Real time monitoring of the combined effect of chlorophyll content and light filtering packaging to virgin olive oil photo-stability using mesh cell-FTIR spectroscopy

Dimitrios Trypidis^{a,b}, Diego Luis García-González^{a*}, Ana Lobo-Prieto^a, Nikolaos Nenadis^b,
Maria Z. Tsimidou^b, Noelia Tena^a

^a Instituto de la Grasa (CSIC), Campus Universitario Pablo de Olavide - building 46,
Ctra. de Utrera, km. 1, 41013 - Sevilla, Spain.

^b Aristotle University of Thessaloniki, School of Chemistry, Laboratory of Food
Chemistry and Technology, 54124, Thessaloniki, Greece.

*corresponding author: dlgarcia@ig.csic.es; Telephone: +34 954611550

ABSTRACT

The present work aimed at examining the combined effect of chlorophyll content and light filtering packaging material on the photo-stability of virgin olive oil (VOO) via mesh cell-FTIR spectroscopy monitoring. Four different VOOs of Koroneiki cultivar differing in total chlorophyll content (12-46 mg/kg) were exposed in parallel to direct visible light (6000 lx, 24 °C, 344 h) and FTIR spectra were recorded periodically with or without applying light protection by an industrial filter used for packaging. Findings suggested that the protective role of light filtering material was more evident in the VOO with the lowest total chlorophyll content. Real time monitoring of VOO by mesh cell-FTIR was found to be a useful tool to follow the combined effect of pro-oxidant chlorophylls and the protective light filtering materials in the photo-oxidation process of VOO employing a minute sample amount.

Keywords: FTIR spectroscopy, mesh cell, photo-oxidation, virgin olive oil, chlorophylls, light filtering packaging material.

1. Introduction.

Among the edible vegetables oils, VOO containing chlorophyll pigments is particularly sensitive to oxidation when stored in transparent bottles exposed to light on the shelves of various marketplaces. Fast degradation of valuable constituents and deterioration of sensory attributes are observed due to the photosensitizing properties of chlorophyll pigments and derivatives such as pheophytin a. These compounds upon light exposure excites and may transfer energy to triplet oxygen, leading to the formation of the highly pro-oxidant singlet oxygen that is then added to double bonds of the unsaturated fatty acids by an “ene” addition mechanism (Frankel, 2005; Tsimidou, 2006). They can also abstract a hydrogen atom from the fatty acids by the carbonyl group of the isocyclic ring (Rawls & Van Santen, 1970). However, singlet oxygen formation can account for approximately 80% of the observed chlorophyll photo-oxidation. It is postulated that proton abstraction by the photo-activated carbonyl group of chlorophyll could account for the remaining 20% of the observed photo-oxidation. The rate of photo-oxidation mainly depends on the concentration of chlorophyll pigments, the type and content of carotenoids and tocopherols that act through physical and chemical quenching mechanisms and on oxygen availability (Choe & Min, 2006; Tsimidou, 2006).

Despite the fact that the light effect in the stability of unsaturated fats, oils and fat-containing products is recognized since many years (Clements, Van den Engh, Frost, Hoogenhout & Nooi, 1973), the photo-oxidation is less investigated in comparison to autoxidation. A particular interest in photo-oxidation studies is to evaluate the effect of the presence of photosensitizer in the matrix. The so far acquired knowledge regarding the underlying mechanism of photo-oxidation of VOO, the role of minor components and their interplay, temperature, oxygen availability and

packaging effect has been acquired using short term experiments, some of them requiring extreme conditions, or using long term experiments in which mild conditions are applied. In the first case, different approaches have been applied. Thus, exposure of a rather large amount of VOO (purified or not), or lipid models (e.g. triolein) at artificial light illumination of varying intensity (e.g. 400-12100 lx) and for a short period (few h to 140 h) has been employed (Tena, Aparicio & García-González, 2018; Psomiadou & Tsimidou, 2002). Whereas in the second approach, when long term experiments are carried out, multiple containers of authentic VOO samples to allow the frequent sampling have been exposed to diffuse daylight and/or low illumination (e.g. 400-800 lx) aiming to simulate real conditions for 3-12 months (Cecchi, Passamonti & Cecchi, 2010; Kanavouras & Coutelieris, 2006; Caponio, Bilancia, Pasqualone, Sikorska & Gomes, 2005). The minimum information obtained was through measuring primary oxidation products with wet chemistry. Nevertheless, in order to have an insight in the changes occurring in minor components, application of expensive and tedious chromatographic techniques was needed (Tena, Lobo-Prieto, Aparicio & García-González, 2018; Cecchi et al., 2010; Psomiadou & Tsimidou, 2002).

FTIR spectroscopy is a useful low cost technique for monitoring food quality parameters (Nenadis, Androulaki & Tsimidou, 2018) which is of particular interest in lipid analysis (Nenadis & Tsimidou, 2017; García-González, Baeten, Fernández Pierna, & Tena, 2013). In search of cost-effective approaches that could provide meaningful information about VOO stability under typical transport/storage conditions, recently, a new accessory, namely mesh cell, coupled with FTIR measurements has been found promising (Tena, Aparicio & García-González, 2017). This analytical approach provides real-time monitoring of VOO chemical changes upon exposure to mild light intensity and/or temperature via amplification of various spectral regions due to the

high surface to oxygen volume ratio. In this way, decision making on selecting conditions that will ensure quality protection during oil handling (transport and storage) is facilitated.

In the present study, a set of VOO samples were exposed as thin films to direct visible light (6000 lx, 24 °C) to examine whether mesh cell-FTIR monitoring could provide information on the effect of different concentration of total chlorophyll content to the stability of virgin olive oil. Furthermore, the capability of mesh cell-FTIR spectroscopy to assess the protective efficiency of light filtering packaging materials in the photo-oxidation process has been studied for the first time. The samples used in this study were selected from a greater pool of VOOs after characterization of their chemical composition and differed in the levels of total chlorophyll content from each other by ~10 mg/kg. Additionally, the protection offered by a light filtering packing material used in the market for olive oil bottling was examined under the same conditions. Thus, the hypothesis of the work is that mesh cell-FTIR can be used to evaluate the protective effect of light filtering material in VOO photo-oxidation and the results can be interpreted in terms of oil composition. Thus, this study expects to increase evidence for the applicability of mesh cell-FTIR spectroscopy as a green reliable tool for fast virgin olive oil quality control with minimal sample quantity.

2. Materials and methods.

2.1. Samples.

Four samples of Koroneiki monovarietal VOOs were used for this study, which were selected from a pool of 57 VOO (crop season 2016-2017). The strategy followed for the sampling is showed in Figure S1 (Supplementary material). VOO samples were

obtained from local producers just after their production and were stored at -22 °C in glass amber bottles till their use.

2.2. Solvents and standards, other materials.

All solvents used in the analytical determinations of this study were of HPLC grade (>95%) without further purification. DL- α -Tocopherol (>98%) and β -carotene (>97.0%) for HPLC were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Chlorophyll a of a declared purity >85% ($\leq 0.4\%$ Chlorophyll b) obtained from spinach and fatty acid methyl esters (FAMES) standard mixture (Supelco, Bellefonte, PA; Part number: CRM47885) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pheophytin a was obtained by 1N HCl acid treatment of the respective diethylether chlorophyll solution (Sievers & Hynninen, 1977).

The light filtering material used in this study is commonly applied to pack VOOs and extra VOOs in plastic bottles of 750 mL (Envases soplados Enso S.L., Jaén, Spain). The type of plastic used in this study is polyethylene terephthalate (PET) colored of green. This material has been tested to check the ability of light protection. Thus, when the material was exposed to 6000 lx, the light transmitted through this material was 40% lower (≈ 3600 lx) as measured with a digital lux meter (Trotec BF05, Heinberg, Germany). Moreover, it has been proven that this percentage of light intensity reduction is constant in the range of 400-8000 lx.

2.3. Methods.

2.3.1. Colorimetric determination of total polar phenol content.

The total phenol content (TPC) was estimated in the polar fraction extracted from 2.5 g of oil using methanol/water (60:40 v/v), using the Folin-Ciocalteu method

(Mastralexi, Nenadis, & Tsimidou, 2014). Absorbance values were recorded in triplicate by a Shimadzu UV 1601 (Kyoto, Japan) spectrophotometer equipped with glass cells of a 10 mm optical path length and 3 mL of volume. The results were expressed as mg caffeic acid/kg oil.

2.3.2 Spectrometric estimation of chlorophyll content.

The content of chlorophyll pigments was estimated with a IUPAC standardized method according to Pokorny, Kalinova and Dyssele (1995). In this method, the oil sample, once homogenized and filtered, is measured at 630 nm, 670 nm and 710 nm in a 5 mm or a 10 mm spectrophotometer cell against air instead of a reference cell. Then, the equation (1) is applied.

$$\text{Content of chlorophyll pigments} = 345.3 \times \left[A_{670} - \frac{A_{630} + A_{710}}{2} \right] / L \quad (1)$$

where the total content of chlorophyll pigments is expressed in mg/kg as pheophytin a, L is the optical path length in mm, and A_{670} , A_{630} and A_{710} are absorbance units at 630, 670 and 710 nm, respectively. Spectra recording in triplicate were carried out in the same spectrophotometer and processed with the aid of the UVProbe 2.31 (Personal Spectroscopy Software, Shimadzu) software facilities.

2.3.3. Color measurements.

The photometric color index (PCI) was calculated to estimate the color of the samples using the equation (2):

$$\text{PCI} = 1.29 \times (A_{460}) + 69.7 \times (A_{550}) + 41.2 \times (A_{620}) - 56.4 \times (A_{670}) \quad (2),$$

based on the corresponding absorbance (A) values obtained from the spectra recorded as described in §2.3.2 according to AOCS CC-13c-50 method (AOCS, 2017).

2.3.4. HPLC analysis of tocopherols and pigments.

The HPLC system used to carry out the concomitant analysis of tocopherols and pigments according to Psomiadou and Tsimidou, (2001) consisted of a pump, model P4000 (Thermo Separation Products, San Jose, CA), a Midas autosampler (Spark, Emmen, The Netherlands) and a UV 6000 LP diode array detector (DAD; Thermo Separation Products) in series with an SSI 502 fluorescence detector (FLD; Scientific Systems Inc., State College, PA, USA). Samples (8% w/v) were diluted in *n*-hexane and filtered through a 0.45- μ m membrane filter (Schleicher & Schuell, Dassel, Germany) just before HPLC analysis. The injection volume was 20 μ L. A gradient elution with *n*-hexane/2-propanol (99:1 v/v) (A) and 2-propanol (B) as eluents was used as follows: 0% B for 10 min; 0-5% B in 4 min; 5% B for 6 min; 5-0% B in 4 min; 0% B for 6 min. Separation was achieved on a 250 $\times$ 4 mm i.d., LiChrospher-Si, 5 μ m column (MZ Analysentechnik, Mainz, Germany) at a 1.2 mL/min flow rate. Care was taken to avoid exposure of samples and standard solutions to sunlight throughout the analytical procedure. Detection and quantification of tocopherols was performed by using a fluorescence detector (FLD) set at 294 nm (excitation) and 330 nm (emission), whereas quantification of pigments was carried out with a diode array detector [410 nm for pheophytin a and 453 nm for carotenoids (β -carotene+lutein)] using external calibration curves of pheophytin a and β -carotene respectively. Samples were analyzed in duplicate. Coefficients of variation (*CV*) are as follows: $CV_{\alpha\text{-toc}}\%=0.3$ ($n=5$), $CV_{\beta\text{-car}}\%=1.0$ ($n=5$), $CV_{\text{pheo a}}\%=0.2$ ($n=5$). Data were processed with the aid of ChromQuest software (version 3.0, Thermo Separation Products).

2.3.5. Determination of fatty acid composition.

The determinations of the fatty acid methyl esters (FAMES) composition were carried out by transesterification with methanolic solution of potassium hydroxide at room temperature (EC Reg. 2568/91 and updates). The fatty acids were identified by comparing their retention times with those of a commercially available calibration standard FAME mixture solution analyzed under the same conditions. The percentages of the individual FAMES were calculated on the basis of the total area of the peaks in the chromatogram. Analysis was carried out in triplicate.

2.3.6. Other quality parameters associated to the oxidation state.

Three quality parameters were determined: peroxide value (PV), free acidity (free fatty acids or FFA) and the absorbance in ultra-violet at 270 nm and 232 nm (K_{270} and K_{232} indices respectively) according to EC Reg. 2568/91 and updates. Measurements were carried out in duplicate ($CV_{PV\%}=5.4$, $n=5$; $CV_{FFA\%}=4.8$, $n=5$; $CV\%_{K_{232}}=4.9$, $n=5$; $CV\%_{K_{270}}=6.2$ $n=5$).

2.3.7. Mesh cell-FTIR spectroscopy.

The mesh cells were manufactured according to the design described by García-González and van de Voort (2009). The mesh material was a stainless steel AISI 304 (Suministro MAM S.L., Madrid, Spain), with a mesh number of 100, with a wire thread diameter of 0.11 mm and a light of 0.144 mm. The sample (20 μ l) was deposited onto this mesh in the aperture intended for sample loading. The sample was homogeneously distributed on the surface of the mesh with the help of a micropipette tip, avoiding any surface uncovered and any excess of oil (Tena et al., 2017).

Two experiments were carried out simultaneously. Thus, a first set of cells, once loaded with the samples, were horizontally positioned and they were directly exposed to visible light at 6000 lx and $24\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ without any light filtering packaging material. A second set of cells, loaded with the same samples, were covered in the aperture with a piece of light filtering packaging material. Thus, the light always passed through this material and is never projected onto the sample directly. These cells were subjected to the same conditions of light intensity (6000 lx) and temperature ($24\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$). The visible light was provided by a led tube of 9 W and the maximum and minimum temperature in the storage compartment was daily monitored. The experiments were run in triplicate to ensure consistency of the results. The two experiments were run for 344 h, and three spectra per day at different time intervals were scanned for each sample.

A Bruker Vertex 70 FT-IR spectrometer (Bruker, Optics, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector were used for the FTIR measurements. During the analysis, the mesh cell was mounted in the transmission cell holder provided with the instrument. All spectra were collected in the range 5000-400 cm^{-1} by co-addition of 32 scans and a resolution of 4 cm^{-1} using weak Norton-Beer apodization.

The spectra were collected and manipulated with OPUS version 7.2 (Bruker Optics, Ettlingen, Germany). All spectra underwent a normalization process by implementing a macro programmed with the Macro/Basic tool provided by Omnic 7.3 (Thermo Electron Inc., Madison, WI) according to Tena et al., 2017. The peaks heights at 3430 cm^{-1} regarding the hydroperoxide group and at (3534 cm^{-1}) assigned to alcohol group were measured relative to a selected single-point baseline (4600 cm^{-1}) and the peak heights corresponding to the isolated *trans* double bonds (968 cm^{-1}) were

measured relative to a selected double-point baseline (978 cm^{-1} , 928 cm^{-1}) by implementing macros programmed with the Macro/Basic tool (García-González & van de Voort, 2009). Spectral data processing was conducted using Statistica 8.0 (Stat Soft Inc., Tulsa, OK) and Excel 2016 (Microsoft, Albuquerque, NM).

In order to determine the moment in which the samples underwent the abrupt changes in their chemical composition due to photo-oxidation and to compare the results from the experiments carried out with the two different conditions (with and without light filtering material at 6000 lx), the following statistical procedure was applied to the spectroscopic data:

(i) The trend lines that describe the continuous change of the spectroscopic bands were modeled by linear regression and two equations were obtained for two segments of the curve.

(ii) The point in the x axis (expressed in h) in which the two lines from the two equations crossed one another was determined per each sample and storage experiment. This time point is when the samples underwent the abrupt changes in their chemical composition due to light effect.

3. Results and discussion

Firstly, the samples were characterized by determining some chemical parameters, which provided information relevant to the initial oxidation state of the samples and the content in minor compounds involved in the photo-oxidation process. The results were compared to those obtained for samples of the same cultivar in the literature. Then, the spectra obtained with mesh cell-FTIR were analyzed to select the best spectroscopic region to study the time-course changes of the chemical composition of the samples when they were exposed in mesh cell at 6000 lx. Finally, these results

were compared with those obtained when the samples were covered with the light filtering material.

3.1. Chemical composition of tested VOOs and interpretation of their initial oxidation states and their resistance to photo-oxidation.

From the pool of 57 VOO samples, the four ones (*cv.* Koroneiki) were selected to cover the natural variability in chlorophyll pigment content in this oil (Psomiadou & Tsimidou, 2001) (Figure S2). Chlorophyll pigment content was the critical factor for examining the filtering effect in this study. Moreover, the samples had low acidity values (<0.4), almost comparable levels of oxidation criteria (PV, K_{232} , K_{270} values) and fatty acid content (Table 1). The values of the quality parameters were in accordance with the specifications set for the extra virgin olive oil category in all the samples (EC Reg. 2568/91 and updates). The % of oleic acid belonged to the lower range reported in the literature for analyzed samples of the same cultivar (Psomiadou, Karakostas, Blekas, Tsimidou, & Boskou, 2003; Kosma, Vatavali, Kontakos, Kontominas, Kiritsakis, & Badeka, 2017). TPC and α -tocopherol content were among the highest reported for oils of the same cultivar grown in Greece (Kalogeropoulos and Tsimidou, 2014; Psomiadou and Tsimidou, 2001; Tsimidou, 2013) in line with the fact that they were fresh and produced according to the best practices as declared in questionnaires filled upon sampling by the producers. The carotenoid content (β -carotene and lutein) falls within the range reported in the literature (1.0-7.9 mg/kg), while the content of pheophytin a was lower in samples C and D than published range (4.9-31.2 mg/kg) (Psomiadou & Tsimidou, 2001). Even so, the spectrophotometrically calculated values of total chlorophyll content (11.73-46.30 mg pheophytin a /kg oil) were representative of the common range (9.1-50.7 mg/kg) measured in Greek VOOs

of the same cultivar (Psomiadou & Tsimidou, 2001). PCI values calculated from spectrophotometric data were in agreement with those of total chlorophyll content trend and covered a wide range of VOO coloration. Calculated ratios of α -tocopherol content/pheophytin a content and carotenoid content to pheophytin a content indicated that differences in the photostability of the four samples could be expected.

3.2. Infrared band selection to monitor VOO photo-oxidation and interpretation of their time-course changes

The next step was to select the spectral region that is more informative regarding the monitoring of photo-oxidation, assessing the protective effect of the light filtering material and establishing differences between samples. In the spectra, three regions assigned to primary and secondary oxidation products showed noticeable changes, which matches with previous works (Tena et al., 2017; García-González & Van de Voort, 2009). These regions were the OH stretching region ($3700\text{-}3100\text{ cm}^{-1}$), which includes hydroperoxides ($\sim 3430\text{ cm}^{-1}$) and alcohols ($\sim 3534\text{ cm}^{-1}$), and the alkene C-H deformation vibration ($1000\text{-}800\text{ cm}^{-1}$), which includes the band assigned to isolated *trans* isomers at $\sim 967\text{ cm}^{-1}$.

In order to assess, in quantitative terms, which spectral regions suffered major changes during light exposure (6000 lx), the ratios between the peak height at the beginning of the storage and at the end (after 344 h) were calculated for these bands. Figure 1 shows the values of these ratios. It was evidenced that major changes corresponded to the bands assigned to the primary oxidation products (hydroperoxides, $\sim 3430\text{ cm}^{-1}$). Thus, the peak height values of the alcohol band -secondary oxidation products-, and the band assigned to isolated *trans* isomers, were at least 4 times lower than the peak height values of hydroperoxide band in all the samples. Consequently,

the band assigned to hydroperoxides was selected as the most discriminatory compared with other spectral bands to study the effect of light in the oil stability.

The next step was to examine the time-course change of the peak height of this band over time (344 h) when no light protection was applied (6000 lx, 24 °C). Figure 2A shows the time-course trend of the peak height of the hydroperoxide band. The sample A presented the lowest oxidative stability and was oxidized faster. Sample B followed in oxidative stability, whereas C was even more stable. The most photo-stable sample was D. This order corresponded to the order in concentration of total chlorophylls and pheophytin a determined in the samples (Table 1). These results are also in agreement with the trend derived on the basis of α -tocopherol content /pheophytin a content ratio. In all the samples, the formation of hydroperoxides started from the first hours of the experiment and the rate of the formation (measured as the slope of the trend line in Figure 2A) was higher during the first 100 h. Sample A presented the highest rate (highest slope), followed by sample B, C and D with a rate of 1.2, 1.5 and 2.4-fold respectively lower than that of sample A for the same period (100 h).

3.3. Time-course changes in hydroperoxides in the presence of a light filtering material.

Once the oxidative stability of VOO samples was evaluated and the relative effect of chlorophylls in photo-oxidation was observed, the next step was to evaluate the protective effect of a light filtering packaging material, commonly applied in VOO bottling. The possible protective effect of the material was tested by covering the sample with it and applying the same storage conditions to those in the experiment without protection for comparison. Thus, a piece of plastic material cut from PET

bottles was fixed on each mesh loaded with the samples in such a manner that the light passed through this material before being projected to the sample. Figure 2B shows the time-course trend of the hydroperoxide band determined by monitoring the peak height at 3430 cm^{-1} during the light filtering experiment. The resulting curves show that the formation of the hydroperoxides in all the samples also started at the beginning of the experiment despite the use of light filtering material. However, comparison of the findings from the two different conditions – Figure 2A and 2B – indicated that the filter reduced the rate of the hydroperoxide formation. In this experiment, based on the amount of hydroperoxides formed in the first 100 h, the photo-stability of the samples was ($A < C \sim B < D$) in a similar order than in the previous experiment ($A < B \sim C < D$), establishing a clear difference in photo-stability between the two samples with the most noticeable difference in total chlorophyll content, A and D. In addition, the use of a light filtering material resulted in greater differences in the rate of hydroperoxide formation between the samples with the highest and the lowest total chlorophyll content. Thus, when the rate of hydroperoxide formation (slope) was calculated from the plotted lines (Figure 2B), sample D presented 5-fold lower slope than that of sample A, whereas this difference was only 2.4-fold when the light filter was not used (Figure 2A).

Another observed difference was the time to reach the plateau in the intensity of the hydroperoxide band in each sample under the two storage experimental conditions (Figure 2A and 2B). These time values (expressed in h) were obtained by determining the intersection of two regression lines estimated with the hydroperoxide peak heights before and after reaching the plateau (subsection 2.3.7). Figure 3 shows the time values obtained with this procedure. Sample D, with the lowest level of chlorophylls, reached the plateau later than the other samples in both experiments.

Comparisons among the four samples showed that sample A, B and C reached the plateau in 103 ± 8 h when they were exposed directly to light at 6000 lx and 153 ± 2 h when the filter was used regardless of the total chlorophyll content. Thus, the three samples (A, B and C) needed approximately the same time to undergo the abrupt change in their chemical composition in both experiments. On the contrary, sample D, with a considerable lower value of total chlorophyll content (11.73 mg/kg), was the most resistant (144 and 184 h respectively) despite its poorer content in quenchers. In general terms, it was observed that the light filtering packaging material provides protection against the selected light conditions (6000 lx) in all the samples delaying the plateau of the hydroperoxide formation at about 48 ± 7 h. Then, the stability measured as the time to reach the plateau in the hydroperoxide band showed that the differences in chlorophyll pigment concentrations (around 20 mg/kg between A and C) (Table 1) was not reflected as a difference in the time needed to reach plateau (Figure 3).

Differences between the two storage experiments could be also observed from the point of view of the progress of oxidation at different time points by calculating the ratios of peak heights of hydroperoxide band from the two experiments (without and with light filtering material) periodically, from 0 to 344 h. Thus, any deviation from 1 in the ratio would indicate an effect of the light filtering material on the oil deterioration. Figure 4 shows the line plots of these ratios. The maximum delay on hydroperoxide formation upon light protection was evidenced during the first hours of the storage. Thus, the maximum ratio value was reached after 28 h for sample A and 52 h for samples B, C and D. The maximum ratio values at these periods of time for samples A and C were 1.72 and 1.76 respectively, while the corresponding values for samples B and D were 2.6 and 2.2. With respect to these maximum ratios, the order of the samples did not fully match to the trend based on α -tocopherol content/pheophytin a content

ratio or the composition of major and minor compounds (Table 1). The values in the ratio described in Figure 4 did not remain constant over time. Their decrease was sample dependent. Thus, the ratio value of sample B decreased more steeply than in the others, and from 2.6 fell down to 1.6 at the end of the experiment, which can be partially due to its slightly higher content in unsaturated fatty acids (Table 1).

The results so far pointed out that the samples differing greatly in their total chlorophyll content presented different rate in the hydroperoxide formation in the absence or presence of light filtering material during light exposure. Sample A, with the highest total chlorophyll (or pheophytin a) content, and the lowest ratios of quenchers to pheophytin a contents, presented an abrupt change in hydroperoxide formation at earlier stage whilst sample D containing the lowest total chlorophyll (or pheophytin a) concentration and the highest ratios of quenchers to pheophytin a contents was more resistant. For samples B and C with intermediate levels of total chlorophylls (20-30 mg/kg) the trend of hydroperoxide ratios was not in line with the values of the ratios of quenchers to pheophytin a contents which indicated that sample C should be more resistant to photo-oxidation. It is evident that more work is needed to elucidate the role of quenchers and their relative ratios to photosensitizers under these conditions.

4. CONCLUSIONS

The present study showed that with a mesh-cell-FTIR methodology a prioritization of different factors in the photo-stability of VOO samples could be achieved. The main factor involved in the acceleration of the photo-oxidation process was found to be the concentration of total chlorophyll content. The experiments carried out with and without a light filtering packaging material demonstrated that the material

used delayed the formation of hydroperoxides for all samples. Thus, the maximum intensity of the hydroperoxide band was observed 48 h later in the experiment with light filtering material in all the samples. The effect of the light filter was found to be time dependent on the course of photo-oxidation which was in line with the complexity of the photo-oxidation phenomena and the expected interactions between quenchers/ photosensitizers. Thus, it has been confirmed the hypothesis that FT-IR mesh cell monitoring can be proposed as an informative method to evaluate the protective effect of light filtering packaging materials on the stability of VOO samples having different content in minor compounds that contribute to photo-oxidation.

ACKNOWLEDGMENT

Financial support by Fundación General CSIC (Programa ComFuturo) is acknowledged. This work was also supported by Spanish State Secretariat of Research, Development and Innovation (AGL2015-69320-R project). DT received funding for an Erasmus+ Internship.

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FIGURE CAPTIONS

Figure 1. Ratio values between peak heights at 344 h and 0 h of VOOs stored in mesh cell exposed directly to visible light at 6000 lx and 24°C. The peaks heights were measured at the bands assigned to hydroperoxides ($\sim 3430\text{ cm}^{-1}$), isolated *trans* isomers ($\sim 967\text{ cm}^{-1}$) and alcohols ($\sim 3534\text{ cm}^{-1}$).

Figure 2. Time-course trend of hydroperoxide band, peak height at 3430 cm^{-1} , during VOOs storage in mesh cell exposed at visible light (6000 lx) for 344 h in the absence (A) and presence of light filtering packaging material (B).

Figure 3. Time needed to reach plateau in the time-course trend of the hydroperoxide peak height at 3430 cm^{-1} for the four samples A-D in the absence (red bars)/presence (green bars) of light filtering packaging material during 344 h exposure at 6000 lx.

Figure 4. Ratio values of peak height of hydroperoxide band in the absence/ presence of light filtering packaging material over time of storage for samples A - D

Table 1. Quality parameters, fatty acid composition (% m/m fatty acids methyl esters), concentration of antioxidants and pro-oxidants and PCI values.

Chemical/physico-chemical parameters	Sample code			
	A	B	C	D
Free fatty acid (FFA) (% m/m of oleic acid) ^a	0.34	0.23	0.39	0.39
Peroxide value (PV) (meq/kg) ^a	11.4	10.7	11.1	9.9
K ₂₃₂ ^a	1.96	1.70	2.00	1.66
K ₂₇₀ ^a	0.15	0.12	0.15	0.12
FAMEs (% m/m) ^b				
16:0	15.19	13.67	15.47	14.80
16:1	0.98	0.79	1.21	0.95
17:0	0.06	0.07	0.23	0.04
17:1	0.12	0.16	0.19	0.06
18:0	2.76	2.41	2.28	2.79
18:1	73.95	74.81	72.82	73.64
18:2	5.42	6.69	6.52	5.99
18:3	0.85	0.67	0.60	0.74
20:0	0.25	0.36	0.34	0.42
20:1	0.25	0.23	0.22	0.24
22:0	0.11	0.10	0.08	0.11
24:0	0.04	0.03	0.03	0.04
Antioxidants and pro-oxidants				
Total polar phenol content (mg caffeic acid/kg oil) ^b	338.7	275.3	359.8	352.1
α -tocopherol content (mg/kg oil) ^a	359.9	266.2	336.2	230.7
Carotenoid (β -carotene+ lutein) content (mg/kg oil) ^a	7.01	2.89	2.44	1.30
Pheophytin a content (mg/kg oil) ^a	8.90	5.61	2.05	1.05
Total chlorophyll content (mg pheophytin a/kg oil) ^b	46.30	36.01	23.08	11.73
α -Tocopherol content /pheophytin a content	40.44	47.45	164.00	219.70
Carotenoid content/pheophytin a content	0.79	0.51	1.19	1.24
PCI ^b	-68.3	-52.4	-20.7	-9.5

^a, mean value of duplicate determinations; ^b, mean value of triplicate determinations

Figure 1

Figure 2A and 2B

Figure 3

Figure 4

Supplementary material

Figure S.1 Strategy followed for the sampling.

Figure S.2 Frequency distribution of total chlorophyll content of the pool of VOOs ($n=57$)

Graphical abstract