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1	3D Front Face fluorescence Spectroscopy as a tool for monitoring the oxidation level of
2	edible vegetable oil during storage at 60 $^{\circ}\mathrm{C}$
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22 Abstract

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Fluorescence landscapes with excitation wavelengths varying between 280 and 400 nm, and 24 25 emission wavelengths in the range of 300 - 650 nm were scanned directly on oil samples throughout storage in capped and uncapped flasks for 15 days at 60 °C. PARAFAC analysis 26 of the fluorescence landscapes showed the presence of three fluorophores in the edible oils, 27 which are strongly dependent on the storage conditions. The fluorescence spectra were 28 29 resolved into excitation and emission profiles of the pure fluorescent compounds, which are ascribed to be oxidation products and vitamin E. The complexity of edible oil data sets was 30 31 suitable for PARAFAC modeling. Problems with scattering were encountered, the use of 32 missing values was tackled before obtaining a validated model with a criterion of similarity measurements of splits reaching the 83.8 % and this allowed us to start getting through the 33 model of interpretation. 34

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Keywords: Vegetable oil; Thermo-oxidation; Fluorescence spectroscopy; excitation-emission
matrix; PARAFAC.

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1. Introduction

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World trends in oil crop production, yield, and growing area over the last 30 years exhibited 44 an increase of 240 %, 82 %, and 48 %, respectively (El-Hamidi & Zaher, 2018). 45 Consequently, the food industry will be faced with challenges to set up reliable and robust 46 methods in the evaluation of edible oils quality during the process and/or storage. Several 47 48 chemical reactions contribute to the chemical, physical and sensorial aging of edible oils. 49 During heating, edible oils undergo degradation and their functional and organoleptic features are significantly modified. The heating induces chemical reactions such as oxidation, 50 polymerization, hydrolysis and cis/trans isomerization, which have a huge impact not only on 51 the nutritional value of oils but may also generate toxic compounds damaging to health 52 53 (Schaich, 2013). Oxidation stability is considered one of the most important quality indicators of edible vegetable oils. It determines their usefulness in technological processes as well as 54 55 shelf life. Several methods enable to determine the oxidative stability of oils. The most 56 reliable one is the aging test at ambient temperature that requires a long time (several months). Therefore, methods that allow the determination of oil stability in the shortest 57 possible time are valuable such as Schaal oven test, swift test or AOM (Active Oxygen 58 59 Method), Rancimat test, Oxidograph, Oxipress, and Rapidoxy. The Schaal oven test is one of the most frequently used accelerated shelf life of edible oils. This accelerated oxidation test 60 consists of storing oil samples at a constant and controlled temperature at ~ 60 °C (Warner, 61 Frankel, & Mounts, 1989). The increase in temperature acts as a gesture and allows oxidation 62 reactions, as well as measuring their evolution, both organoleptically (color, smell, taste) and 63 by chemical analyses. The Schaal oven test has some advantages such as its simplicity and its 64 standardization among the methods referenced in the AOCS database. The oxidation, thermal 65

stability, chemical composition, and quality of edible oils under various conditions have been 66 previously investigated by applying different techniques such as infrared and Raman 67 spectroscopy (Saleem et al., 2020), 1D and 2D NMR (Cordella, Tekye, Rutledge, & Leardi, 68 2012; Dugo et al., 2015), differential scanning calorimetry (Qi et al., 2016) and front face 69 fluorescence spectroscopy (FFFS) (Cordella et al., 2012, Ali, Iqbal, Atta, Ullah, & Khan, 70 2020). Despite of the current progress in the development of these analytical methods; 71 sensory evaluation, chemical analyses (determination of free acidity, acid value, peroxide 72 value, p-anisidine value, totox, thiobarbituric acid (TBA), iodine value) and chromatographic 73 techniques remained the reference methods used in the different regulations such as AFNOR, 74 75 ISO, and AOCS recommended practice, in edible oil quality control applied during process and storage. These methods are often time-consuming, and require qualified operators. 76 Moreover, chemical analyses and the use of liquid chromatography are incompatible with a 77 78 reagent and/or organic solvent-free strategy. Indeed, these analyses are performed to get informations from the use of different markers for monitoring the edible oil oxidation state. 79 Each marker provides partial information of the whole phenomenon, but is unable to predict 80 by itself the future stability of the edible oil (Cuvelier and Maillard, 2012). In real-world 81 application, these limitations present some constraints from industrial point of view such as 82 duration, cost and environmental impact of analyses that should be overcome. It would be 83 economically and environmentally profitable and viable to find a promising alternative, which 84 is more compatible with the current state of the art in tehnological development. Many food 85 fluorophores can be used as marker for monitoring the quality of edible oil throughout storage 86 (Sikorska, Khmelinskii, & Sikorski, 2019a). Thus the development of FFFS instruments, 87 methods of data analysis, and availability of suitable software should promote wider usage of 88 fluorescence in food research and real-world application. 89

Although FFFS is a method, which has been exploited extensively for monitoring food 91 92 quality (Hassoun & karoui, 2016; Karoui, Cartaud, & Dufour, 2006; Karoui, Hammami, Rouissi, & Blecker 2011; Karoui, Nicolaï, & de Baedemaeker, 2008; Ullah, Khan, Shah, Ali, 93 & Bilal, 2018; Ullah, Khan, Bilal, Ali, & Khalil, 2019), this non-destructive technique has not 94 been fully explored in the determination of the quality of oil products and the comprehension 95 of physico-chemical interactions of lipids with other component occurring within food 96 matrices during storage. In line with this objective, FFFS was used, for instance, to monitor 97 changes in sponge cakes during aging (Botosoa, Chèné, Karoui, 2013a, 2013b). The 98 technique was, recently, presented among high-potential innovative technologies for food 99 100 quality evaluation (Sikorska et al., 2019a). In addition to its higher sensitivity and selectivity, its multidimensional character, and its usefulness for studying minor and trace components in 101 complex food matrices, FFFS is benefiting from recent technology development to improve 102 103 its performance in reducing significantly acquisition time of EEM and monitoring specific fluorophores (NADH or tryptophan) and bioprocesses. Several achievements can be cited in 104 105 that direction such as replacing photomultiplier tubes (PMT) detectors with charge-coupled device (CCD) ones, using compact portable devices with diode lasers or LEDs for food 106 samples measurements, or more advanced sensor like the filter-based Bio View sensor, and 107 usage of optical light guides (Sikorska et al., 2019a). FFFS provides several analytical 108 informations in a non-destructive manner; in addition, sample treatment (extraction or 109 digestion) is not required. This is in line with the view of green chemistry (Gredilla, Fdez-110 Ortiz de Vallejuelo, Elejoste, de Diego, & Madariaga, 2016). Moreover, the use of FFFS 111 coupled with chemometric tools presented numerous advantages in terms of going in-depth in 112 the comprehension of physico-chemical interactions between fluorescent molecules and their 113 environment within food samples. Several studies have explored the use of FFFS coupled 114 with multiway data analysis, such as PARAFAC. Concerning food research, PARAFAC was 115

applied in investigating the relationship between sugar impurities and its quality at the
molecular level (Bro, 1999), screening method for dioxin contamination in fish oil (Pedersen,
Munck, & Engelsen, 2002), monitoring chemical changes in dry-cured Parma ham during
maturation (Moller, Parolari, Gabba, Christensen, & Skibsted, 2003), and the stability of
processed cheese, yogurt (Christensen, Miquel Becker, & Frederiksen, 2005), and edible oils
during storage (Guimet, Ferré, Boqué, Vidal, & Garcia, 2005; Christensen, Nørgaard, Bro, &
Engelsen, 2006; Sikorska et al., 2019b).

To the best of our knowledge, up to date; no research study was conducted to determine the 123 oxidative stability of the following four selected vegetable edible oils together, namely 124 125 rapeseed oil (RO), linseed oil (LO), sunflower oil (SO), and extra virgin olive oil (EVOO) by using 3D Fluorescence spectroscopy coupled with PARAFAC. Due to its importance in the 126 human diet, olive oil (OO) was the most studied in understanding the chemical changes 127 undergone after thermal oxidation (Tena, Aparicio, & García-González, 2012; Saleem et al., 128 2017; Domínguez Manzano, Muñoz de la Peña, & Durán Merás, 2019). Recently, Sikorska et 129 al., (2019b) depicted, by applying PARAFAC to EEM, the presence of four fluorophores in 130 cold-pressed rapeseed oil that showed different evolution during storage at ambient room 131 temperatures (18 - 25 °C) for 6 months. 132

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The objective of the present study was to determine the impact of heating at 60 °C for 15 days on the quality of RO, LO, SO, and EVOO. Structural changes were monitored at the molecular level by applying 3D FFFS coupled with PARAFAC.

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139 **2. Material and methods**

140 2.1 Oil sampling and oil thermo-oxidation procedure

A set of 4 edible oils was purchased from a local supermarket and a local producer (Arras,
France). The sample collection included 1 sample of 1L of: i) RO; ii) SO; iii) EVOO, and iiii)
unrefined and cold-pressed LO.

Oil samples (45 mL) were poured into flasks (VWR-France) of 26 mm in diameter, with the 145 surface-to-volume ratio of 0.47 cm² / mL and placed into a convection oven (Air Concept, 146 FIRLABO, Emerainville, France), heated and maintained at 60 °C during experimentation, 147 except the sample aged 0 day (fresh oil). No mechanical stirring was applied during the 148 heating period and the flasks were covered with aluminum foil to avoid photo-oxidation. The 149 dishes were introduced into the oven "with" and "without" lids to compare the impact of the 150 151 presence of air in the headspace of flasks. The samples were analyzed at 0, 3, 6, 9, 13, and 15 day(s). One flask of each oil was taken out of the oven and used for 3D fluorescence 152 spectroscopy analysis. 153

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155 **2.2 Fluorescence measurements**

Fluorescence spectra were recorded using a Fluoromax-4 spectrofluorimeter (Jobin Yvon, 156 Horiba, NJ, USA). The spectrofluorimeter was equipped with xenon lamp source used to 157 excite fluorescence, excitation and emission monochromators, a photomultiplier (PM) (range 158 200 - 850 nm) used as emission detector to measure the intensity of fluorescence, a 159 thermostated front-face sample-cell holder and the temperature was controlled by a Haake 160 A25, AC 200 temperature controller (Thermo-Scientific, France). The incidence angle of the 161 excitation radiation was set at 60° to ensure that reflected light, scattered radiation and 162 depolarization phenomena were minimized. The sample is illuminated by the photons of 163 excitation (light beam: ~ 3 mm in height and ~ 0.3 mm width) in its center during 2-3 164 minutes. The PM responds to individual photons, and the pulses can be detected as an average 165 signal or counted as individual photons. The PM is useful for low-level light detection due to 166

its capacity to amplify low-noise and its sensitivity depends on the incident wavelength. 167 168 Fluorescence intensity is a simultaneous function of the excitation and emission wavelengths. The intensity values of a fluorescence emission spectrum are determined by keeping the 169 170 excitation wavelength (λ ex) constant while the emission wavelengths (λ em) are scanned. Similarly, the intensity values of a fluorescence excitation spectrum are determined by 171 remaining the λ em constant, while the λ ex are scanned. When measuring several emission 172 173 spectra at different λ ex (or vice versa), a three-dimensional fluorescence landscape, the socalled fluorescence excitation-emission matrix (EEM), is obtained. The spectra of oil samples 174 were scanned at ambient temperature in a 10 x 10 mm quartz cuvette for a volume of 3 mL. 175 Raw non-smoothed data were recorded. For every oil samples, an EEM was obtained by 176 measuring the emission spectra from 300 to 650 nm at 1 nm intervals with excitation at every 177 1 nm from 280 to 400 nm. The measurements started with the highest excitation wavelength 178 and ended with the lowest to minimize the photodecomposition of the oil samples. The three 179 180 main components of the spectrofluorimeter (lamp, monochromators and PM) are wavelengthdependent, therefore, they cause distorsions in the fluorescence spectra. All spectra were 181 corrected for instrumental distortions in excitation using a rhodamine cell in the reference 182 183 channel. For correcting the excitation spectra, the output \mathbf{R} of the PM is directly proportional to the flux of photons emitted by the excited sample. This relationship can be written as $\mathbf{R} \propto \mathbf{R}$ 184 $I_0 \varepsilon \phi_i$, where I_0 is the excitation intensity, ε is the molar absorptivity, and ϕ_i is the quantum 185 yield of fluorescence efficiency of the selected fluorophor (i.e. the ratio between the number 186 of photons emitted and the number of photons used to excite the system). However, the 187 quantum efficiency of the detector, the bandwidth of the monochromator, and the 188 189 transmission efficiency of the monochromator must be taken into account for determining the true emission spectrum. Based on these elements, the dependence of the uncorrected emission 190 spectrum can be expressed as follows 191

192
$$\frac{dF}{d\lambda} = \left(\frac{dI}{d\lambda}\right) P_{\lambda} B_{\lambda} M_{\lambda} = \left(\frac{dI}{d\lambda}\right) S_{\lambda}$$

193 where $dF / d\lambda$ is the apparent or observed intensity of fluorescence emission at wavelength λ ; 194 $dI / d\lambda$ is the true intensity at λ ; $P\lambda$, $B\lambda$, and $M\lambda$ represent the relative quantum efficiency of 195 the PM, the relative bandwidth of the monochromator, and the fraction of light transmitted by 196 the monochromator, respectively, at λ . These last three factors were combined into a single 197 factor in $S\lambda$, which is called spectral sensitivity factor of the monochromator-PM 198 combination. The true emission spectrum $dI / d\lambda$ can be determined from the apparent 199 emission spectrum by dividing each ordinate $dF / d\lambda$ by the corresponding value of $S\lambda$.

200

201 **2.3 Mathematical analyses of data tables**

The fluorescence data of oils can be presented in an $I \times J \times K$ three-way data array, which will be trilinear. The first index (*I*) refers to the samples, the second (*J*) to the emission wavelengths and the third (*K*) to the excitation wavelengths. In order to model these data, PARAFAC was used.

206 The PARAFAC model can be put in the equation as follow:

207
$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} - e_{ijk}$$

208 i = 1,...,I; j = 1,...,J; k = 1,...,K

209

where x_{ijk} is the intensity of the *ith* sample at the *jth* variable (emission mode) and at the *kth* variable (excitation mode) a_{if} , b_{jf} and c_{kf} are parameters describing the importance of the sample / variables to each component and the residuals, e_{ijk} , contain the variation not captured by the model (Andersen & Bro, 2003). The PARAFAC components estimate the signals from the individual fluorophores if the data are approximately low-rank trilinear and when the correct number of components is used. In that case, the scores in a_{if} may be interpreted as the relative concentration of analyte f in sample *i*. The *j*-vector b_f with elements b_{if} (j = 1, ..., J) is the estimated emission spectrum of this analyte and likewise c_f is the estimated excitation spectrum. However, some mathematical conditions must be fulfilled to allow the decomposition to be unique and to provide meaningful estimates (Andersen & Bro, 2003), namely:

- the uniqueness of the decomposition depending on the linearly independence of the spectraand concentration profiles;

- the assumption of the validity of the additivity and linearity of the signal.

The disturbance of the trilinearity of the data can be caused by the inner-filter effects, which may come from high concentrations, scattering and quenching. Furthermore, uncertain estimates can be created because of the abundance of missing data and spectral similarities. Consequently, chemical interpretation should be done with care. Moreover, PARAFAC model can be validated using fit values, visual assessment of the loadings, residual analysis, core consistency diagnostic, jack-knifing and especially by a split-half analysis.

All analyses of the data in this work were performed with PLS Toolbox 7.9 and 8.0 (Eigenvector Research Inc., Wenatchee, WA, USA) and Matlab version R 2013b (The MathWorks, Natick, MA, USA).

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235 **3. Results and discussion**

236 **3.1** Analysis of edible vegetable oils fluorescence landscapes

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Fig. 1a presents the EEM spectra in the form of a contour map of fresh EVOO as an example. 238 239 The fluorescence landscapes of four edible vegetable oils namely RO, SO, EVOO and LO are shown in Figs. 1(b and c) for capped samples and in Figs. 1(d and e) for uncapped ones, 240 during 15 days of storage. For the reason of clarity, the present analysis is focused only on the 241 eight samples that represent the extreme in the experimental plan, i.e. four fresh edible oil 242 samples (RO0, SO0, EVOO0, and LO0) and four uncapped edible oil samples (RO-L15, SO-243 L15, EVOO-L15, and LO-L15) stored for 15 days at 60 °C. The highest fluorescence peaks 244 for the fresh edible oil samples are seen with $342 < \lambda_{ex} < 400$ nm and $403 < \lambda_{em} < 518$ nm for 245 RO, 338 < λ_{ex} < 386 nm and 408 < λ_{em} < 484 nm for SO, 343 < λ_{ex} < 360 nm and 514 < λ_{em} < 246 531 nm for EVOO, and 340 < λ_{ex} < 356 nm and 519 < λ_{em} < 542 nm for LO; whereas the 247 highest fluorescence peaks for the uncapped edible oil samples are observed with $\lambda_{ex} = 349$ 248 nm and λ_{em} = 442 nm for RO, 344 < λ_{ex} < 387 nm and 414 < λ_{em} < 481 nm for SO, 345 < λ_{ex} 249 < 372 nm and 512 < λ_{em} < 531 nm for EVOO, and 340 < λ_{ex} < 356 nm and 519 < λ_{em} < 542 250 nm for LO. 251

252

253 The excitation and emission characteristics indicate that the fluorescence peaks arise from oxidation products, polyphenols, and tocopherols, which are expected to be present in edible 254 vegetable oils. Indeed, the peak observed in the region $\lambda_{ex} = 300 - 400$ nm and $\lambda_{em} = 400 - 100$ 255 500 nm (Figs 1b, c, d and e) was attributed to the oxidation products contained in vegetable 256 oils (Tena et al., 2012). Some previous works reported that polyphenols can present peaks in 257 the region of $\lambda_{ex} = 260 - 310$ nm and $\lambda_{em} = 310 - 370$ nm (Zandomeneghi, Carbonaro, & 258 Caffarata, 2005; Ali et al., 2020). Moreover, the same peak may correspond to the 259 tocopherols. But this attribution was considered to be erroneous according to Christensen et 260 al., (2006). Actually, this component does not agree with the fluorescence characteristics of 261 tocopherol found in the literature (Duggan, Bowman, Brodie, & Udenfriend, 1957). Recently, 262

it has been reported that the emission of some fluorescent components at 525 nm was not
fully resolved and this fluorescent compound corresponds to the more than one chemical
compound present in vegetable oil (Sikorska et al., 2019b).

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3.2. Application of PARAFAC on excitation-emission matrix obtained from edible
vegetable oils during oil aging at 60 °C

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As shown in **Fig. S1a and 1b**, the presence of the massive band of peaks (in the dashed circle) in the place of the first Rayleigh scatter is the result of masking the 1st and 2nd Rayleigh scatter options available on the Fluoromax-4 spectrofluorimeter. For the edible oil data set, non-trilinear parts were removed by using EEM filter (**Fig. S2a and 2b**) or flucut function. An attempt was performed by removing the top part of the 3D spectra which is > $9.2*1e^{6}$ CPS) and replacing it with NaN but no reliable model was obtained.

276

277 To resolve the profile of fluorophores in edible oils during storage, PARAFAC was applied to the raw EEM to fit the array. Two, three, four, five components models were performed. The 278 explained variance for each number of components is given in Table 1. The relative change 279 in explained variances increases from 1 to 5 components model. Although the 4 and 5 280 components models exhibited the highest percentage of explained variance, only the 3 281 components model obtained the highest scores of similarity measure of splits (83.8 %) and the 282 highest number of components. Therefore, the split-half analysis and investigation of 283 residuals suggested that the evolution of edible vegetable oils EEM fluorescence spectra 284 during their storage were best-fitted by a three-component model (Table 1). This model 285 exhibited the optimal value of the number of components, explained variance and similarity 286 measure of splits. Sikorska et al., (2019b) reported an optimal PARAFAC model with four 287

components (explained variance 99.1 %, core consistency value 70) during investigating the
EEMs of different RO samples during storage. The authors used core consistency and a visual
inspection of both the residuals and the loadings to validate their model.

Fig. 2 provides the split-half model estimates for all three components in the emission and excitation modes, respectively. It is noticeable that the spectra were very similar to each other and had the same peak positions, from which it can be concluded that the three-component PARAFAC model is appropriate for edible oil samples studied in the present case.

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Fig. 3 shows the emission (a) and excitation (b) spectra of three components present in edible 296 297 vegetable oil samples obtained through the decomposition of the EEM spectra using the three component PARAFAC models. These two modes, emission and excitation spectra, were the 298 same for the four edible oils studied despite the difference of their EEM profile and 299 300 represented the underlying pure spectra of fluorophores characteristic of RO, SO, LO, and EVOO samples during 15 days of storage at 60 °C. The first mode of the PARAFAC model 301 302 contained information about the specific concentration of these fluorophores in every measured edible oil samples. The estimated excitation spectra presented maxima at 395 nm 303 (component 1), 344 nm (component 2) and 355 nm (component 3) (Fig. 3b). This trend 304 corresponds to the peaks in the raw fluorescence landscapes (Fig. S1). While their 305 corresponding estimated emission spectra exhibit their maxima at 487 nm, 420 nm and 528 306 nm, respectively (Fig. 3a). 307

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The pair of excitation/emission wavelength (395/487 nm) corresponds to the maximum fluorescent intensity of the first component. According to previous findings, these spectra are similar to those of oxidation products (Guimet et al., 2005). An intensive band in the range 450 – 650 nm was attributed to the oxidation products while studying chemical changes of thermo-oxidized virgin olive oil (Tena, Garcia-Gonzalez, & Aparicio, 2009; Tena et al., 2012). Based on these findings, the first component can be assigned to secondary oxidation products such as ketones, aldehydes and other carbonyl compounds in agreement with the findings of Guimet et al., (2005).

The second pair of excitation/emission wavelength (344/420 nm) corresponds to the 317 maximum fluorescent intensity of the second component. Rodriguez Delgado, Malovana, 318 319 Perez, Borges, & Garcia Montelongo (2001) reported that phenolic compounds show maximum excitation between 265 and 335 nm, and maximum emission in the 358 - 426 nm 320 range. Although the maximum emission (420 nm) of this excitation/emission wavelength is 321 322 comprised between 358 and 426 nm, its maximum excitation (344 nm) is not included 323 between 265 and 335 nm. Again, according to Guimet et al., (2005), oils at early degradation stages exhibit strong fluorescence between $\lambda_{ex} = 315 - 370$ nm; $\lambda_{em} = 415 - 460$ nm. 324 Moreover, this component, which was ascribed to compounds formed during cold-pressed 325 rapeseed oil oxidation, appeared at 320/420 nm in excitation/emission (Sikorska et al., 326 2019b). In accordance with those findings, the second component could be hypothesized to 327 correspond to primary oxidation compounds, such as conjugated hydroperoxides (Guimet et 328 329 al., 2005), present in edible oil samples.

The third pair of excitation/emission (355/528 nm) corresponds to the maximum fluorescent 330 intensity of the third component. Despite several identification attempts, the assignment of 331 this component to its real chemical structure remained uncertain. Moreover, the spectral 332 ranges of excitation between 300 and 400 nm and emission between 400 and 695 included 333 chlorophylls, which peak is used to be much more intense than those of other components 334 (Guimet, Ferré, Boqué, & Rius, 2004; Guimet et al., 2005). However, the peak is usually 335 detected at 695 nm (Guimet, 2005), but has already been detected at 681 nm (Kyriakidis & 336 Skarkalis, 2000), and even at 668 nm (Christensen et al., 2006; Sikorskaet al., 2019a). These 337

wavelengths are outside of the range of emission wavelength fixed in the present study to 338 339 such an extent that this hypothesis can be excluded. As it was previously reported, the peak at $\lambda_{em} = 525$ nm was also attributed to vitamin E from EVOO (Guimet et al., 2004). This 340 hypothesis was confirmed by the same authors who observed an increase in the fluorescent 341 342 intensity at 525 nm after the addition of vitamin E to EVOO. Previously, the same experiment 343 has already been performed to attempt the same peak identification (Kyriakidis & Skarkalis, 2000). However, Christensen et al., (2005) reported that this component was erroneously 344 assigned to tocopherol based on the results obtained by Kyriakidis and Skarkalis (2000). 345 346 Recently, the pair of excitation/emission corresponding to 360/530 nm was considered as one of the components detected in studying the evolution of fluorescence of cold-pressed rapeseed 347 oil during storage (Sikorska et al., 2019a). The authors found the origin of this component less 348 obvious as well. The loading of its emission profile presented a similarity with the present 349 profile which exhibits additional broad emission with low-intensity on the short-wavelength 350 351 side. This was explained by the fact that the emission of some fluorescent components was not fully resolved, and therefore this component corresponded to more than one chemical. 352 The score of the model are estimates of the relative concentration of the three fluorophores in 353

354 every edible oil samples identified by the loadings and are therefore measures of the amount of the fluorophores as shown in Fig. 4. Oil samples were grouped according to their botanical 355 origins for easier interpretation. For every botanical group, the concentrations of the three 356 components can be calculated. For example, it can be observed that the concentrations of the 357 first and second components, corresponding hypothetically to primary and secondary 358 oxidation products showed the following tendency RO > SO > EVOO > LO. Indeed, refined 359 oils, namely RO and SO, exhibited the highest score of relative concentrations of both 360 oxidation products compared with unrefined ones (EVOO and LO). This implies that the 361 evolution of these oxidation compounds throughout the 15 days of storage depends on the 362

previous refining step undergone by the edible oils. This profile is, to our best knowledge the first time, obtained with PARAFAC model. It is well known that refined oils are more reliable to undergo oxidation processes when compared with unrefined oils (Guimet et al., 2004). The main reason would be due to the refining process, which may decrease the natural antioxidants (phenolic compounds, tocopherols and beta-carotene) contained in edible vegetable oils.

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Regarding the third component, the tendency showed the following order LO > EVOO > RO 370 > SO during the 15 days of storage. Unrefined oils are supposed to contain less natural 371 antioxidant compounds (vitamin E) since the refining process drastically decreased its 372 content. This tendency may be explained by the difference between vitamin E and/or its 373 derivative contents in both categories of edible oils. Regarding the profile of tocopherol, the 374 order is in agreement with the contents of $(\beta + \gamma)$ tocopherols in the investigated oils which 375 values are approximatively 363 mg/kg, 281 mg/kg, and 25.2 mg/kg for cold-pressed LO, 376 refined RO, and refined SO, respectively (Gliszczyńska-Świgło, Sikorska, Khmelinskii, & 377 Sikorski, 2007). The value for EVOO (12.3 mg/Kg) is normally the lowest between the four 378 edible oils studied; however, the presence of the important peak corresponding to chlorophyll 379 close to 525 nm may interfere and could present an impact in the increase of fluorescence 380 intensity of EVOO at this wavelength. In fact, only EEM of EVOO presented an increase of 381 fluorescence intensity between 625 and 650 nm that should correspond to the beginning or the 382 tail of the chlorophyll's peak. Guimet (2005) has already reported that, in EVOOs, the 383 chlorophyll peak was much more intense than those of the other fluorescent molecules and 384 can influence strongly the fluorescence intensity of the peak at 525 nm. Consequently, the 385 different magnitude of this peak may cause problems when handling the data and, for this 386 reason, the fluorescent region of chlorophylls has been often removed. 387

389 The contributions of each of the three PARAFAC components were shown in Fig. 5 (a-l). The score values obtained in the PARAFAC decomposition were plotted against the storage 390 time to explore the impact of thermo-oxidation throughout the storage period. The systematic 391 variations of the score values, corresponding to the respective components were observed 392 except for component 1 for LO and component 3 for SO. Regarding the evolution of 393 oxidation products, RO showed a decrease of primary products during storage (Fig. 5a) while 394 the secondary products showed the highest score at 9 days of storage for capped samples and 395 decreased until the 13th day for uncapped ones (Fig. 5b). For EVOO samples (Fig. 5c-d), the 396 397 primary oxidation products reached the highest score on day 9 for capped samples and only on day 6 for uncapped ones, while the secondary products exhibited a progressive increase 398 during 15 days of storage. For LO samples (Fig. 5e-f), the primary oxidation products 399 400 progressed in a less systematic way for capped and uncapped samples; however, no compounds were detected before 13 days for both storage conditions. It is probably due to the 401 402 fact that LO samples were the freshest among the four studied oils. For SO, Fig. 5g shows a slight decrease of component 2 during storage but the highest score of component 1 was 403 reached at 3 days for capped and uncapped samples (Fig. 5h). 404

Regarding vitamin E (tocopherols), Fig. 5i-j showed a systematic and slight decrease for RO and EVOO during storage, while the level of vitamin E in LO samples remained stable throughout storage (Fig. 5k). The most spectacular diminution was observed with SO reaching the lowest score after 9 days of storage (Fig. 5l). It can be attributed to the fact that SO was a refined oil and contained the lowest value of tocopherols it seems that these tocopherols were more thermosensitive.

411

412 **4.** Conclusion

This study aimed to investigate the potential of fluorescence spectroscopy coupled with 413 PARAFAC for monitoring the thermo-oxidation at 60 °C, in the frame of the Schaal oven 414 test, undergone by RO, SO, EVOO and LO samples during 15 days. The PARAFAC applied 415 to fluorescence excitation-emission matrix scanned on RO, SO, EVOO, and LO samples 416 separated three fluorophores components, which had different progression dynamics 417 throughout storage. The results revealed, for the first time, the complexity of interpretation 418 when PARAFAC is applied on EEM obtained from four oil fluorescence data sets. Indeed, the 419 application of PARAFAC as multiway analysis method allowed to: (i) comprehend the 420 evolution of primary and secondary oxidation products during storage; (ii) monitor the 421 422 thermo-oxidation effect on the evolution of vitamin E depending on the refining process during their aging time; and iii) study a large number of edible oils including LO, with 423 different profiles of mono-unsaturated and poly-unsaturated fatty acids, tocopherols, and 424 425 chlorophylls.

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427 Declaration of competing interest

The authors declare that they have no known competing financial interests or personalrelationships that could have appeared to influence the work reported in this paper.

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554 List of Tables:

555

556 Table 1: Explained variance as a percentage versus the number of components for PARAFAC

557 models of the fluorescence data with 1-5 components

PLS Toolbox	Number of components				
	1	2	3	4	5
Explained variance (%)	95.4	97.5	99.3	99.5	99.317
Similarity measure of splits (%)	98.7	40.9	83.8	59.5	0.1

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561 Legend to the Figures:

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Fig. 1 Excitation-emission matrix contour plots of rapeseed oil (RO), sunflower oil (SO),
extra virgin olive oil (EVOO) (a), and linseed oil (LO) stored up to 15 days at 60 °C in
capped (b, and c) and uncapped (d, and e) flasks

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Fig. 2 Result from a split-half analysis performed by dividing oil samples into random groupswith emission and excitation modes

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Fig. 3 Emission (a) and excitation (b) mode loading vectors obtained from the three component PARAFAC model calculated on the EEMs of the 44 oil samples in the excitation range of 280 - 400 nm and emission range of 300 - 650 nm. Component 1 (—), component 2 (- · - · -), and component 3 (- - -).

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Fig. 4 PARAFAC model concentration mode loadings for rapeseed oil (RO), extra virgin olive oil (EVOO), linseed oil (LO), and sunflower oil (SO). Component 1 (—), component 2 $(-\cdot - \cdot -)$, and component 3 (- - -).

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Fig. 5 Scores versus storage time (a – l): scores on component 1 (b, d, f, and h), scores on component 2 (a, c, e, and g), and scores on component 3 (i, j, k, and l) for rapeseed oil (RO) (a, b, and i), extra virgin olive oil (EVOO) (c, d, and j), and linseed oil (LO) (e, f, and k), and sunflower oil (SO) (g, h, and i), stored under 60 °C during 15 days in capped (empty bar) and uncapped (checkered bar) flasks.

Figure 1a:

















Figure 1e:













Figure 5 (a – d):



Figure 5 (e – h):





Figure 5 (i – l):

