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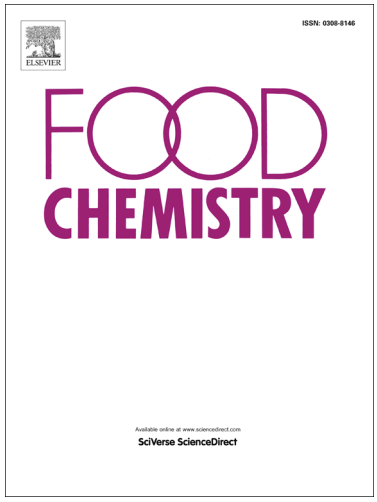

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Quality evolution of extra-virgin olive oils according to their chemical composition during 22 months of storage under dark conditions

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Running title: Effects of 22 months storage on virgin olive oil quality

ABSTRACT

The quality evolution of 14 extra-virgin olive oils (EVOOs), with different initial polyphenol and oleic acid (64.6–77.7%) levels, was determined during real-time storage in dark conditions at room temperature for 22 months. EVOOs with low (<20–200 mg/kg), medium (450–700 mg/kg), and high polyphenolic levels (750–1400 mg/kg) were used. We found high correlations among peroxide values, K_{232}, K_{270}, and storage time. Oleuropein derivatives decreased by 98%, 89%, and 85% in EVOOs with low, medium, and high polyphenolic levels, respectively, with the highest depletion occurring in those with the lowest initial concentrations. Besides having higher α-tocopherol protection from oxidative phenomena, EVOOs with the highest phenolic fractions showed lower head space accumulations of off-flavour volatile substances and thus demonstrated the best retention of sensory and health benefits. We propose that the shelf life of EVOOs can be determined from their initial levels of oleuropein derivatives.

Keywords: Dark storage; Shelf life simulation; Phenolic compounds; Fatty acid composition; Off-flavour volatile compounds; K_{232}

1. Introduction

Although the oxidation of extra-virgin olive oils (EVOOs) occurs very slowly under room temperature and dark storage conditions, it is the principal cause of the deterioration of productb quality and thus determines its shelf-life (Morales & Przybylsky, 2013; Frankel, 2014). Oxidative stability can be compromised even within 9 months after production, depending on the specific chemical composition of the product and the conditions under which it is stored. In fact, the chemical composition of virgin olive oils (VOOs) in terms of their concentrations of antioxidants (tocopherols and secoiridoid derivatives, in particular) and fatty acids, as well as the extraction methods involved, packaging processes and materials used, and the light and temperature conditions maintained during storage, markedly influence the period of oxidative stability, or the shelf life of these products (Bendini, Cerretani, Salvador, Fregapane, & Lercker, 2010; Stefanoudaki, Williams, & Harwood, 2010; Dabbou, Gharbi, Brahmi, Nakbi, & Hammami, 2011; Esposto et al., 2017; Fregapane & Desamparados Salvador, 2017). Fatty acids are susceptible to autoxidation, whereas minor compounds such as polyphenols and tocopherols provide resistance to VOO deterioration (Choe & Min, 2005, 2006). Furthermore, the sensory and nutritional values of EVOOs are directly related to their contents of minor substances, and mainly volatiles and polyphenols, which hence determine their shelf life stability (Angerosa, 2002; Cicerale, Conlan, Barnett, & Keast, 2013; Servili et al., 2015).

Over the past decade, many different systems involving several different methods have been developed to estimate virtual shelf lives (Li & Wang, 2018). However, very often, these studies have not considered that accelerated methods are not representative of true conditions because they often use extreme experimental conditions that vastly differ from those characteristic of real-life EVOO storage conditions. Moreover, most researchers have not considered the usual variability in the composition of antioxidants and fatty acids that characterise EVOOs produced throughout the growing zones of olive trees. Further, only a very limited number of studies have reported the

evolution of EVOO quality following real-time storage in olive mill tanks or under market conditions.

Gõmez-Alonso, Mancebo-Campos, Desamparados Salvador, and Fregapane (2007) evaluated the autoxidation stability of several EVOOs over 21 months of storage in darkness at room temperature and demonstrated that the extinction coefficient K_{232} was most correlated with product oxidation, which was directly influenced by the concentration of antioxidants. In another study conducted by Fregapane, Gomez-Rico, Inarejos, and Salvador (2013), Spanish EVOOs and OOs (olive oils) stored in market conditions at room temperature were monitored and only slight oxidation was observed after 1 year of shelf life. In contrast, we demonstrated in our previous study (Esposto et al., 2017) that EVOOs with different chemical compositions, exposed to light for 11 hours per day over 12 months of storage at room temperature in closed UVA grade bottles, underwent different levels of photooxidation, which were especially rapid in samples with lower levels of oleuropein derivatives. A high correlation between K_{270} and the photooxidation progression was also found.

Based on these studies, it can be assumed that the shelf life of an EVOO can be monitored by cheap and easy analyses (i.e. extinction indexes) and predicted by studying its initial antioxidant composition, and particularly, its polyphenol fraction. In this context, the aim of this study was to monitor the evolution of 14 EVOOs with different concentrations of polyphenols and oleic acid percentages for 22 months of storage in darkness and at room temperature. In addition to the most important market parameters [acidity, peroxide value (PV), and spectrophotometric constants], the evolution of antioxidant and volatile compounds was also evaluated.

2. Materials and methods

2.1. Materials

High-performance liquid chromatography (HPLC)-grade methanol was purchased from Fluka (Milan, Italy), and acetic acid, anhydrous sodium sulphate, ethanol, ethyl acetate, methanol, n-hexane, and 2-propanol solutions were purchased from Sigma-Aldrich (Milan, Italy). To cover

differences in commercial EVOOs in terms of olive cultivar, geographic origin, and mechanical extraction method used, already highlighted by a previous publication (Servili et al., 2015), we followed previously described procedures (Esposto et al., 2017) to obtain 14 different EVOOs with a range of polyphenolic contents and oleic acid percentages (as reported in Table 1). In brief, by stripping the polyphenols of two EVOOs, characterised by a high level of polyphenols (1476.7 mg/kg) and a high oleic acid percentage (77.7%) (Sample A) and by a medium level of polyphenols (682.5 mg/kg) and a low oleic acid percentage (64.6%) (Sample B), two other EVOOs were obtained, namely Sample C, characterised by a low polyphenol level (128.3 mg/kg) and high oleic acid percentage (77.7%), and Sample D, characterised by a very low polyphenol level (18.1 mg/kg) and low oleic acid percentage (64.6%).

Polyphenol stripping was conducted by repeating the following procedure three times: oil and water (1:1, v/v) were immediately mixed by vortexing for 3 min. The mixture was centrifuged in a basket centrifuge at 1,600 rpm for 8 min. The oily phase of the supernatant was recovered and filtered with sodium sulphate to remove any trace water. The application of central composite design (CCD), allowed us to know the exact percentage we had to use to obtain EVOOs with a range of polyphenols and oleic acid percentages as large as those present in the EVOOs in the market. Following this, the first four samples were placed at the hedge of the square to prepare another 10 EVOOs; specifically, A, B, C, and D samples were mixed as proposed by the statistical CCD. These 10 samples were named as follows: C+A, D+B, B+A, D+C, A+D, C+B, DC+DB, CA+BA, DC+CA, and DB+BA.

2.2. Experimental set-up to simulate storage

Twenty-two 750-mL green-glass bottles were prepared for each of the 14 EVOOs (A, B, C, D, C+A, D+B, B+A, D+C, A+D, C+B, DC+DB, CA+BA, DC+CA, and DB+BA). All bottles were placed in constant darkness (24 h per day) in a climate chamber with a set room temperature of 22 °C. A bottle from each sample was withdrawn from storage each month and labelled accordingly as follows: T0m, T1m, T2m, T3m, T4m, T5m, T6m, T7m, T8m, T9m, T10m, T11m, T12m, T13m,

114m, 115m, 116m, 117m, 118m, 119m, 120m, 121m, and 122m, these were stored at 12 °C until analyses, which were carried out 1 week after they were withdrawn.

2.3. Analytical determinations

All analyses were conducted for each of the 14 EVOOs at all 22 time points (T0m–T22m), with the exceptions clearly stated herein.

2.3.1. Merchandise parameters

The official methods of the European Commission [Commission Delegated Regulation (EU) 2015/1830] were used to determine the free acid content (g of oleic acid/100 g of oil), PVs (amount of hydroperoxides expressed as milli-equivalents of O_2/kg), and K_{232} and K_{270} extinction coefficients of all EVOO samples.

2.3.2. α-Tocopherol determination

The α-tocopherol contents were determined using HPLC equipped with a diode array and fluorescence detectors (HPLC-DAD-FLD; Agilent Technologies, Santa Clara, CA, USA), as described by Esposto et al. (2015).

2.3.3. Determination of phenolic compounds

Phenols were extracted following the methods described by Esposto et al. (2013) and evaluated by HPLC-DAD (Agilent Technologies, Santa Clara, CA, USA) analysis.

2.3.4. Determination of volatile compounds

The EVOO head space compositions of volatile compounds were determined using headspace solid-phase micro-extraction followed by gas chromatography/mass spectrometry (HS-SPME-GC/MS; Agilent Technologies, Santa Clara, CA, USA), as described by Esposto et al. (2013).

2.4. Statistical analysis

A priori one-way analysis of variance, using the Tukey test, was performed with SigmaPlot software package, version 12.3 (Systat Software Inc., San Jose, CA). The Modde 9.1 package was used to create a CCD for two factors and the SIMCA 13.0 chemometric package was used to

conduct the principal component analyses (PCA) and partial least squares (PLS) regressions. Both packages were purchased from Umetrics AB (Umeå, Sweden).

3. Results and discussion

3.1. Initial EVOO composition at T0m

An initial exploration of the qualitative parameters investigated in this experiment, including free fatty acid percentage, indicators of primary (PV and K_{232}) and secondary (K_{270}) oxidation products, showed that all 14 EVOO samples belonged to the "extra-virgin" category according to the current EU Regulation (2015/1830) for these parameters (data not shown). Regarding their initial acidic, antioxidant, and fatty acid compositions (Table 1), and also according to Servili et al. (2015), the EVOOs showed sufficient variability to conclude that all typologies of commercial EVOOs were represented. Specifically, the EVOOs showed the following ranges: oleic acid percentage, 64.5–77.7%; polyphenols, 18.1–1476.7 mg/kg; tocopherol, 173.8–220.5 mg/kg (Table 1). Furthermore, 70% of the polyphenol constituents were represented by the oleuropein derivatives 3,4-DHPEA, 3,4 DHPEA-EDA, and 3,4-DHPEA-EA.

3.2. Evolution of the overall quality of EVOOs during dark storage

The autoxidation phenomenon, possibly the most important variable when transporting EVOO or storing EVOO in the dark, was determined by evaluating the evolution of the legal, health-promoting, and sensory parameters, for 22 months. In particular, the acidity, the PV, extinction coefficients K_{232} and K_{270} product parameters, and acidic composition were considered. Regarding the health-promoting status, all phenolic compounds of secoiridoid derivatives were evaluated and considered. Furthermore, in addition to the secoiridoid compounds, which are responsible for bitter and pungent sensations, the sensory properties were also evaluated by measuring the head-space volatile compounds. The results were assessed using different multivariate statistical methods. Initially, PCA was conducted to observe the object dispositions in a two-dimensional space (Supplementary figure).

With the aim of evaluating the correlation between the time of storage in darkness (dependent variable) and the evolution of all aforementioned parameters (independent variables), the relative results were collected and analysed by a PLS (partial least of square regression of Latent variables) statistical elaboration. The relative score plot of the PLS model (Figure 1), which explained 94% of the total variance, showed an evident separation of the samples according to their phenolic concentrations. Specifically, the first component analysis revealed that the EVOOs were divided into three different classes as follows: those with the highest level of polyphenols (HPLEVOOs: A, B+A, CA+BA, C+A, and A+D samples), which were located on the left of the score plot, and those with the lowest levels of polyphenols (LPLEVOOs: D, D+C, C, and DC+DB samples), which were located on the opposite side. EVOOs with medium levels of polyphenols (MPLEVOOs: C+B, DC+CA, B, DB+BA, and D+B samples) were located in the middle of the score plot. Along the second component of the same plot (Figure 1a), the discrimination of the samples was presumably attributed to the time of storage in the dark; specifically, the samples were distributed from T0m to T22m, from the bottom to the upper part of the figure.

The relative loading plot (Figure 1b) displayed important information regarding the chemical parameters (independent variables, X) most responsible for distribution of the EVOO score plot and the correlation between samples and the time of storage in the dark (dependent variable Y). The secoiridoids such as 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA and the relative sum of them (total polyphenols), as well as the lignans, were quite distant from Y, demonstrating a negative correlation with storage duration. However, positive correlations between the storage duration and legal parameters including the spectrophotometric coefficients K_{270} and K_{232}, as well as PV, were evident. High correlations between PV and extinction indexes K_{270} and K_{232} increases, and time of storage were also observed by other authors in their research through PCA analyses (Ben-Hassine et al., 2013).

Furthermore, a strict relationship, given by the short distance in the loading plot with the dependent variable Y, was observed between the volatile compounds generally recognised as

responsible for the rancid defect (Kalua, Allen, Bedgood, Bishop, Prenzler, & Robards, 2007). These compounds include (*E*)-2-heptenal, 2,4-decadienal, as well as pentanal and hexanal, of which the last two are often associated with the "green fruity" sensation in fresh EVOOs but also with the "rancid" off flavour in fatty foods undergoing oxidation (Kalua et al., 2007).

Fatty acid evolution of the 14 EVOO samples, reported as the sum of the saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) and oleic acids, did not demonstrate a specific correlation with storage duration. These findings indicated their marginal involvement in the evolution of the quality of EVOOs, as previously observed by Esposto et al. (2017), when the starting samples were characterised by specific compositions (Table 1). These preliminary results suggested that the time of storage in the dark was negatively correlated with the antioxidant content of EVOOs and positively correlated with the most important parameters used to evaluate oxidative status (i.e. PV, K_{270}, and K_{232}) and the volatile compounds associated with oil rancidity. A deep analysis of the evolution of these substances was therefore conducted.

3.3. Evolution of PV, K_{232}, and K_{270}

Table 2 shows the evolution of the more correlated parameters (such as the PV, K_{270}, and K_{232}) during dark storage according to the PLS results; the data are expressed as the time required to exceed the current EU regulation legal limit and the increase rate, which was determined every 2 months of storage in the dark. The PV, K_{270}, and K_{232} parameters for the samples collected for the three different groups classified in the PLS model, including HPLEVOOs, MPLEVOOs, and LPLEVOOs, showed high and direct correlations with the storage duration, as respectively shown by the high R^2 values and the positive slopes of the regression equations (Table 2). Furthermore, the initial polyphenol content of the samples was shown to have a strong influence on the evolution of these parameters.

An analysis of single parameters revealed that the PVs increased rapidly when the initial polyphenol concentration was low (as seen in D+C and D), and exceeded the legal limit of 20 meq O_2/kg in 10 and 8 months, respectively. In the same LPLEVOO classification, the other two

samples (C and DC+DB) reached the legal limit in 14 and 16 months, respectively. In contrast, most A-containing EVOOs (A, B+A, and CA+BA) never reached the legal limit, and thus remained classified in the "extra-virgin" category until the end of the 22 months of storage in the dark. However, the time taken for C+A and A+D EVOOs such HPLEVOOs, which contained lower contents of polyphenols, to exceed the maximum PV was equal to or greater than 20 months, respectively. Samples included in the MPLEVOOs group reached the PV legal limit between 16 and 20 months. Among them, D+B and DC+CA exceeded 20 meq O_2/kg after 16 months of storage. The same trend was observed with regards to the rate of increase that was evaluated every 2 months, whereby higher levels (2.60–4.92) characterised the samples with the lowest polyphenol content. For the A-containing EVOOs, augmentation of the PV was contained between 0.53 and 1.60, with the lowest value observed for the HPLEVOO samples. The range of the rate increase was 1.49–1.78 for MPLEVOOs, whereas the range of R^2 was 0.53–0.93, relative to A+D and D samples, respectively. Furthermore, a general good relationship between the values of the slopes of the regression equations (Table 2) and the relative increasing rates was observed. In fact, sample A which was characterised by the lowest increasing rate (0.53) also showed the lowest slope value (0.3367). In contrast, in the sample with the highest increasing rate (sample D, with a value of 4.92), we found the highest slope level (equal to 5.0865). Similar results in terms of the evolution of the oxidative status of the 14 samples was observed by analysing the results of the spectrophotometric coefficients K_{270} and K_{232}. Specifically, K_{270} revealed that the time required to exceed the relative EVOO legal limit (0.220) was positively correlated with the storage time (R^2 range 0.72–0.96, corresponding respectively to A and DB+BA samples).

Furthermore, even for this parameter, a discrete correlation between the value of the regression equation slope and the relative increasing rate was found. In particular, in those EVOOs with the lowest level of K_{270} increases over the 22 months of storage, such as A, C+A, and C+B (0.008), the regression equations were characterised by the lowest slope values registered for this parameter and corresponding to 0.065, 0.078, and 0.076, respectively. Nevertheless, EVOOs

characterised by a higher K_{270} increasing rate also showed higher slope values, such as sample C where an increasing rate of 0.026 corresponded to a 0.241 slope value.

However, in sample D, where the coefficient K_{270} increased to 0.028 in each of 2 months, the relative regression equation was characterised by a low slope (0.0094), but at the same time, by the highest value of the intercept (0.1685). Moreover, the higher the initial content of the secoiridoid derivatives, the longer the time needed to reach "virgin" status (i.e. 18–22 months for HPLEVOOs, 14–18 months for MPLEVOOs, and 6–14 months for LPLEVOOs). In addition, according to this parameter, the richest HPLEVOOs in terms of the secoiridoid content, such as A and B+A, never reached the maximum limit and thus remained in the EVOO category until the end of the experiment, as was also observed for the PV value.

More interesting results were gathered by monitoring the K_{232} spectrophotometric constant evolution during the 22 months of darkness, specifically, as revealed by the highest correlation with the shelf life duration of the 14 EVOOs (R^2 = 0.77–0.97, belonging respectively to DC+CA and B). Particularly, as observed for K_{270} and even more clearly for the PV t, K_{232} also showed a good correlation between the increasing rate and the regression equation slope values. Nevertheless, since the differences in K_{232} increasing rates among samples were lower than those revealed for the other two legal parameters, little differences among slopes values were observed. In EVOO sample A, as an example, at an increasing rate of 0.095, a slope value of 0.0707 was correlated; in contrast, at the highest increasing rate registered for this parameter and corresponding to sample D, a value of 0.156 for the regression equation slope was associated.

The same evolutionary trend in this parameter was observed for the PV and K_{270}, but in all cases, the time required to exceed the maximum level for the "extra-virgin" status (2.50) was always less. Even for those HPLEVOOs that remained in the "extra-virgin" category according to the PV and K_{270} values, they showed a loss of this status at the end of storage according to the K_{232} parameter, within 16–22 months (Table 2). These findings suggest a higher sensibility of the K_{232} measure in monitoring the evolution of EVOO shelf life. Specifically, the D samples were declassed

from "extra-virgin" to "virgin" status in a range of 6–8 months, 6–14 months, and 8–16 months according to the K_{232}, K_{270}, and PV measures, respectively. This higher sensibility was better revealed in samples DC+DB, C, and D+C, which were declassed from the "extra-virgin" status in 10, 8, and 6 months, respectively, hence 4, 8, and 4 months before that revealed by PV and 4, 2, and 2 months before that revealed by K_{270}, respectively (Table 2).

In the D sample, which was characterised by a polyphenol concentration significantly lower than that of the other 13 samples (Table 1, $p < 0.05$), no differences in time were observed between K_{270} and K_{232}, which were exceeded only after 6 months instead of 8 months, if the PV value was considered (Table 2). These findings were confirmed by the results of previous studies, whereby a strict relationship between the EVOO oxidation evolution and the initial polyphenol concentration was demonstrated. Specifically, Esposto et al. (2017) used similar samples but exposed them to light conditions for 11 hours per day for 165 days. According to all measured merchandise parameters, the samples richest in polyphenols took the longest to lose legal "extra-virgin" quality status. In this case, the authors found that K_{270} was mostly correlated with the oxidative status of the samples and is hence more applicable to monitor EVOO shelf life evolution (Esposto et al., 2017).

Moreover, our results confirmed the observations of Gõmez-Alonso et al. (2007), who also reported a high correlation between the concentration of secoiridoids in the EVOOs from Spain, belonging to several cultivars and olive maturation states, and their resistance to oxidation during 21 months of dark storage at room temperature. Furthermore, a prediction of the time required to exceed the legal classification upper limit according to the marketing standard for an EVOO was built, using the K_{232} parameter as the best predictor. Even Psomiadou and Tsimidou (2002) demonstrated the higher sensibility of this index in monitoring the evolution of the oxidative status with Greek EVOOs stored under dark conditions longer than their 24-month shelf life, in addition to a strong correlation between higher polyphenol content and lower levels of this parameter.

Furthermore, Kalua, Bedgood, Bishop, and Prenzler (2006) compared the effect of several storage conditions on the real-time shelf life of VOOs, and highlighted K_{232} as one of the three most

important freshness markers, together with *E*-2-hexenal and K_{270}. Fregapane and Desamparados Salvador (2017) also confirmed that K_{232} is often the first oxidation index to exceed the upper limit for commercial-grade EVOO.

3.4. Changes in hydrophilic phenols

Figure 2a shows the evolution of the polyphenol content of the EVOOs, measured every 2 months and expressed as the sum of oleuropein and ligstroside derivatives and lignans, from the beginning to the end of the 22 months of storage in the dark. The results demonstrated that the loss of these substances was significant in all samples, but that the rate of the decrease clearly differed among the three groups. Indeed, for the HPLEVOOs, with initial concentrations of 1500 mg/kg and 655 mg/kg, the decrease ranged between 59.8% and 80.4%, with a mean of loss of 73.4% (i.e. the strongest loss in this group) in samples A+C and A+D, which had initial polyphenol values of 800 mg/kg and 780 mg/kg, respectively.

In contrast, for the LPLEVOOs with initial concentrations of 350 mg/kg and 18 mg/kg, the decrease was the most consistent (74.5–100.0%). In fact, in samples C and D, no HPLC detection of these antioxidants was observed after 22 months of storage. However, even for samples D+B, DC+DB, and D+C, the residual concentration of these substances was less than 50 mg/kg at the end of the experiment. For this set of samples, the loss mean was established as ~90%.

For MPLEVOOs (level of polyphenols: 661–503 mg/kg), the mean decrease in the concentration was 74.5%, and the decrease ranged from 71.0% to 75.6% (minimum to maximum). These results suggested that a loss of the principal antioxidants in EVOOs was always evident, but at different levels according to the initial concentration of the antioxidants. Specifically, a higher initial concentration of these substances at the beginning of storage was associated with a lower loss until the end of the simulated shelf life experiment. Our findings were in contrast to those observed by Gõmez-Alonso et al. (2007), in which the highest loss of polyphenols was observed for the EVOOs with higher level of secoiridoids.

However, Esposto et al. (2017) confirmed the specific trend observed in the present study, wherein the highest decrease in the sum of antioxidants was registered in the samples with the lowest initial concentration of secoiridoids. As suggested by Esposto et al. (2017), polyphenols can limit the oxidative phenomena that occur in EVOOs via several mechanisms. In fact, even in dark conditions, polyphenols are capable of limiting the radical oxidation of lipid peroxyl radicals and hydroperoxides (Khan, 1955; Choe & Min, 2005, 2006, 2009; Roche, Dufour, Mora, & Dagles, 2005). Moreover, in all EVOOs characterised by higher initial polyphenol concentrations, reduced polyphenol losses (Figure 2a) and lower PV and K_{232} values (Table 2), which describe the primary phase of oxidation, were already evident. Additionally, the EVOOs with the highest polyphenol contents, which rapidly limit or interrupt the oxidative process, had the highest residual polyphenol content after the oxidative processes. Therefore, the consumption of such EVOOs would confer benefits (from their biological activities) (Cicerale et al., 2013; Covas, Fitò, & de la Torre, 2015). Furthermore, the observed decreasing trend for each group of samples revealed an almost constant trend for LPLEVOOs throughout the experimental storage period; however, in the other two groups, in particular for HPLEVOOs, the decrease was very slow during the first 6 months of simulated shelf life, but increased during the last period of shelf life.

The specific contents of and changes in oleuropein and ligstroside-derivatives, as well as lignans and *α*-tocopherol, were also investigated over the 22 months of storage. The sum of 3,4-DHPEA, 3,4-DHPEA-EA, and 3,4-DHPEA-EDA (Figure 2b), which represented the oleuropein derivatives, fully retraced the degradation trends observed for the total hydrophilic phenols (Figure 2a). At the beginning of storage, they represented 70% of the total hydrophilic phenols in all EVOOs, and specifically, 578–1144 mg/kg in the HPLEVOOs, 298–532 mg/kg in the MPLEVOOs, and 8–258 mg/kg in the LPLEVOOs. The percentage decrease in oleuropein derivatives was 70.0–92.9% for HPLEVOOs, 81.8–92.3% for MPLEVOOs, and 94.3–100.0% for LPLEVOOs. In all samples the decrease in oleuropein derivatives always exceeded 70% of the initial level, even if the maximum losses were registered in the EVOOs with the poorest oleuropein derivative contents (i.e.

where the level of the decrease was higher than 94% and reached 100% loss in almost all samples belonging to this group). In fact, only in D+B, which had the richest oleuropein content among the LPLEVOOs, was a very small quantity of these substances was found at the end of the experiment. The mean loss in oleuropein derivative content in this group was 98%. In the HPLEVOOs, however, all samples had minimum levels higher than 30 mg/kg after 22 months of storage, with the higher contents characteristic of those that initially had the highest concentrations of oleuropein derivatives, such as samples A and B+A. In the MPLEVOOs, the mean losses were established at approximately 89%. However, traces of these hydrophilic phenol classes were also observed, in particular in DB+BA and B, where at the end of the shelf life, the concentrations of oleuropein derivatives were higher than 30 mg/kg.

A comparison of Figure 2a and Figure 2b shows very similar trends in terms of the decrease in oleuropein derivatives, not only with regards to the total quantity of loss but also the temporal effect. Specifically, the EVOOs richest in oleuropein derivatives showed a significant decrease in these compounds after the first 6 months of storage, and the higher the initial content of oleuropein derivatives, the later the significant loss occurred. This was particularly clear in sample A, where these compounds decreased from 600 mg/kg to 400 mg/kg only after 16 months of shelf life.

In contrast, a progressive and constant diminution occurred in EVOOs with lower levels of oleuropein derivatives, including those that belonged to the MPLEVOO group such as DC+CA and C+B and all samples belonging to the LPLEVOO group. These results were consistent with those of another previous study by Morellò, Motilva, Tovar, & Romero (2004), who observed significant decreases in secoiridoid derivatives, and especially 3,4-DHPEA-containing substances, in commercial VOOs of the Arbequina cultivar after 12 months of storage. Even Esposto et al. (2017) found higher losses of 3,4-DHPEA, 3,4-DHPEA-EA, and 3,4-DHPEA-EDA, especially in those samples characterised by lower phenolic and oleuropein derivative concentrations. Particularly, the authors observed that the trend in the evolution of these substances was strictly dependent on their initial concentration and highlighted that the lower the initial quantity of oleuropein derivatives, the

more rapidly they were lost within the first phases of shelf life. Collectively, these results clearly demonstrate the direct involvement of the oleuropein derivatives as "delayers of the oxidation phenomena based on several antioxidant mechanisms, which are capable of protecting the EVOOs from alterations in various cases such as accelerated oxidation conditions, high temperatures adopted during specific cooking systems (i.e. pan frying), prolonged light exposure, or, as evidenced in this last experiment, when stored in darkness at room temperature (Mancebo-Campos, Desamparados, Salvador, & Fregapane, 2014; Servili et al., 2015; Fregapane & Desamparados Salvador, 2017).

Different trends were observed for both ligstroside derivatives and lignans. A decrease in ligstroside derivatives, determined as the loss of both *p*-HPEA and *p*-HPEA-EDA (Figure 2c), was very different according to the initial concentrations of total polyphenols. Specifically, the percent loss in HPLEVOOs and MPLEVOOs ranged between 23.6% and 45.6% (with 36.6% as the mean) and 32.7% and 47.9% (with 39.7% as the mean), respectively, after 22 months of storage. In contrast, the LPLEVOOs showed a mean loss of 81.3%, or a minimum decrease of 58.6% for those with the highest initial polyphenol levels, such as samples D+B and DC+DB, and a maximum decrease of 100.0% for those with the lowest initial polyphenol levels, such as samples C and D. The ligstroside derivatives thus underwent a constant, progressive, and important decrease in the EVOOs characterised by very poor heritage in terms of hydrophilic phenols and oleuropein derivatives. When the concentration of these substances was higher than 200 mg/kg, the content of ligstroside derivatives was less depauperated. As already observed by Esposto et al. (2017), it can be assumed that ligstroside derivates have more involvement in preventing oxidative phenomena when the EVOOs contain a very low initial quantity of oleuropein derivatives. Less decreases in the content of lignans [sum of (+-pinoresinol and 1(+)-acetoxypinoresinol)] were also observed in HPLEVOOs and MPLEVOOs, specifically 7.9%–25.9% and 10.3%–35.5%, respectively, with a mean of ~22.0% for both groups. Higher losses were observed in LPLEVOOs, with a minimum loss

of 15.3% for D+B samples and up to a 100.0% loss for C and D samples (with a mean loss of 52.8%) (Supplementary Figure).

These results were validated by previous studies (Esposto et al., 2015; Esposto et al., 2017), which identified lignans as substances that are less involved in opposing the oxidative phenomena, both in accelerated or in real-time shelf life scenarios. Nevertheless, a higher decrease, reaching the total loss of these substances, occurred in EVOOs with low polyphenol contents, specifically, those low in antioxidant sources that are involved in opposing such deterioration. Owen, Mier, Giacosa, Hull, Spiegelhalder, and Bartsch (2004) demonstrated the primary importance of polyphenols as antioxidant compounds by studying this property in EVOOs characterised by low quantities of oleuropein and ligstroside derivatives (Owen et al., 2004).

3.5. Evolution of α-tocopherol

α-tocopherol HPLC analyses conducted over the entire shelf life period demonstrated a constant and progressive decrease in all samples (Figure 2d), but the decrease was inversely correlated with the initial content of hydrophilic phenols. Indeed, at the beginning of the experiment, the level of this vitamin (vitamin E) was 173.8–220.5 mg/kg (Table 1), and, even if no correlations were found between the initial polyphenols and tocopherol concentration, the preservation until the end of the storage period was determined by the presence of the sum of polyphenols. Specifically, the mean percent decrease was 45.0% (with a minimum–maximum range of 17.5–55.6%), 59% (with a minimum–maximum range of 47.3–66.6%), and 87.0% (with a minimum–maximum range of 67.7–100.0%), respectively, for HPLEVOOs, MPLEVOOs, and LPLEVOOs. None of the EVOOs belonging to the first and the second group showed a total loss of tocopherol at the end of the simulated shelf life period, whereas the EVOOs with the poorest content of hydrophilic phenols, such as samples C and D, had lost 100.0% of their vitamin E content at the end of the storage period; in sample D+C, only a residual quantity (10 mg/kg) remained. Only those LPLEVOOs that started with higher hydrophilic phenol contents ended the storage period with a residual tocopherol content exceeding 50 mg/kg. In contrast, after 22 months of shelf life, the

HPLEVOOs were found to have concentrations higher than (sample A) or equal to 100 mg/kg of tocopherol (B+A, CA+BA, C+A, and A+D). Further, in the MPLEVOOs, good preservation of this substance was observed. In fact, in all the EVOOs of this group, tocopherol levels were higher than 70 mg/kg.

These results confirmed the marked capacity of hydrophilic phenols to delay or inhibit α-tocopherol degradation, specifically via their primary involvement in autoxidative phenomena and thus their ability to preserve the loss of this important substance. The preservation of α-tocopherol at the end of 22 months of dark storage is suggested to extend the shelf life of EVOO in other aspects after the storage period (i.e. light exposure, cooking use). It also guarantees a higher nutritional characteristic of the product because of its antioxidant effect and vitamin properties (Morellò, Motilva, Tovar, & Paz Romero, 2004; Esposto et al., 2015; Esposto et al., 2017).

3.6. Evaluation of the evolution of volatile compounds

The group of volatile compounds specifically monitored during this experiment comprised C_7–C_{11} aldehydes [2-heptenal, (*E*)-2-heptenal, (*E*)-2,4-heptadienal, (*E*, *E*)-2,4-heptadienal, (*E*, *E*), 2,4-nonadienal, (*E*, *E*)-2,4-decadienal, (*E*, *E*)-2,4-decadienal, and (*E*, *E*)-2-undecenal] because of their known involvement in the oxidative process as final products of hydroperoxide fragmentation (Angerosa, 2002; Kalua et al., 2007; Bendini et al., 2010; Fernandes, Ellis, Gámbaro, & Barrera Arelland, 2018; Oueslati, Krichene, Manaï, Taamalli, Zarrouk, & Flamini, 2018). As shown in Figure 3, all T0m samples showed the presence of off-flavour aldehydes at < 0.1 mg/kg, confirming the initial fresh aroma of all 14 EVOOs. Nevertheless, changes in the concentrations of these substances in the head spaces of the samples after this time were evaluated at 6 months (T6m), 12 months (T12m), 18 months (T18m), and 22 months (T22m). This demonstrated that the entire dark storage (i.e. simulated shelf life) was characterised by large variability in the accumulation of these compounds according to their initial antioxidant levels. Specifically, the increase in volatile compounds was evidently much higher in LPLEVOOs than in the other two EVOO groups from initial monitoring (T0m–T6m), and especially in samples D+B, DC+DB, and D, where the levels

reached 3–4 mg/kg. In contrast, the A-containing samples, which represented those in the HPLEVOO group, showed a lower accumulation of negative aldehydes (0.21–0.39 mg/kg). Very similar levels were also registered in the MPLEVOOs, where the sum of the negative aldehydes did not exceed 0.7 mg/kg and this only occurred with sample DB+BA, whereas all other EVOOs belonging to this group did not accumulate more than 0.3 mg/kg of aldehydes (Figure 3).

Between the 6th and the 12th months, the LPLEVOOs showed a lower increase in aldehydes when compared to that during first period. Except for DC+DB, C, and D+C samples, which accumulated > 1 mg/kg, 0.1 mg/kg, and 0.5 mg/kg, respectively, no increase in aldehydes was observed for LPLEVOOs. In contrast, augmentation was observed in the HPLEVOOs, except samples A and B+A, when compared to that in the previous intervals. The increase in aldehyde levels ranged from 0.3 to 0.5 mg/kg. However, after 1 year of storage, the sum of the C_7–C_{11} aldehydes remained at < 1 mg/kg in this set of EVOOs. In the MPLEVOOs, this phase was the most important in terms of the accumulation over the entire period of dark storage, with all MPLEVOOs exceeding 1 mg/kg of C_7–C_{11} aldehydes.

Except for that in HPLEVOOs, the phase between the 12th and 18th months was characterised by a very rapid increase in negative volatiles for all other EVOOs. As highlighted by Figure 3, the accumulation was 0.65, 0.91, 1.1, 1.8, and 2.4 mg/kg in LPLEVOO samples DB+BA, B, DC+CA, C+B, and D+B, whereas the accumulation was 1.5, 1.1, 2.1, and 2.3 mg/kg in MPLEVOO samples DC+CB, C, D+C, and D, respectively. In contrast, the HPLEVOOs showed the opposite results because the lowest level of accumulation in that period was 0.25–0.7 mg/kg. Even after 18 months, samples A and B+A did not reach 1 mg/kg of C_7–C_{11} aldehydes. For this last group of samples, the final period of dark storage was the most insightful in terms of the accumulation of these volatile compounds since a substantial increase was revealed, except for in sample A, with a final level of almost 3 mg/kg in sample A+D (the lowest EVOO in terms of polyphenol content) and an accumulation of 2 mg/kg of off-flavour aldehydes in samples CA+BA and C+A.

Between the 18th and the 22nd months of storage, samples belonging to MPLEVOOs and LPLEVOOs did not show any important changes when compared to the marked increases registered during the first 6 months and for the 12th to the 18th months. These data confirmed the observations of Esposto et al. (2017) (who conducted a light-exposure shelf life experiment using similar EVOOs over a period of 11 months), wherein samples with higher concentrations of polyphenols showed lower accumulation of negative aldehydes, following a similar trend as described herein, with a rapid decrease in sensory quality due to the accumulation of off-flavour aldehydes soon after the first period of experimentation. In this regard, as observed with photo-oxidative stress, the antioxidant fraction of the EVOOs represented by oleuropein derivatives seemed to immediately oppose the oxidative phenomenon of the autoxidation, and hence, the first phase represented by the hydroperoxide formation before C_7–C_{11} aldehyde production (Choe & Min, 2005, 2006). This was confirmed by the lower levels of K_{232} spectrophotometric indexes, which serve as an indirect measure of hydroperoxide accumulation. Over the 22 months of storage, the hydroperoxides in the HPLEVOOs absorbed light at a wavelength of 232 nm, or the reference wavelength considered for the K_{232} spectrophotometric index measurement. These findings suggest that K_{232} is both capable of monitoring product quality better than free acidity, K_{270}, and PV, as previously demonstrated, and also monitoring sensory qualities in terms of predicting the generation of off-flavour volatile compounds, according to the initial concentration of polyphenols. Indeed, with the exception of DC+DB, all EVOOs characterised by a low polyphenol content, such as D and D+C in particular, reached the legal K_{232} limit of 2.50 (EU Reg. 2015/1830) after 6 and 8 months of storage (shelf life) in the dark, which represents the critical period wherein a substantial accumulation of C_7–C_{11} aldehydes was also revealed by head space analysis.

4. Conclusion

The real-time shelf life of food is not easily determined due to constraints in the time and funding needed to fully investigate them; however, studies on important aspects of product evolution can better clarify changes in the product quality, as well as the actual “best before” dates.

Furthermore, these kinds of experiments also afford the opportunity to understand the most influential factors and chemical variables that govern important changes in product quality, and hence, their further potential involvement in predictive models can be considered. In the present study, we investigated 14 EVOOs, which largely differed in the chemical parameters that mostly influence the quality of EVOOs, such as fatty acids, oleic acid in particular, and antioxidants. After statistical exploration based on PCA and PLS modelling, we further investigated specific parameters that were highly negatively and positively correlated with the evolution of the shelf life of EVOOs.

Compared to other legal parameters usually measured, the K_{232} spectrophotometric index demonstrated a higher positive correlation with the oxidative status of the EVOOs, which highlighted its potential use as an effective and cheap measure to monitor the legal quality evolution of such products during storage in dark conditions. This study also confirmed that the higher the initial quantity of polyphenols (i.e. oleuropein derivatives, in particular), the longer duration of EVOO quality stability. These phenols react immediately with the dissolved oxygen in the oil, as well as other free radicals, indicating their O_2-quenching and radical scavenging properties, and thus limiting or avoiding autoxidation evolution. As a result, EVOOs with higher initial quantities of polyphenols remained preserved for longer durations, retained a higher antioxidant level, and better delayed the production of off-flavour volatile compounds.

Indeed, in the present study, the HPLEVOOs and EVOOs with higher contents of polyphenols in the MPLEVOO group underwent the minor loss of secoiridoid derivatives and α-tocopherol, and the limited accumulation of volatile C_7–C_{11} substances responsible for the rancid defect was observed. These results, which confirmed our previous findings (Esposto et al., 2017), indicated that the potential shelf life of EVOOs stored under determined conditions (i.e. light/dark conditions, room temperature), can be predicted by studying their initial phenolic compositions.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Table 1
Initial fatty acid (%) and antioxidant (mg/kg) compositions of the 14 extra-virgin olive oils used in this study[1].

	SFA	MUFA	Oleic acid	PUFA	Polyphenols (sum)	Oleuropein derivatives	Ligstroside derivatives	Lignans	α-Tocopherol	C5-C6 Aldehydes	C7-C11 Aldehydes
	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	µg/kg	µg/kg
A*	13.2 ± 1.0a	78.6 ± 4.9a	77.7 ± 4.9a	8.0 ± 0.6a	1476.7 ± 4.4a	1143.9 ± 3.0a	274.3 ± 1.0a	58.5 ± 0.2a	173.8 ± 3.1ai	652.5 ± 3.1a	173.8 ± 3.1ag
B	19.9 ± 1.0b	67.6 ± 5.5a	64.6 ± 5.5a	12.5 ± 1.0b	682.5 ± 8.2b	508.7 ± 1.7b	135.4 ± 0.9b	38.4 ± 1.5b	220.5 ± 7.8b	865.5 ± 7.8b	220.5 ± 7.8b
C	13.4 ± 0.7a	78.6 ± 4.6a	77.6 ± 4.6a	8.0 ± 0.6a	128.3 ± 1.7c	66.1 ± 0.6c	55.9 ± 0.05c	6.2 ± 0.1c	172.6 ± 1.6a	279.5 ± 1.6c	172.6 ± 1.6a
D	20.0 ± 1.1b	67.4 ± 5.5a	64.5 ± 5.5a	12.5 ± 1.0b	18.1 ± 0.8d	8.1 ± 0.5d	9.1 ± 0.5d	0.9 ± 0.2d	218.6 ± 0.4bg	346.0 ± 0.4d	218.6 ± 0.4bh
C+A	13.5 ± 0.3a	78.5 ± 4.6a	77.7 ± 4.6a	8.0 ± 0.2a	802.1 ± 10.4e	605.0 ± 0.4e	164.7 ± 10.0e	32.4 ± 0.05e	172.7 ± 2.6a	475.0 ± 2.6e	172.7 ± 2.6a
D+B	19.9 ± 0.9b	67.6 ± 4.2a	64.6 ± 4.2a	12.5 ± 0.6b	350.3 ± 6.5f	258.4 ± 0.3f	72.3 ± 6.4f	19.7 ± 0.03f	220.5 ± 1.0b	644.0 ± 1.0a	220.5 ± 1.0b
B+A	16.8 ± 1.4cde	73.0 ± 4.7a	71.0 ± 4.7a	10.3 ± 0.9cd	1059.8 ± 2.6g	811.0 ± 1.1g	201.8 ± 0.5g	47.0 ± 0.2g	197.3 ± 2.2cd	719.0 ± 2.2f	197.3 ± 2.2ce
D+C	16.5 ± 1.4cde	73.4 ± 2.5a	71.7 ± 2.5a	10.0 ± 0.9ade	70.9 ± 3.9h	35.4 ± 0.3h	31.9 ± 3.2h	3.6 ± 0.3cd	195.2 ± 2.2ce	320.0 ± 2.2g	195.2 ± 2.2cf
A+D	16.5 ± 0.6cde	73.1 ± 4.3a	71.3 ± 4.3a	10.4 ± 0.4cde	655.7 ± 5.5i	578.0 ± 1.8i	48.3 ± 1.3c	29.4 ± 1.9h	197.4 ± 4.7ch	574.0 ± 4.7h	197.4 ± 4.7ce
C+B	16.6 ± 0.5cef	73.2 ± 2.5a	71.3 ± 2.5a	10.1 ± 0.3cde	503.9 ± 6.5j	298.3 ± 4.2j	182.8 ± 0.6i	22.7 ± 2.0i	197.2 ± 4.5cd	533.0 ± 4.5i	197.2 ± 4.5ce
DC+DB	17.4 ± 0.4bde	70.8 ± 5.0a	68.3 ± 5a	11.7 ± 0.28bce	246.6 ± 2.9k	143.9 ± 0.3k	91.0 ± 0.4j	11.6 ± 0.7j	207.7 ± 2.8dgh	527.0 ± 2.8i	207.7 ± 2.8e
CA+BA	15.2 ± 1.2ae	75.7 ± 6.6a	74.1 ± 6.6a	9.1 ± 0.8ad	872.7 ± 1.8l	694.8 ± 0.9l	136.7 ± 0.4b	41.2 ± 1.4b	185.3 ± 0.4ef	603.0 ± 0.4j	185.3 ± 0.4fg
DC+CA	15.5 ± 1.0ade	75.3 ± 3.4a	74.0 ± 3.4a	9.1 ± 0.7ad	483.4 ± 6.6m	314.7 ± 5.8m	140.7 ± 0.1b	28.0 ± 0.02h	183.3 ± 3.2f i	366.0 ± 3.2k	183.3 ± 3.2g
DB+BA	18.1 ± 0.9bdf	70.6 ± 3.5a	68.0 ± 3.5a	11.2 ± 0.6bce	661.2 ± 6.4i	531.3 ± 4.2n	96.6 ± 0.2j	33.3 ± 0.1e	209.0 ± 4.8g	1025.0 ± 4.8l	209.0 ± 4.8h

[1] Data are the means of two independent experiments analysed twice. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Identical uppercase and lowercase letters indicate no significant differences between the values of the different samples, in the same column, at time 0 ($p < 0.05$).

Table 2
Evolution of the legal quality parameters of 14 extra-virgin olive oils (EVOOs) during 22 months of storage in the dark at room temperature

	Peroxide value (PV)				K_{232}				K_{270}			
EVOOs	Regression equation	Determination coefficient R^2	Time required to exceed legal limit (months)	Increasing rate (meq O_2/kg oil)	Regression equation	Determination coefficient R^2	Time required to exceed legal limit (months)	Increasing rate	Regression equation	Determination coefficient R^2	Time required to exceed legal limit (months)	Increasing rate
			(range of 2 months)				(range of 2 months)				(range of 2 months)	
A	y = 0.3367x + 10.253	0.68	-	0.53	y = 0.0707x + 1.6383	0.88	22	0.095	y = 0.0065x + 0.1149	0.72	-	0.008
B+A	y = 0.6866x + 8.6112	0.92	-	0.88	y = 0.1255x + 1.7017	0.89	16	0.135	y = 0.0074x + 0.112	0.74	-	0.011
CA+BA	y = 0.9371x + 6.7614	0.92	-	0.99	y = 0.1316x + 1.6677	0.91	16	0.127	y = 0.0089x + 0.1095	0.92	22	0.013
C+A	y = 1.1707x + 4.4011	0.92	22	1.36	y = 0.0827x + 1.8937	0.69	18	0.136	y = 0.0078x + 0.1442	0.74	18	0.008
A+D	y = 0.8641x + 11.364	0.53	20	1.60	y = 0.1411x + 1.621	0.88	12	0.153	y = 0.0138x + 0.0768	0.95	18	0.013
DB+BA	y = 1.1449x + 8.7798	0.84	20	1.49	y = 0.0915x + 1.7989	0.96	14	0.119	y = 0.009x + 0.1274	0.96	18	0.010
B	y = 1.239x + 7.5114	0.90	18	1.34	y = 0.1652x + 1.6395	0.97	10	0.156	y = 0.0142x + 0.1035	0.90	18	0.018
DC+CA	y = 2.1526x + 4.9771	0.62	16	1.80	y = 0.1258x + 2.0632	0.77	10	0.142	y = 0.0113x + 0.1197	0.82	14	0.011
C+B	y = 1.3697x + 8.7868	0.83	18	1.70	y = 0.1046x + 2.0354	0.93	8	0.126	y = 0.0076x + 0.1512	0.82	16	0.008
D+B	y = 1.2394x + 12.06	0.64	16	1.78	y = 0.1176x + 1.9013	0.94	8	0.138	y = 0.0116x + 0.1282	0.86	14	0.013
DC+DB	y = 3.0083x + 4.1548	0.81	14	3.21	y = 0.111x + 2.0701	0.80	10	0.151	y = 0.0192x + 0.1175	0.91	14	0.018
C	y = 1.3358x + 9.8684	0.90	16	2.60	y = 0.1382x + 2.0144	0.82	8	0.131	y = 0.0241x + 0.0174	0.87	10	0.026
D+C	y = 2.6353x + 7.1944	0.79	10	3.37	y = 0.123x + 1.7535	0.79	6	0.166	y = 0.0158x + 0.1227	0.80	8	0.019
D	y = 5.0865x + 2.1242	0.97	8	4.92	y = 0.156x + 1.7221	0.86	6	0.169	y = 0.0094x + 0.1685	0.86	6	0.028

Maximum values of EVOO quality parameters (EU Reg. 2015/1830): PV, 20 meq O_2/kg; K_{270}, 0.22; K_{232}, 2.50.

FIGURE CAPTIONS

Fig. 1. (**a**) Score and (**b**) loading plots of the partial least squares (PLS) model built using all analytical determinations (representing the independent variable X) for 14 extra-virgin olive oils stored in the dark at room temperature for 22 months (representing the latent variable Y, defined as TIME). Total explained variance of Y: 95%, with three latent significant variables. Legend: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22: number of months of EVOO storage under dark conditions.

Fig. 2. Changes in the concentrations (mg/kg) of the sum of the (**a**) hydrophilic phenols (oleuropein and ligstroside derivatives and lignans), (**b**) oleuropein derivatives (3,4-DHPEA, 3,4-DHPEA-EA, and 3,4-DHPEA-EDA), (**c**) ligstroside derivatives (*p*-HPEA, *p*-HPEA-EDA), and (**d**) α-tocopherol in 14 extra-virgin olive oils during 22 months of storage in the dark at room temperature; samples were measured at different intervals (months) of storage. Bars and error bars represent means + standard errors, respectively.

Fig. 3. Evolution of the concentrations of volatile compounds (μg/kg) expressed as the sum of 2-heptenal, (*E*)-2-heptenal, (*E*)-2,4-heptadienal, (*E*, *E*)-2,4-heptadienal, (*E*, *E*), 2,4-nonadienal, (*E*, *E*)-2,4-decadienal, (*E*, *E*)-2,4-decadienal, and (*E*, *E*)-2-undecenal) in 14 extra-virgin olive oils during 22 months of storage in the dark. Head space analyses were conducted every 2 months of storage. Bars and error bars represent means + standard errors, respectively.

Supplementary Figure 1. (**a**) Score and (**b**) loading plots of the partial least squares (PCA) model built using all analytical determinations for 14 extra-virgin olive oils stored in the dark at room temperature for 22 months. Total explained variance: 77%, with three principal components. Legend: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22: number of months of EVOO storage under dark conditions.

Supplementary Figure 2. Changes in the concentrations (mg/kg) of the sum of the lignans [(+-pinoresinol and 1(+)-acetoxypinoresinol)] in 14 extra-virgin olive oils during 22 months of storage in the dark at room temperature; samples were measured at different intervals (months) of storage. Bars and error bars represent means + standard errors, respectively.

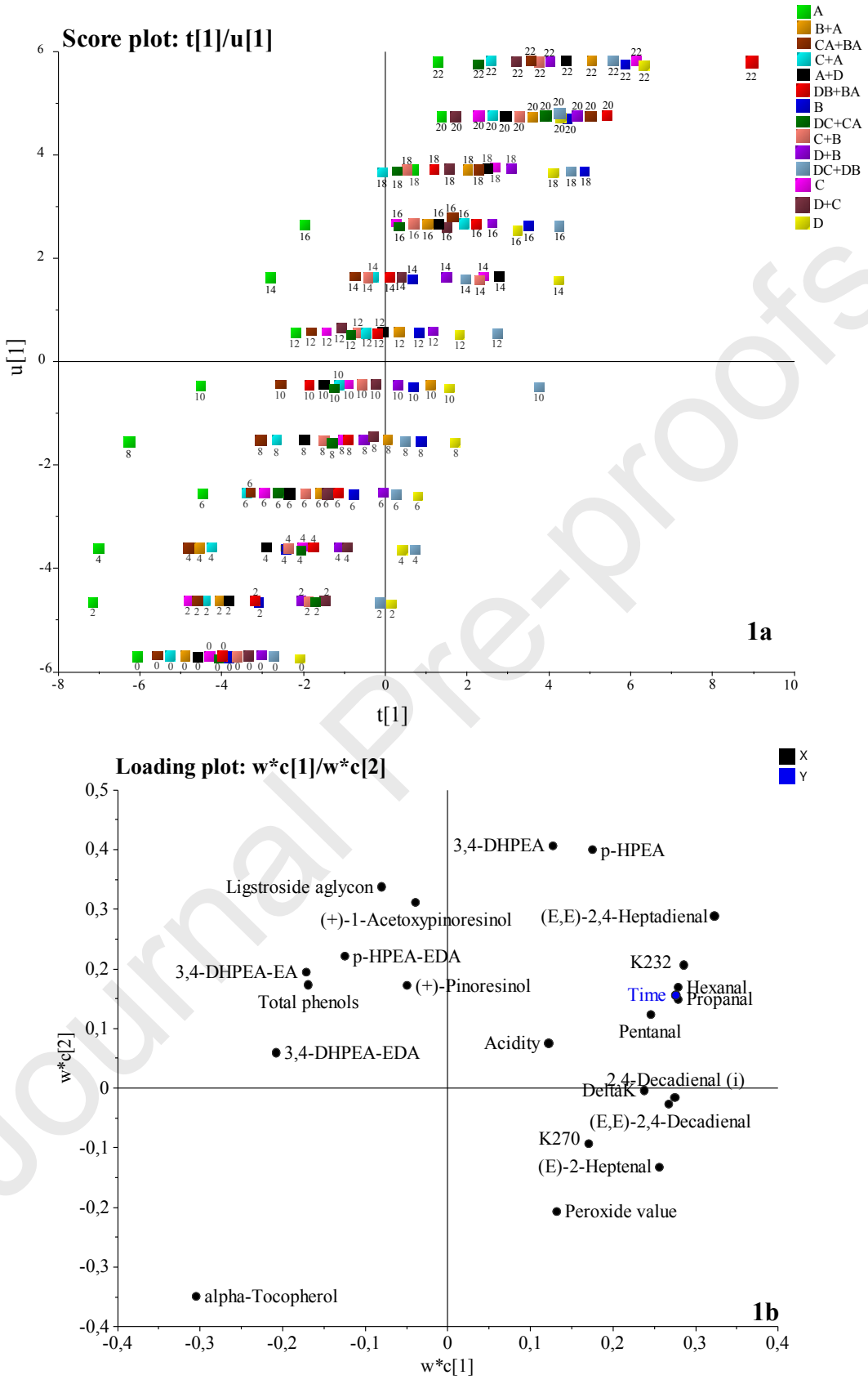

Fig. 1. *Esposto et al.*

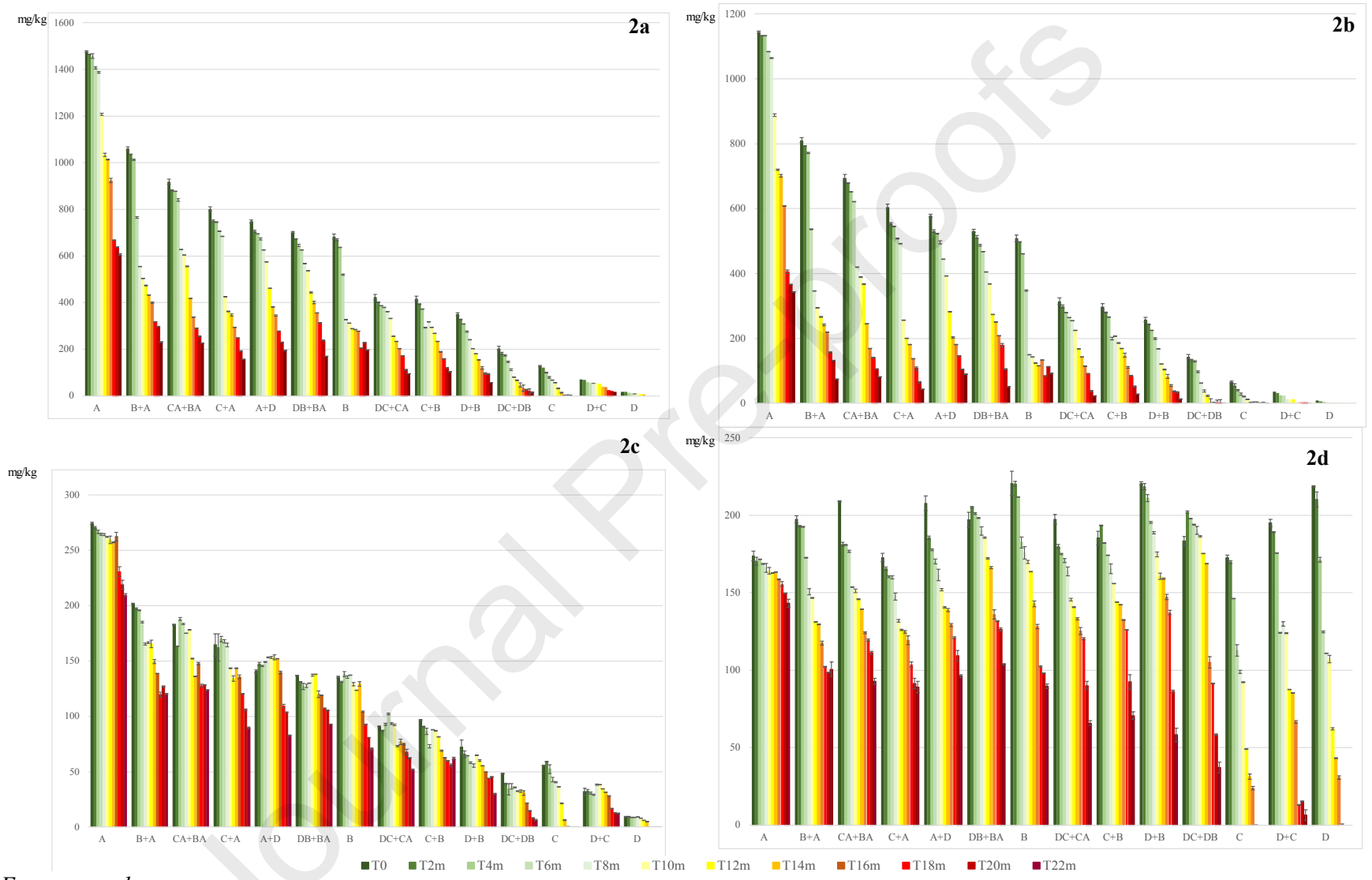

Fig. 2. *Esposto et al.*

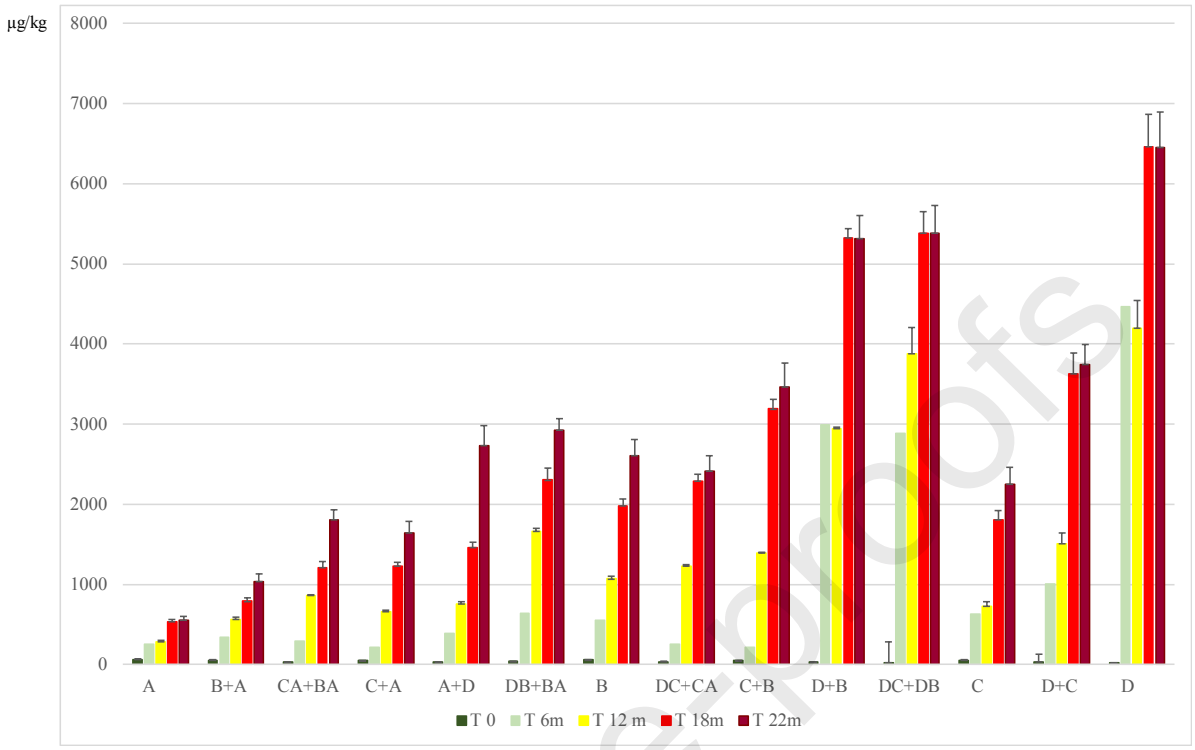

Fig. 3. *Esposto et al.*

Table 1
Initial fatty acid (%) and antioxidant (mg/kg) compositions of the 14 extra-virgin olive oils used in this study[1].

	SFA	MUFA	Oleic acid	PUFA	Polyphenols (sum)	Oleuropein derivatives	Ligstroside derivatives	Ligna
	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/
A*	13.2 ± 1.0a	78.6 ± 4.9a	77.7 ± 4.9a	8.0 ± 0.6a	1476.7 ± 4.4a	1143.9 ± 3.0a	274.3 ± 1.0a	58.5 ±
B	19.9 ± 1.0b	67.6 ± 5.5a	64.6 ± 5.5a	12.5 ± 1.0b	682.5 ± 8.2b	508.7 ± 1.7b	135.4 ± 0.9b	38.4 ±
C	13.4 ± 0.7a	78.6 ± 4.6a	77.6 ± 4.6a	8.0 ± 0.6a	128.3 ± 1.7c	66.1 ± 0.6c	55.9 ± 0.05c	6.2 ±
D	20.0 ± 1.1b	67.4 ± 5.5a	64.5 ± 5.5a	12.5 ± 1.0b	18.1 ± 0.8d	8.1 ± 0.5d	9.1 ± 0.5d	0.9 ±
C+A	13.5 ± 0.3a	78.5 ± 4.6a	77.7 ± 4.6a	8.0 ± 0.2a	802.1 ± 10.4e	605.0 ± 0.4e	164.7 ± 10.0e	32.4 ±
D+B	19.9 ± 0.9b	67.6 ± 4.2a	64.6 ± 4.2a	12.5 ± 0.6b	350.3 ± 6.5f	258.4 ± 0.3f	72.3 ± 6.4f	19.7 ±
B+A	16.8 ± 1.4cde	73.0 ± 4.7a	71.0 ± 4.7a	10.3 ± 0.9cd	1059.8 ± 2.6g	811.0 ± 1.1g	201.8 ± 0.5g	47.0 ±
D+C	16.5 ± 1.4cde	73.4 ± 2.5a	71.7 ± 2.5a	10.0 ± 0.9ade	70.9 ± 3.9h	35.4 ± 0.3h	31.9 ± 3.2h	3.6 ±
A+D	16.5 ± 0.6cde	73.1 ± 4.3a	71.3 ± 4.3a	10.4 ± 0.4cde	655.7 ± 5.5i	578.0 ± 1.8i	48.3 ± 1.3c	29.4 ±
C+B	16.6 ± 0.5cef	73.2 ± 2.5a	71.3 ± 2.5a	10.1 ± 0.3cde	503.9 ± 6.5j	298.3 ± 4.2j	182.8 ± 0.6i	22.7 ±
DC+DB	17.4 ± 0.4bde	70.8 ± 5.0a	68.3 ± 5a	11.7 ± 0.28bce	246.6 ± 2.9k	143.9 ± 0.3k	91.0 ± 0.4j	11.6 ±
CA+BA	15.2 ± 1.2ae	75.7 ± 6.6a	74.1 ± 6.6a	9.1 ± 0.8ad	872.7 ± 1.8l	694.8 ± 0.9l	136.7 ± 0.4b	41.2 ±
DC+CA	15.5 ± 1.0ade	75.3 ± 3.4a	74.0 ± 3.4a	9.1 ± 0.7ad	483.4 ± 6.6m	314.7 ± 5.8m	140.7 ± 0.1b	28.0 ±
DB+BA	18.1 ± 0.9bdf	70.6 ± 3.5a	68.0 ± 3.5a	11.2 ± 0.6bce	661.2 ± 6.4i	531.3 ± 4.2n	96.6 ± 0.2j	33.3 ±

[1] Data are the means of two independent experiments analysed twice. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Identical uppercase and lowercase letters

indicate no significant differences between the values of the different samples, in the same column, at time 0 ($p < 0.05$).

Table 2
Evolution of the legal quality parameters of 14 extra-virgin olive oils (EVOOs) during 22 months of storage in the dark at room temperature

Peroxide value (PV)				K_{232}				K_{270}		
Regression equation	Determination coefficient R^2	Time required to exceed legal limit (months)	Increasing rate (meq O_2/kg oil)	Regression equation	Determination coefficient R^2	Time required to exceed legal limit (months)	Increasing rate	Regression equation	Determination coefficient R^2	Time required to exceed legal limit (months)
		(range of 2 months)				(range of 2 months)				(range o
y = 0.3367x + 10.253	0.68	-	0.53	y = 0.0707x + 1.6383	0.88	22	0.095	y = 0.0065x + 0.1149	0.72	-
y = 0.6866x + 8.6112	0.92	-	0.88	y = 0.1255x + 1.7017	0.89	16	0.135	y = 0.0074x + 0.112	0.74	-
y = 0.9371x + 6.7614	0.92	-	0.99	y = 0.1316x + 1.6677	0.91	16	0.127	y = 0.0089x + 0.1095	0.92	22
y = 1.1707x + 4.4011	0.92	22	1.36	y = 0.0827x + 1.8937	0.69	18	0.136	y = 0.0078x + 0.1442	0.74	18
y = 0.8641x + 11.364	0.53	20	1.60	y = 0.1411x + 1.621	0.88	12	0.153	y = 0.0138x + 0.0768	0.95	18
y = 1.1449x + 8.7798	0.84	20	1.49	y = 0.0915x + 1.7989	0.96	14	0.119	y = 0.009x + 0.1274	0.96	18
y = 1.239x + 7.5114	0.90	18	1.34	y = 0.1652x + 1.6395	0.97	10	0.156	y = 0.0142x + 0.1035	0.90	18
y = 2.1526x + 4.9771	0.62	16	1.80	y = 0.1258x + 2.0632	0.77	10	0.142	y = 0.0113x + 0.1197	0.82	14
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Maximum values of EVOO quality parameters (EU Reg. 2015/1830): PV, 20 meq O_2/kg; K_{270}, 0.22; K_{232}, 2.50.

Highlights

The quality evolution of extra-virgin olive oils during real-time storage in darkness was determined

Legal, health & sensory extra-virgin olive oils parameters were monitored during 22 months of storage

K_{232} index can be used to monitor extra-virgin olive oils legal quality parameters during dark storage

High initial antioxidant contents better opposed quality loss during dark storage

Higher extra-virgin olive oils polyphenol fractions reduced quality and antioxidant heritage losses

Declaration of interests

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

Esposto Sonia: Conceptualization, Methodology, Writing-Original draft preparation Writing-Reviewing and Editing; **Roberto Selvaggini**: Methodology, Validation, formal analysis, **Agnese Taticchi**: Data curation. ***Gianluca Veneziani*** *Investigation****; Beatrice Sordini*** *Investigation****; Maurizio Servili:*** Conceptualization, Project administration, Supervision.