavour quality of fried food (Choe and Min, 2007).

Edible oils are also characterized by the polymorphism, i.e., by the ability to crystallize in different modifications. The formation of crystalline modifications is affected by the composition and rate of oil cooling. The gradual transition of crystalline modifications proceeds from the least stable modification  $\gamma$  (amorphous) to the most stable  $\beta$  modification of grainy crystal structure. For that reason, it is very important to keep the right temperature storing conditions (Widlak et al., 2001).

For the consumer, it is important to know functional properties of an edible oil, to find whether it can be used for specific purposes such as frying or just a salad oil at normal temperatures, and whether it decomposes at higher temperatures with the subsequent release of potentially toxic products or, on the other hand, with the loss of health benefit substances. For instance, phenols and tocopherols (vitamins E) decompose in olive oil at temperatures above 180 °C (Hammer, 2008).

### Scientific hypothesis

There were used spectrophotometric methods of edible oil analysis for detection of their adulteration and thermal degradation. There were evaluated concentrations of oxidation products and chlorophyll pigments in studied olive oil to confirm the hypothesis that the effect of thermal stress significantly changed its chemical composition. Additionally, the study was focused on the evaluation of olive oil quality according to the procedure of the „International Olive Council“ (EEC 702/2007). The compliance of oil qualitative parameters (UV/VIS data) with the criteria defined for an extra virgin olive oil were assessed.

## MATERIAL AND METHODOLOGY

### Materials

Olive, peanut, rapeseed, soybean and sunflower oils were purchased in the Czech market. Olive oil was declared as oil of extra virgin quality.

In accordance with the manufacturer’s recommendation, the oils were stored in a dry, dark place at temperatures up to 15 °C. The relative humidity was about 40% (vol.).

The chemical composition of untreated oils is stated in Table 1.

The chemicals used as non-polar solvents to dilute the samples of oils to a specified concentrations were delivered by the suppliers; heptane 99% (v/v) of spectrophotometric grade, by Sigma Aldrich (USA); cyclohexane 99.99% (v/v) of analytical grade, by IPL (Czech Republic).

### Methods

#### UV/VIS absorption spectrometry

UV-VIS spectrophotometric measurements were realized by UV/VIS spectrophotometer CECIL, CE 1021, series 1000 (Germany). The cuvettes of quartz glass, type 6030-UV of light path 10 mm (Hellma Analytics, Germany) were employed in the experiments. All measurements were performed at laboratory temperature (ca. 25 °C).

The samples of oils were diluted in ratio 1:3, 1:5 and 1:10 with heptane and measured in the wavelength range 300 – 800 nm. UV/VIS spectra were recorded and the concentration dependence of absorbance on wavelength was evaluated. Based on maximum absorbance values, the chemical components in the oils were specified.

UV/VIS data were used to evaluate the quality of extra virgin olive oil using the method by the „International Olive Council“ (EEC 702/2007). The principle of this method is based on the fact that an olive oil of lower quality contains the conjugated dienes and trienes formed as a result of oxidative degradation processes in oil. The conjugated carbon-carbon double bonds absorb UV light in the wavelength range 200 – 300 nm. In contrast to this, non-conjugated double bonds, which are present in an extra virgin olive oil (e.g., unsaturated fatty acids), do not absorb light within the spectral range. Consequently, low absorption in the spectral range of 200 – 300 nm indicates high quality extra virgin olive oil and high absorption oils of lower quality (De Caro and Schubnell, 2015).

The "International Olive Council" (EEC 702/2007) defined three criteria that must be valid for an extra virgin olive oil; the criteria consists in several extinction coefficients  $K_{\lambda}$  at specified wavelengths  $\lambda$  (232 nm, 266 nm, 270 nm and 274 nm). An extra virgin olive oil diluted

**Table 1** Characteristics of edible oils by the producers.

Sample of oil	Producer	Chemical composition (g 100g <sup>-1</sup> )		
		FA saturated	FA monounsaturated	FA polyunsaturated
Olive extra virgin	Kreolis	12.8	70.5	8.3
Peanut	Topvet	11.0	28.0	52.0
Rapeseed	Lukana	7.4	-	-
Soybean	Country Life	14.0	-	-
Sunflower	Viviol	14.0	-	-

Note: The hyphen means not stated value.

with cyclohexane to the concentration 1% (v/v) must meet the following relations:  $K_{232} \leq 2.7$ ,  $K_{270} \leq 0.4$  and  $\Delta K \leq 0.01$ .

$K\lambda$  is calculated by the Eq. 1:

$$K\lambda = A\lambda / (c \cdot L) \quad (1)$$

where  $A\lambda$  is the absorbance at the wavelength  $\lambda$ ,  $c$  – the concentration of oil in solvent used, and  $L$  – the light path of measuring cuvette.

The equation of  $\Delta K$  has the following form:

$$\Delta K = K_{270} - ((K_{266} + K_{274})/2) \quad (2)$$

where  $K_{266}$  and  $K_{274}$  are the extinction coefficients at 266 nm and 274 nm, respectively (De Caro and Schubnell, 2015; Commission Regulation (EC) No. 1989/2003; Commission Regulation (EC) No. 702/2007).

According to the standard, the extra virgin olive oil was diluted to 1% (v/v) solution in cyclohexane, and the qualitative parameters were evaluated by the procedure described above.

#### Fluorescence spectroscopy

Spectrophotometric measurements were realized using spectrophotometer RF-1501, Shimadzu Corporation (Japan), enabling the setting (counting) of individual nanometric units (nm). The precision cells made of quartz glass with light path 10 mm, type 6030-UV (Hellma Analytics, Germany) were used for the analysis.

The fluorescent spectra of vegetable oils were measured in the range of excitation wavelengths 220 – 400 nm, and emission wavelengths 300 – 800 nm. The samples of oils were diluted with heptane in proportions 1 : 50, 1 : 100, 1 : 500 due to the observed concentration dependences. The mixtures of oils were prepared in several tested proportions about 1 : 10 of soybean to another oil in order to distinguish the individual compounds (i.e., the specific oils) by spectrophotometric method, and by this mode to detect adulterated oils.

#### Thermal aging of oils

Thermal aging was simulated by heating extra virgin olive oil at 110 °C for 25 hours. Before the heating process, the samples of oil were diluted with heptane in ratio 1 : 100. Then the solutions were successively heated for a 5-h, 10-h, 15-h, 20-h and 25-h period. One sample of olive oil was used in a thermally untreated (natural) state. Each sample was measured 3 times and the mean value of fluorescence emission intensity (in arbitrary units) was calculated from the results. The samples of oil were analysed by the spectrofluorometer in excitation wavelength range 220 – 400 nm and following emission range 300 – 800 nm.

#### Statistical analysis

All measurements were performed three times for each type of oil. Data were analyzed by ANOVA nonparametric statistics using SigmaPlot (Systat Software, USA). Where statistical differences among the data were determined, the significance was defined at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Samples of selected oils were investigated using UV/VIS spectrometry and fluorescent spectroscopy. The blends of an oil with soybean oil as a cheap substitute were analysed in order to find the detection limit of fluorescence spectroscopy method for the accurate distinction of individual oils in the case of their adulteration. Thermal properties of olive oil were studied by the method of thermal stress simulation to describe the influence of temperature and time on oil chemical composition.

#### UV/VIS absorption spectrometry

The quality of oils can be evaluated according to the content of specific substances such as tocopherols, chlorophylls and carotenoids which influence the oxidative stability of oils (Gonçalves et al., 2014). UV/VIS spectra were used to evaluate the chemical composition of oils, i.e., the maximum absorbance values were related to specific substances of oils, dependent on the wavelength recorded; the values were statistically significant ( $p < 0.05$ ).

The highest absorbance values of all samples were detected in a wavelength range 300 – 550 nm; the peaks between 400 – 500 nm can be related to unsaturated fatty acids present in the oils. In the case of soybean oil, the most intense peak was determined around 450 nm. The maximum absorbance around 670 nm found in olive oil spectrum confirmed the presence of chlorophyll pigments in relatively high amount in this oil. The comparison of UV/VIS spectra of soybean and olive oil at various dilutions is illustrated in Figure 1.

All samples showed an intense peak at wavelength about 290 nm related to the content of tocopherols. This is in accordance with the results by Zou et al. (2018) who detected  $\alpha$ - and  $\beta$ -tocopherols at 295 nm in wheat germ oil by HPLC method. The presence of tocopherols in olive oil in our study indicates the high quality of oil examined because tocopherols protect the oil at elevated temperatures, and their content is reduced in the course of refining process, i.e., the refined oils of lower quality are less oxidation-stable than virgin oils of higher antioxidants content (Gharby et al., 2016).

The qualitative parameters of olive oil obtained by UV/VIS spectrometry were assessed by the extinction parameters  $K\lambda$  which are conventionally used to evaluate the quality of olive oils in the food industry.

In Table 2, there are stated the criteria for an extra virgin olive oil according to the "International Olive Council" (EEC 702/2007) which are compared with the parameters calculated for the olive oil tested in our study.

It can be summarized from the results, that olive oil in the present study significantly meets all postulated criteria ( $p < 0.05$ ) and can be declared as oil of the highest (extra virgin) quality.

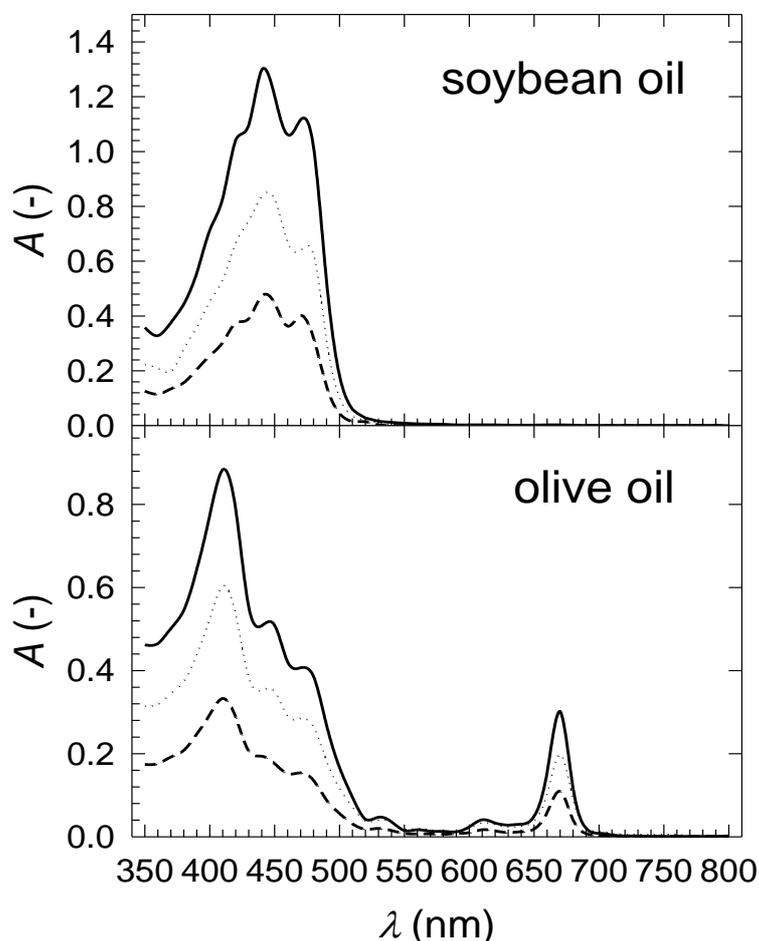
#### Fluorescence spectroscopy

Using fluorescence spectroscopy, the edible oils were characterized according to the presence of specific substances (fluorophores), which provide the characteristic emission spectra to each type of oil (Xu et al., 2016).

**Table 2** Criteria and calculated parameters for extra virgin olive oil.

Criteria (extinction coefficients)	Values according to IOC	Values calculated for olive oil
$K_{232}$	$\leq 2.7$	$2.05 \pm 0.05$
$K_{270}$	$\leq 0.4$	$0.21 \pm 0.02$
$\Delta K$	$\leq 0.01$	$0.00 \pm 0.00$

Note: IOC, the "International Olive Council";  $\pm$  values indicate the standard deviation. The number of replicates, 3.



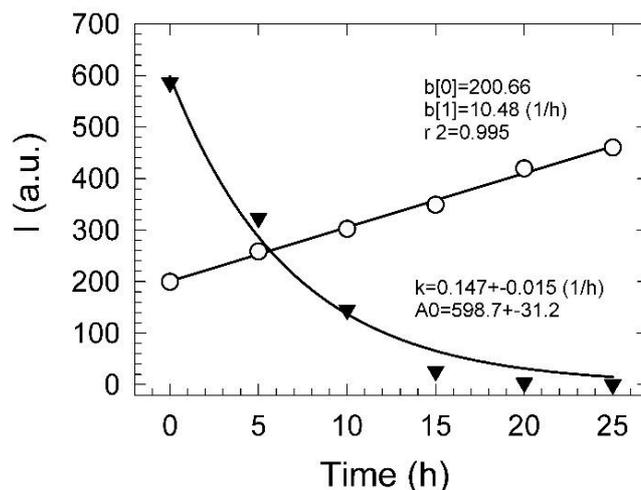
**Figure 1** UV/VIS absorption spectra of soybean and olive oil diluted with heptane in the ratio 1 : 3 (full line), 1 : 5 (dotted line) and 1 : 10 (dashed line):  $A$  - absorbance,  $\lambda$  - wavelength.

Relatively intense signal was observed in emission range 300 – 350 nm which can be attributed to tocopherols and tocotrienols. For olive oil, fluorescence signal was distinctly intensive in emission range 660 – 700 nm related to chlorophyll pigments. Since extra virgin olive oil is not considerably technologically modified, the chlorophyll content is relatively unchanged. Thus, the intensity of fluorescence signal can indicate the quality of edible oil and the mode of its technological treatment. The signal recorded in emission range 440 – 455 nm of varying intensity for each type of oil and its concentration (dilution) correspond to the content of monounsaturated fatty acids. The most pronounced signal was detected for rapeseed oil indicating the highest amount of monounsaturated FA among all samples. As expected, the signal was increasing with rising oil concentration. On the other hand, olive oil provided signal of relatively low intensity at wavelength about 450 nm, suggesting relatively low content of monounsaturated FA. This intensity was minor in comparison to the fluorescence intensity recorded at about 670 nm (chlorophyll content) in the same oil. The results of the

present study can be compared to the investigation by **Sikorska et al. (2005)** who observed the emission spectra of tocopherols and chlorophylls pigments at the same wavelengths for various types of oils.

The mixtures of oils were measured by fluorescence spectroscopy to differentiate the oils in the blends with soybean oil, i.e., to detect the adulterated oils. Because fluorescence spectroscopy is a cost-effective and non-destructive technique, it is particularly used for the quality control of oils (**Guzmán et al., 2015**).

Based on the previous results, the characteristic excitation-emission spectra of individual oils were compared with the spectra of blends and the fluorescence intensity was assessed. It can be summarized that the peaks of all blends showed higher intensity compared to the individual oils, i.e., the addition of soybean oil to another oil was successfully detected. The data of fluorescence intensity were evaluated as statistically significant ( $p < 0.05$ ). The adulteration of oils was revealed at concentration of 9% (v/v) soybean oil, which is in accordance with the study by **Li et al. (2015)** who determined the detection limit of soybean oil (added



**Figure 2** Dependence of fluorescence emission intensity  $I$  on time during thermal stress load (at 110 °C) of extra virgin olive oil detected at 450 nm (empty circles) and 667 nm (full triangles) wavelengths: a.u. – arbitrary units, h – hours. Insets: parameters of the linear regression  $y = b(1) * x + b(0)$  and 1. order formal kinetics nonlinear calculations  $y = A0 * \exp(-k * x)$ .  $y = I$  (a.u),  $x = \text{time}$  (h).

into another oil) equal to 10% (v/v). The sensitivity of the method used seems to be adequate for the requirements of successful detection of the illegal commercial oils adulteration.

#### Thermal aging of oils

As investigated, the most suitable oil for frying and other thermal treatment is refined olive oil, which can be heated to 210 °C because it is stable due to the presence of a large amount of antioxidants and a lower fatty acids (FA) content. High-grade rapeseed oil shows also suitable properties for long-term processing. In opposite to these oils, soybean and sunflower oils with a relatively high content of unsaturated FA are less appropriate for longer thermal treatment (Choe and Min, 2007).

For the above reasons, an extra virgin olive oil was chosen to perform the effect of a relatively long-term thermal stress on the oxidation stability of oil system. Time dependence of the formation of oxidation products and chlorophyll pigments at thermal stress was evaluated by fluorescence emission intensity. In all cases, the chlorophyll concentration was decreased at 667 nm and, on the other hand, there was an increase in concentration of oxidation products at a wavelength of 450 nm. The increasing concentration of oxidation products is consistent with the increase amount of free FA during thermal treatment of olive oils (Gharby et al., 2016). With increasing time of heating procedure, the differences in fluorescence intensity indicating the concentrations of oxidation products and chlorophyll pigments became more statistically significant ( $p < 0.05$ ), as can be seen in Figure 2.

The results of the present study are in accordance with the results by Guzmán et al. (2015) who found a strong fluorescence emission band at 430 – 450 nm for oxidised olive oils which can be related to oxidation intermediates. The authors also identified a medium intensity band at 681 nm, representing a chlorophyll content, which can be compared to the emission wavelength 667 nm of chlorophylls in our study. Gonçalves et al. (2014) investigated the thermal aging of several oils by UV/VIS

measurement. As in our study, the authors determined the increase in the concentration of oxidation intermediates during the heating procedure (by gradual increase of temperature). In the case of olive oil, they detected the change in oxidation products level already at 70 °C.

Because oil is a complicated system, there can be a correlation in the results of thermal aging with different commercial products. Moreover, the oxidation intermediates are often present in untreated oils stored at room temperature, which can be associated with using of transparent oil packaging, effect of light and other storing conditions (Gonçalves et al., 2014). Therefore, the other investigation of oil thermal properties is needful and desirable.

#### CONCLUSION

The results of the present study demonstrate that the methods of UV/VIS absorption spectrometry and fluorescence spectroscopy can be successfully used to characterize selected oil samples with good sensitivity.

The study was performed on 5 commercial oils: olive, peanut, rapeseed, soybean and sunflower oils. Using qualitative analysis, it was possible to distinguish individual types of oils, determine the adulteration of oils in the mixture, and evaluate thermal aging of oils.

The unsaturated fatty acids in oils were detected by fluorescence spectroscopy, providing relatively high intensity in 400 – 500 nm emission area, which can be attributed to unsaturated fatty acids. For olive oil, the intensity was pronounced in the range 660 – 700 nm emission area attributable to the chlorophyll content in the oil.

Using UV/VIS absorption spectrometry, all samples examined showed an intense peak, which occurs at a wavelength of 290 nm assigned to tocopherols. Olive oil had a second peak, occurring around 670 nm, belonging to chlorophyll pigments. Moreover, the UV/VIS parameters of olive oil met the criteria defined by the "International Olive Council" (EEC 702/2007) and can be declared as oil of extra virgin quality.

The results demonstrate the ability of spectrophotometric techniques to characterize and differentiate vegetable oils. In addition, a study of the effect of thermal stress on olive oil was realized with fluorescence spectroscopy. By constant heating, the chlorophyll concentration in oil was reduced at 667 nm of emission area and, on the other hand, there was an increase in the concentration of oxidation products at emission wavelength of 450 nm. With increasing time of heating, the difference in concentrations was more significant.

The adulteration of high-quality oils by soybean oil was also studied. The results proved a sufficient sensitivity of the spectrophotometric method to distinguish individual oils; the adulteration was revealed even when added only 9% (v/v) of false (soybean) oil to the original one. The fluorescence signals were assigned to specific fluorophores according to which the individual oils can be successfully distinguished or monitored due to the oxidation changes.

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