Application of accelerated shelf-life test (ASLT) procedure for the estimation of the shelf-life of extra virgin olive oils: a validation study

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Abstract

The shelf-life (SL) estimation of extra virgin olive oil is a timely concern for food producers to comply with the EU regulations throughout product commercialization up to consumption, but also to maintain consumer trust in the producers. The application of accelerated shelf-life testing (ASLT) procedures could allow to speed up the process. In this study, three freshly made extra virgin olive oils having increased total polyphenol content (156, 273 and 507 mg/kg) were stored at increasing temperatures (25, 40, 50 and 60 ° C) in the dark in glass containers under reduced oxygen content to simulate market storage. The changes of K_{270} and % of pyropheophytin a (PPP) was found to be the best indicators to monitor product behaviour during storage. The rate constants of the changes of K_{270} and %PPP over time showed a temperature dependence that can be described with the Arrhenius model with activation energies (E_a) in the range of 49-65 kJ/mol and 115-122 kJ/mol for K_{270} and %PPP, respectively. These values confirmed the significantly higher susceptibility of the parameter %PPP to temperature changes during storage, as also demonstrated by the estimated shelf-life values and relevant confidence intervals. Interestingly, the initial quality characteristics of the oils and especially the polyphenols content did not affect the temperature dependence of the rate constants of these indexes. It was concluded that %PPP could be used as a "rapid alert" indicator of product performances on the market and K_{270} as indicator to compute the compulsory value of "best before" date.

Key words

Accelerated test, Extra virgin olive oil; Polyphenols, Pyropheophytin, Modelling, Shelf-life

1. Introduction

European Regulation on food labelling requires that the majority of packed foods displays a date mark accompanied with indications explaining whether the date signals a threshold in the product's safety ("use by") or its quality ("best before") (Reg. (EU) 1169/2011). The date mark informs consumers but also food chain operations and official controllers about the status of the product. Based on the final report "Market study on date marking and other information provided on food labels and food waste prevention" (European Commission, 2018) up to 10% of the 88 million tons of food waste generated annually in the EU are linked to date marking. Considering that waste reduction is one of the priority of the EU reported in the "Farm to fork strategy" (European Commission, 2020), the capability for food companies to precisely define the date mark appears not only important to accomplish food law and maintain consumer loyalty but also contribute to the reduction of food waste. Recently, EFSA panel on Biological Hazards released a guidance on date marking and related food information subdivided in two parts: the first one develops a risk based approach to be followed by the food business operators (FBO) when deciding the type of date marking and of shelf life to ensure food safety (EFSA, 2020) and the second the risk based approach when deciding the food information relating to storage conditions and/or time limits for consumption after pack opening (i.e. secondary shelf life) (EFSA, 2021). In both documents, the main focus is food safety. Besides these documents, in our knowledge, no further indications are available to take decision on date marking for microbiologically stable foods with a long life, such as those undergoing oxidation during storage. One high-value shelf stable commodity, for which the procedures for the definition of the "best before date" are highly demanded, is extra virgin olive oil (EVOO). Based on EU Regulation (CEE) 2568/91 (1991) and following amendments as well as International Olive Oil Council (IOC) trade standard (COI/T.15/NC No 3/ Rev.16/2021) (2021), the oil extracted from olives by mechanical methods must comply with a number of quality indices to be included in the extra virgin category. It is a matter of fact that the compliance with these parameters must be guaranteed throughout the product shelf-life

until the reaching of the labelled *best before date*. The definition of the date marking for this EVOO could be commercially critical for producers because the failure in just one of the compulsory quality indicators could lead to the product downgrading in the virgin oil category. This situation could not only cause the possible negative impact on brand reputation but also the possible engagement in food frauds. Many of the EVOO quality indicators reported in the food law might sharply change during product storage due to the development of oxidative reactions. Typical examples are peroxide number, absorbance values in UV region at 232 and 270 nm (K_{232} and K_{270}) and the sensory profile. In the case of EVOO, the SL acceptability limit – that is the value of a selected quality indicator discriminating products that are still acceptable for the consumption from those no longer unacceptable (Manzocco, 2016) - is compulsory defined and it is the quality index among those listed in the Regulation 2568/91 (1991) firstly reaching the compulsory threshold.

Beside these compulsory indexes, other indicators, mainly related to oil freshness profile, such as phenols, tocopherols and pigments content, are generally monitored during product storage to evaluate product quality changes. However, since mandatory limits for these parameters have not been yet established, they cannot be considered as shelf life indicators usable to take decisions on the "*best before date*" to be reported on EVOO labels.

Company managers dealing with shelf-life of EVOO are looking for accurate and easily applicable tools allowing to predict the EVOO shelf life in the shorter possible time and take decisions on date marking. In this context the application of real time shelf life testing, during which the conditions suffered by the product mimic those experienced by the product on the shelf, is not profitable (Nicoli, 2020). The application of an *accelerated shelf-life testing* (ASLT) procedure is thus frequently proposed to speed up the shelf-life assessment process (Nicoli, 2020, Calligaris et al., 2019). If properly applied, ASLT procedure allows the estimation of the product shelf-life under the storage conditions usually experienced by the product on the market by modelling data acquired under accelerated storage conditions. Temperature is surely the most common accelerating factor used in ASLT and the shelf life at the interested temperature is predicted by using the Arrhenius equation that

finally can be regarded as shelf life predictive model (Calligaris et al., 2019, Labuza & Schmidl, 1985). The Arrhenius equation has been successfully used to estimate the temperature dependence of oxidation rate for different product categories (Calligaris et al., 2016). However, the successful application of the Arrhenius model requires that food is able to withstand the increase in temperature without causing the changes in the reaction pathway. As stated by Frankel (2005), the use of temperatures higher than 60 °C is questionable since responsible for the selection of different oxidation pathways. Samples develop excessive level of rancidity which are not relevant to what happens under normal storage conditions. Similarly, when temperature causes oil phase transitions, the Arrhenius behaviour is no longer expected to be fulfilled (Calligaris et al., 2016).

Surprisingly, the ASLT approach based on temperature as accelerating factor and Arrhenius model as predictive tool has been scarcely considered for EVOO, even thought different literature studies focused on the development of prediction models applicable for EVOO shelf-life, as reviewed by Li and Wang (2018). In these studies, the changes of many indexes are frequently studied contemporarily during storage without applying a kinetic study. It should be pointed out that not all the reported experiments can be listed as shelf-life studies, but only as stability tests since SL is not associated to the reaching of compulsory acceptability limits. Moreover, the variety of environmental (e.g. temperature, light) and packaging conditions (packaging material, oxygen concentration) considered in these studies further hinders a correct assessment of shelf life and a comparison among published data.

Recently, Conte et al. (2020) applied the ASLT approach to obtain a shelf life prediction model of EVOO packed in closed amber glass containers with reduced headspace, simulating commercial storage. It was demonstrated that the K_{270} can be considered the best early shelf-life indicator among compulsory quality indexes applicable in ASLT. In fact, this was the sole compulsory quality index showing a good rate temperature dependence during storage fulfilling the well-known Arrhenius equation (Eq. 2).

The ASLT approach has been also efficaciously applied by Macebo-Campos et al. (2008, 2022) to study the quality evolution of EVOO stored in open amber glass containers at increasing temperatures from 25 to 60 °C. Also in this case, mathematical modelling based on the Arrhenius equation have been developed to predict product shelf life and K_{232} has been selected as the best shelf-life index. It should be pointed out that, being the EVOO containers open during storage, these studies deal with the computation of the secondary shelf life – defined as the shelf life of the product once opened (Nicoli and Calligaris, 2019)- rather than primary shelf life. Thus, the diversity in the critical indicator proposed in literature can be mainly attributed to the packaging conditions adopted during the test (open vs closed containers), evidencing once again the critical role of oxygen concentration in the headspace in determining the oxidation pathways and rate (Iqdiam et al., 2020).

Interestingly, some other Authors (Aparicio-ruiz et al., 20012, 2014 and 2017; Conte et al., 2020) recognised the formation of pyropheophytins as a possible indicator to be used in ASLT to predict product freshness. Pyropheophytins in olive oil are formed due to degradations of chlorophyll pigments and this reaction begins soon after the oil is extracted. The chlorophyll pigments break down due to a process that involves the decarbomethoxylation of chlorophyll and pheophytins to form pyropheophytins (Gertz & Fiebig, 2006). Being EVOO freshness recognised by FBO as well as by consumers as a parameter of paramount importance to maintain highest standard levels on the market, the limit of 17%of phyropheophytin (%PPP) has been proposed to guarantee product quality as well as some trade standards (Standards Australia, 2011; CFDA, 2016). Being the formation of phyropheophytin more susceptible to temperature than K_{270}, the reaching of %PPP critical limit is expected to occur earlier as compared to other compulsory indicators associated to oxidation (Aparicio-Ruis et al., 2014; Conte et al., 2020). Thus, this index, even though not listed as shelf life indicator, could be regarded as an early indicator of oil quality change, just a sort of “a rapid alert” advising that EVOO is approaching the end of compulsory shelf-life. Thus, in the case of EVOO two different “lives” of the product could be defined: a “high-quality life” (SL_{HQ}) defined as the length of

time needed to reach the acceptability limit for the freshness indicator and a "compulsory shelf-life (SL_C)" associated with to the time needed to reach the compulsory acceptability limit.

In main aim of this study was the validation of the previously developed ASLT methodology for the prediction of EVOO shelf-life by considering three freshly made extra virgin olive oils with different compositional characteristics. This is because there is the need to understand the SL computation variability as well as the possible effect of initial oil quality characteristics. In particular, oils with three different initial level of polyphenols (156, 273, 507 mg/kg) were stored at temperatures from 25 to 60 °C and selected quality indexes (K_{232}, K_{270}, polyphenol content, tocopherol content and %PPP) were monitored. After conventional kinetic modelling, a statistical bootstrap procedure was applied for the first time to estimate the shelf-life uncertainties. It is a matter of fact that different sources of uncertainties (intrinsic variability of the food product, analytical methodology and mathematical modelling) could affect the final shelf-life value begetting a wide confidential interval. Its computation may be challenging from a mathematical point of view and is generally not performed in the available literature on the same topic. The possibility to estimate the product shelf life and the relevant confidential interval appears particularly interesting in an attempt to develop predictive tools for food companies.

2. Material and methods

2.1 Materials

2.1.1 Chemicals

Acetone, acetonitrile, isopropanol, ethanol, methanol and *n*-hexane (all HPLC grade) were purchased from Sigma–Aldrich (Milano, Italia). Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade. Tocopherol (α, β+γ and δ-tocopherols), phenolic compounds (syringic acid, tyrosol and hydroxytyrosol) and chlorophyll A standards were purchased from Sigma–Aldrich Milano, Italia.

2.1.2 Olive oil samples

EVOO (*Olea europaea* L.) samples were kindly provided by three different Italian producers. Samples were selected based on their initial total polyphenols content from about 156 (*a* sample) to 273 (*b* sample) and 507 mg/kg (*c* sample). Each of the three sample was part of a homogeneous batch of the product produced in 2019 just after harvesting and packed within one month after EVOO production. Aliquots of 250 mL of the EVOO samples were packed into glass bottles with metal cap, made of polytetrafluoroethylene (PTFE) as internal septum, and with 2 cm of headspace, mimicking the commercial conditions. A total of 40 bottles for each type of EVOO was considered for each storage condition.

2.2 Storage conditions

Samples were stored in incubators (FTC 90I Refrigerated Incubator, Monza, Italy) at the following controlled temperatures 25, 40, 50 and 60 °C, under dark for up to 300 days. At different lengths of time during storage, one bottle of each oil was taken from the selected incubator and subjected to analytical determinations. The sampling plan was not fixed in advance, but defined after obtaining analytical results. This is due to the different oxidation kinetics expected as storage temperature increased.

2.3 Analytical determinations

2.3.1 Fatty acids composition

In order to determine fatty acid composition (%), the methyl-esters were prepared according to the IOC method (International Olive Council, 2017) and analysed by Thermo Trace 1300 gas

chromatograph equipped with a FID detector and an auto-sampler. A fused silica column, SP-2330 (60 m length × 0.32 mm i.d. × 0.20 μm film thickness), was used. Helium was employed as carrier gas, with a flow through the column of 1 ml/min. The temperatures of the injector (split) and detector (FID) were both set at 250 °C. An injection volume of 1 μl was used. The operating conditions were as follows: oven temperature was held at 165 °C for 5 min, then increased by 3°C min^{-1} to 210 °C and held for 10 min. Split ratio was 1:50. Results were expressed as percentage of relative area.

2.3.2 Total phenolic compounds

The determination of total amount of phenolic compounds was obtained using the official IOC method (International Olive Council, 2017).

2.3.3 Tocopherols

UHPLC analysis was realized using a Shimadzu Nexera (Shimadzu, Kyoto, Japan) coupled with the same components used for polyphenols analysis and a fluorescence detector RF-20Axs with double acquisition channels and a 12 μL cell. The detector was set at 296 nm and 325 nm for exciting and emission wavelengths, respectively. Oil samples were diluted in 2-propanol for reaching a 100 mg/mL concentration and 1μL injected on the column as a compromise between sensibility and column capacity.

The chromatographic separation was performed following the procedure already reported elsewhere (Lucci et al., 2020). Briefly, an Agilent Eclipse PAH column (1.8 μm particle size, 4.6 x 50 mm) was used under isocratic conditions with solvent A (methanol) and B (acetonitrile) in the ratio 60/40 (v/v) and a total flow of 600 μL min^{-1}. The oven temperature was set to 30 °C. The injected volume for each sample was 1 μL. Tocopherols were quantified using a calibration curve for δ, β+ϒ and α respectively in the range 0.05–100 ng injected on the column with R^2 values higher than 0.999.

2.3.4 Pyropheophytin a

Pyropheophytin a was measured using method ISO 29841:2009 (2009). Pigments were isolated by used an SPE SiOH column 6 mL/1 g (Chromabond Macherey-Nagel GmbH & Co, Düren, Germany) using firstly 10 mL of a petroleum ether/ethyl ether solution in the ratio 90:10 (v/v) for the elution of non-polar compounds and then 10 mL of acetone as elution solvent for chlorophylls fraction. The eluate was then analysed by reverse-phase Spherisorb ODS2 C18 HPLC column and the separated components were monitored at 410 nm using a photometric detector. The results were expressed as relative proportions (pyropheophytin a, %PPP) of the analyses (pyropheophytin a and pheophytin a and a'), in relation to the sum of pyropheophytin a and pheophytin a+a'.

2.4 Data elaboration

2.4.1 Kinetic modelling - step 1

Data were elaborated by using a zero-order reaction model and the rate constants values were computed from the following equation:

$$I = kT + I_0 \quad (1)$$

where I is the selected indicator, k is the zero-order rate constant, t the storage time in days and I_0 the intercept. No lag phase was detected and only the increasing part of the curves was considered.

The order of the reaction was evaluated by visual inspection of the plots of I, $ln(I)$ and $1/I$ against storage time.

Differences between reaction rates at different temperatures and for different EVOOs were evaluated comparing regression models with and without temperature and oil effects with ANOVA test.

2.4.2. Temperature dependence of the reaction rate - step 2

The relationship between reaction rate and temperature has been separately estimated for each oil according to the Arrhenius equation:

$$k_T = k_0 \exp\left(-\frac{E_a}{RT}\right) \quad (2)$$

where k_0 is the pre-exponential factor, R is the molar gas constant (8.31 J/K/mol) and E_a is the apparent activation energy (J/mol).

We used the reparametrized version of the equation

$$k_T = k_{ref} \exp\left(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad (3)$$

where T_{ref} is a reference temperature (318 °K in our case that is the intermediate value in the range of considered temperature). The reparameterization is recommended because it enhances the statistical properties of the estimates of the unknown coefficients (Van Boekel, 2009).

The equation was linearized by applying the logarithm to both sides of the equation and then coefficients k_{ref} and E_a were estimated using linear regression.

2.4.3 Shelf-life prediction - step 3

Shelf life (SL) for a given Kelvin temperature T^* was estimated according to

$$\mathrm{SL}(T^*) = \frac{I_{lim} - I_0}{k_{ref} \exp\left(-\frac{E_a}{R}\left(\frac{1}{T^*} - \frac{1}{T_{ref}}\right)\right)} \quad (4)$$

where I_{lim} is the acceptability limit for the critical indicator, I_0 is the value of the critical indicator at time 0 and the quantity in the denominator is the reaction rate at temperature T^* predicted using the Arrhenius equation previously estimated (eq 3).

The quantification of the uncertainty was estimated by using a bootstrap procedure. This term refers to a broad set of resampling techniques widely applied when model complexity makes it difficult to apply standard inferential techniques (Efron & Tibshirani, 1993). In general, bootstrap is based on pseudo-datasets created by resampling with replacement the original observations. In our case, each

pseudo-dataset was constructed from regression analysis in step 1 by resampling the residuals of the regression. The pseudo-values for the critical indicator were then computed according to:

$$\hat{I}_t = a + k_T t + \hat{\varepsilon} \quad (5)$$

where $\hat{I}_t$ is the pseudo-value of the critical indicator at storage time t and $\hat{\varepsilon}$ is the value of the resampled residual. Step1, step 2 and step 3 were then applied to the pseudo-dataset and the resulting shelf life was stored. The process was iterated 1000 times. It was then used the sequence of pseudo-estimates of the shelf life to construct a confidence interval. It was applied the so-called Bias Corrected and accelerated (BCa) confidence interval (Efron, 1987).

2.5 Statistical analysis

Data were expressed as the mean of at least two analytical determinations on two replicated samples and relative standard deviation. All the computations were carried out using R ver. 4.0.3 (R Core Team (2020 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Wien, Austria, URL http://www.R-project.org/.). Bootstrap computations were based on the boot package (Canty, Ripley, 2021. Boot: Bootstrap R (S-Plus) Functions. R package version 1.3-26.).

3. Results and discussion

3.1 Chemical characteristics of oils

Table 1 shows the main chemical characteristics of the considered oils. As expected, all the samples complied with the quality indexes reported by IOC and EU regulation No. 2568/91 (1991). These samples were selected mainly based on their total polyphenol content ranging from the lowest value 156 to the highest 507 mg/kg. The majority of EVOOs available on the market falls within this range (López-Huertas et al., 2021, Piscopo et al., 2016). As well known, these differences are associated not only to the olive variety but also to the agronomic and technological variables applied during harvesting and processing. Considering tocopherols, the total content was in the range of 225-268 mg/kg, thus not so different. It should be pointed out that, due to the aim of the study, the total polyphenol and tocopherol content was considered in the shelf-life study instead of the concentration

297 of the different compounds belonging to these component families. Observing the indexes referring
298 to the oxidative status of the samples, in all cases these parameters are well below the Regulation
299 limits with a limited variability among samples.
300
301 3.2 *Changes of the quality indicators during storage*
302 The changes of some selected quality indicators (peroxide value, K_{232}, K_{270}, polyphenols, tocopherols,
303 and pyropheophytins) were monitored during storage at 25, 40, 50 and 60 °C for increasing time.
304 In agreement with our previous results (Conte et al., 2020), PV, K_{232}, polyphenols and tocopherol
305 content did not significantly change during storage at any considered temperatures, never approaching
306 the compulsory limit for PV and K_{232} (data not shown). These results confirm that primary oxidation
307 products did not further develop during storage under reduced oxygen content in the headspace of the
308 bottles. This is in agreement with Iqdiam et al. (2020), evidencing the impact of oxygen concentration
309 on EVOO oxidation kinetics: as oxygen content decreased the rate of oxidation also decreased. It can
310 be hypothesised that, in our experimental conditions designed to simulate EVOO market storage, the
311 oxygen content resulted the limiting factor to the development of oxidation due to the reduced
312 headspace volume in contact with the product. Thus, under limited oxygen content, the oxidative
313 reactions did not generate additional primary oxidation products, preserving in this way the naturally
314 occurring antioxidants. In fact, the content of both polyphenols and tocopherols did not show a
315 significant reduction during storage even at the highest storage temperatures (data not shown). It
316 should be noted that the opposite results were detected by Macebo-Campos et al. (2008 and 2022)
317 when considering EVOO stored in open containers. In these studies, PV and K_{232} significantly
318 increased in concomitance with the decrease of polyphenol and tocopherol contents. The comparison
319 of literature results with those here described clearly highlighted the importance of the simulation of
320 the storage conditions under which shelf-life would be predicted. When oxygen is not the limiting
321 factor, oxidation proceeds in its propagation step accumulating peroxides and involving polyphenols
322 and tocopherols in the oxidation pathway.

323 Moving to the formation of secondary oxidation products, the K_{270} showed a progressive increase
324 during the storage (**Figure 1 a, b, c**). As expected, the rate of K_{270} changes also increased as storage
325 temperature also increased. This result seems to indicate that secondary oxidation products are formed
326 by the decomposition of primary oxidation products already present in the oil at bottling time.
327 Finally, the changes of the content of pyropheophytin a during storage at 25, 40, 50, and 60 °C were
328 also monitored (**Figure 2 a, b, c**). As previously mentioned, despite no compulsory indications are
329 available for this parameter in the Regulations on EVOO, some Authors (Aparicio-Ruiz et al., 2014,
330 2017) as well as some trade standard (Standards Australia, 2011; CDFA, 2016) proposed this
331 parameter as freshness indicator. In agreement with previous results (Conte et al., 2020), a linear
332 increase of this index was observed as a function of time, before a steady state was reached. It is also
333 well evident the temperature dependence of the changes of this parameter.
334
335 *3.3 Data modelling*
336 The kinetics of the changes of K_{270} and %PPP were modelled by using a pseudo zero reaction order
337 and apparent zero-order rate constants were computed by linear regression analysis. Results of the
338 kinetic analysis were reported in **Table 2** along with the relevant standard error and the coefficient of
339 determination. In all cases, the selected reaction order well described the evolution of the selected
340 indexes ($R^2 > 0.80$; $p < 0.05$). The reaction rates at different temperatures are significantly different
341 both for K_{270} ($p<0.001$) and for %PPP ($p<0.001$) as well as the temperature evolution of reaction rates
342 was significantly different among the three EVOO ($p<0.001$ in both cases).
343 To study the temperature dependence of K_{270} and %PPP, the values of *k* reported in **Table 2** were
344 analysed according to the reparametrized Arrhenius model (eq.4). **Table 3** shows the acquired results.
345 In all cases, the Arrhenius behaviour was fulfilled in the entire range of temperatures considered ($R^2 >$
346 0.97, $p < 0.05$) and the relevant E_a values were calculated (**Table 3**). It can be noted that the E_a
347 relevant to %PPP resulted significantly higher and almost double than those obtained for K_{270},
348 confirming the highest temperature sensitivity of this index in comparison to the formation of

secondary oxidation products. This result is in agreement with those previously reported by Conte et al. (2020) and Aparicio-Ruiz et al. (2010, 2012, 2014) suggesting that the changes of temperature during EVOO storage causes a higher acceleration of chlorophyll and pheophytins degradation rate as compared to the formation of secondary oxidations products. On the other hand, the E_a values of K_{270} are consistent with those present in literature moving from 60 to 76 kJ/mol (Conte et al., 2020, Mancebo-Campos et al., 2008). Based on these data, it should be stressed that %PPP resulted an early indicator of product freshness and can be considered like a "rapid alert" that compulsory limits could be reached in short time.

It should be also noted that the E_a values of both indexes obtained by considering the different oils were comparable despite the different initial polyphenol content. This result suggests that the polyphenol content cannot be use as convenient parameter to predict product stability during storage, in agreement with previously described results highlighting that polyphenols probably did not intervene during the formation of secondary oxidation products or the degradation of pigments, remaining constant during storage of the oil under dark and reduced oxygen content.

3.4 Shelf-life estimation

In the final part of the research, the estimated Arrhenius equations were used as predictive tools to estimate EVOO shelf-life at temperatures below 60 °C. To this aim, the acceptability limits were chosen equal to 0.22 for K_{270}, being the threshold value for the EVOO category (Regulation 2568/91, 1991), and 17% for %PPP as limit reported in the Australian trade standard (Standards Australia, 2011). The following equations were used to compute the product compulsory SL (SL_c) and the high-quality life (SL_{HQ}):

$$SL_C = \frac{I_{\lim} - I_0}{k_T} \quad (6) \qquad \text{for } K_{270}$$

373 $$SL_{HQ} = \frac{I_0 - I_{\lim}}{k_T} \quad (7) \qquad \text{for \%PPP}$$

374

375 where I_0 is the initial value of the selected index, $I_{\lim}$ is the value of the index defined as acceptability
376 limit and k_T the rate constant at the temperature at which the SL would be defined. Considering $I_{0,}$ it
377 should be noted that in the further calculations the experimental value was used (data reported in
378 **Table 1**) instead of the calculated value of the intercept of Eq (1). Regarding the *k* values in Eq. (6)
379 and (7), they were computed by using the Arrhenius equations reported in **Table 3** inserting the value
380 of the temperature of interest.
381 The estimated shelf lives, together to the bootstrap confidence intervals, for the three EVOOs were
382 computed at different temperatures from 20 to 60 °C (**Table 4 and 5**). In this contest, the
383 quantification of the uncertainty of the estimate is quite complex for two reasons: i) uncertainty from
384 the first linear regression (Eq 1) does not propagate to the second regression that is the reparametrized
385 Arrhenius model (Eq 3) (indeed predicted values from the first linear regression are used as fixed
386 quantities in the second linear regression); ii) the relationship between shelf life and the parameters
387 of the second linear regression is highly non-linear, and only approximate results can be obtained
388 with error propagation formulas. For these reasons we decided to apply a bootstrap procedure. By
389 applying this procedure, it is possible to obtain a mean value of SL and an estimation of the variability
390 of the SL value. This variability is generally quite high at the actual storage conditions but allows to
391 obtain an estimation of what could happen during the commercial storage of the product to predict if
392 the best before date reported on the label would be appropriated.
393 The SL_C estimates at 25 °C moved from around 686 days to 870 days and the SL_{HQ} from 341 to 432
394 days considering %PPP. Based on the EU regulation (1991), it is expected that all the selected oil will
395 not overcome the compulsory acceptability limit before the expected shelf life of 18 months (540
396 days) at 25 °C. However, possible temperature fluctuation over 30 °C during storage could
397 significantly impact product shelf life, reducing shelf-life value below the 18 months “best before”

398 date. It can be also noted that SL_C values resulted in any case strictly dependent on the initial value
399 of K_{270} highlighting the importance of monitoring this parameter at the bottling time.
400 Considering the parameter % PPP as early freshness indicator (Gertz & Fiebig, 2006, Aparicio-Ruiz,
401 2012 and 2014; Conte et al., 2020), the SL_{HQ} predicted by using %PPP resulted significantly shorter
402 than that calculated by considering K_{270} and in all cases shorter than 18 months. It should be
403 remembered that we used 17 %PPP as acceptability limit reported on some trade standard (Standards
404 Australia, 2011; CDFA, 2016), but this value is not mandatory and universally accepted and other
405 limits might be applied. In any case, this index remains a precocious indicator of product quality
406 changes being its temperature dependence much higher than that of the K_{270}, based on E_a values.
407
408 **4. Conclusions**
409 The results here reported confirmed the feasibility of the ASLT methodology to predict the shelf-life
410 of EVOOs by using K_{270} as the best quality indicator for the estimation of the *best before date*. At the
411 same time, %PPP resulted a valuable predictive index of product freshness useful to compute a so
412 called high-quality shelf life (SL_{HQ}) of the product. Moreover, the % PPP can be regarded as a "rapid
413 allert" advising that compulsory limits could be reached in short time. This is due to the higher
414 sensitiveness to temperature changes of %PPP than K_{270}, as demonstrated by the E_a values ranging
415 from 115 to 122 kJ/mol and 49 to 65 kJ/mol, respectively. These E_a values clearly highlighted the
416 different effect of temperature on the kinetics of the two indexes: the increase of 10 °C of temperature
417 caused the approximate halving of the shelf life for the first index, while the same temperature
418 increases led to a four times reduction of the shelf life for the second one. However, it should be said
419 that a point of discussion on the use of %PPP in shelf life studies for the scientific community but
420 also business operators is the value of the %PPP acceptability limit to be used.
421 Considering that neither primary oxidation products nor antioxidant content significantly changed
422 during storage, these chemical characteristics of the fresh produced oils resulted not critical in
423 determining the temperature dependence of the changes of both selected indexes and thus the final

shelf life value for EVOO stored under dark and reduced oxygen content. Finally, it should be added that based on the acquired results, the ASLT methodology can be applied also to estimate the shelf life of other vegetables oils or lipid containing foods. To this task, the open issues to be investigated are the availability of acceptability limits to be applied to estimate product shelf life as well as the understanding of the temperature dependence of the oxidation rate depending on the lipid phase composition.

Abbreviations used

ASLT, accelerated shelf-life testing; $E_{a,}$ activation energies; PPP, pypropheophytin A; $E_{a,}$ activation energies; EVOO, extra virgin olive oil; SL, shelf-life

Declarations of interest:

None

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Figure captions

Figure 1. Changes of K_{270} of extra virgin olive oils containing increasing polyphenol content (a:156, b: 273 and c: 507 mg/kg) and stored at 25, 40, 50 and 60 °C

Figure 2. Changes of %PPP of extra virgin olive oils containing increasing polyphenols content (a:156, b: 273 and c: 507 mg/kg) and stored at 25, 40, 50 and 60 °C.

Table 1. Initial values of official quality parameters and fatty acid composition of samples used for the development of shelf-life predictive model and its validation.

Qualitative Characteristics	**a**	**b**	**c**
PV ($meqO_2/kg$)	7.5±0.3	5.3±0.5	4.0±0.5
K_{232} (e_x, 1%, 1cm)	1.82±0.05	1.74±0.05	1.78±0.08
K_{270} (ex, 1%, 1cm)	0.11±0.03	0.11±0.01	0.15±0.02
Total Polyphenols (mg/kg)	156.2±8.9	273.4±3.7	507.3±7.8
Total Tocopherols (mg/kg)	284±4	245±2	268±2
Fatty Acids %			
Palmitic acid (16:0)	12.8	11.4	9.3
Stearic acid (18:0)	1.7	1.7	2.4
Oleic acid (18:1 w9)	72.1	77.1	79.2
Vaccenic acid (C18:1 w7)	2.7	1.8	1.0
Linoleic acid (18:2)	8.0	5.9	6.1
Linolenic acid (18:3)	0.5	0.5	0.6
Others	2.2	1.5	1.3

560 **Table 2.** Apparent zero-order reaction rate (estimate ± SE) of K_{270} and %PPP of EVOO stored at 25,

561 40, 50 and 60 °C having increasing polyphenol content (156, 273 and 507 mg/kg)

562

Polyphenol content	**Temperature (°C)**	**K_{270}**		**%PPP**	
		k_{270}	R^2	k_{PPP}	R^2
mg/kg		(D.O.day^{-1}·10^{-3})		(%PPP day^{-1})	
156	25	0.12±0.01	0.96	0.033±0.002	0.98
	40	0.44±0.01	0.99	0.297±0.026	0.94
	50	1.21±0.04	0.99	2.275±0.185	0.98
	60	1.83±0.08	0.99	4.616±0.598	0.94
273	25	0.14±0.03	0.88	0.027±0.001	0.99
	40	0.40±0.02	0.99	0.344±0.019	0.98
	50	0.80±0.06	0.96	1.925±0.259	0.95
	60	1.04±0.10	0.91	3.801±0.702	0.94
507	25	0.12±0.03	0.80	0.035±0.001	0.99
	40	0.26±0.03	0.93	0.332±0.023	0.97
	50	0.86±0.08	0.94	1.694±0.242	0.92
	60	1.59±0.08	0.98	4.231±0.811	0.93

563

564

565 **Table 3.** Activation energy (E_a) and pre-exponential factor (estimate ± SE) of K_{270} and %PPP in the

566 three EVOO samples analysed.

Polyphenols content mg/kg	**Index**	***E_a*(kJ/mol)**	**log**	**R^2**
156	K_{270}	66.07±5.38	-7.30±0.09	0.99
	%PPP	121.60±11.47	-0.31±0.18	0.98
273	K_{270}	49.29±4.87	-7.57±0.08	0.98
	%PPP	120.90±11.31	-0.42±0.18	0.98
507	K_{270}	63.91±7.94	-7.57±7.94	0.97
	%PPP	115.43±6.19	-0.37±0.10	0.99

567

568

Table 4. Compulsory shelf-life (SL_C) estimated data in days considering K270 as SL index.

	Polyphenols content in oil								
	156 mg/kg			273 mg/kg			507 mg/kg		
	Predicted SL_C (days)								
Storage temperature (°C)	Mean	Lower limit Upper limit		Mean	Lower limit	Upper limit	Mean	Lower limit	Upper limit
20	1371	1114	1847	1048	776	1536	1064	745	1653
25	870	734	1113	747	578	1026	686	515	986
30	561	487	681	538	438	694	448	357	600
40	243	224	270	288	259	332	199	173	233
50	111	104	116	160	149	173	93	85	103
60	53	48	58	92	82	106	46	40	54

579 **Table 5.** High-quality **s**helf-life (SL_{HQ}) estimated data in days considering %PPP as SL index.

580

	Polyphenols content in oil								
	156 mg/kg			273 mg/kg			507 mg/kg		
	Predicted SL_{HQ} (days)								
Storage temperature (°C)	Mean	Lower limit	Upper limit	Mean	Lower limit	Upper limit	Mean	Lower limit	Upper limit
20	788	632	1050	1200	785	2260	956	708	1447
25	341	283	428	522	366	856	432	339	614
30	152	131	181	234	178	346	200	166	266
40	33	29	36	51	42	61	46	41	55
50	8	7	9	12	11	14	12	10	14
60	2	1	2	3	2	4	3	3	4

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583 Figure 1

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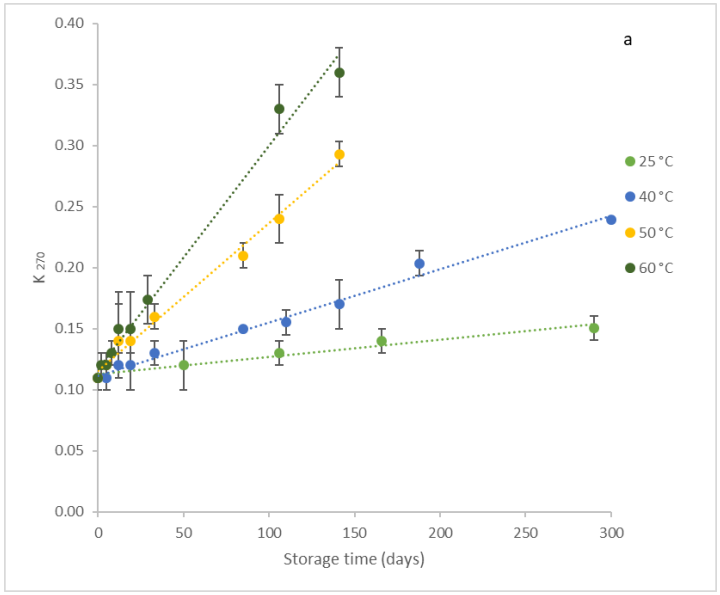

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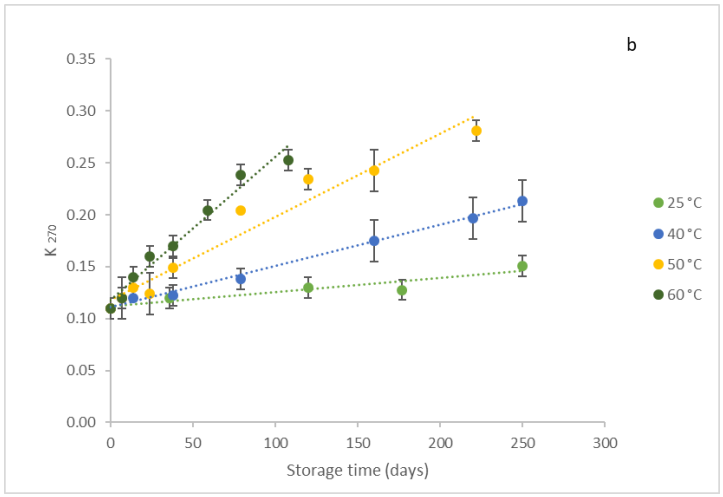

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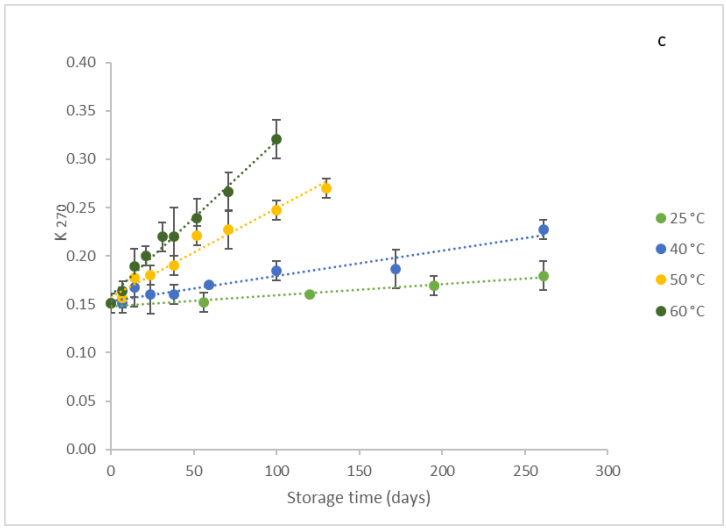

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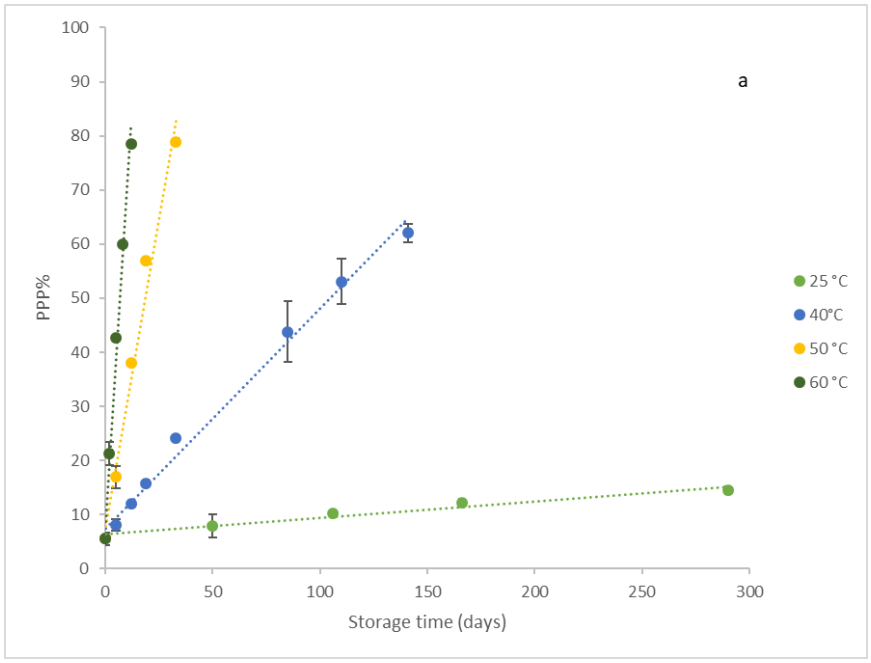
589 Figure 2



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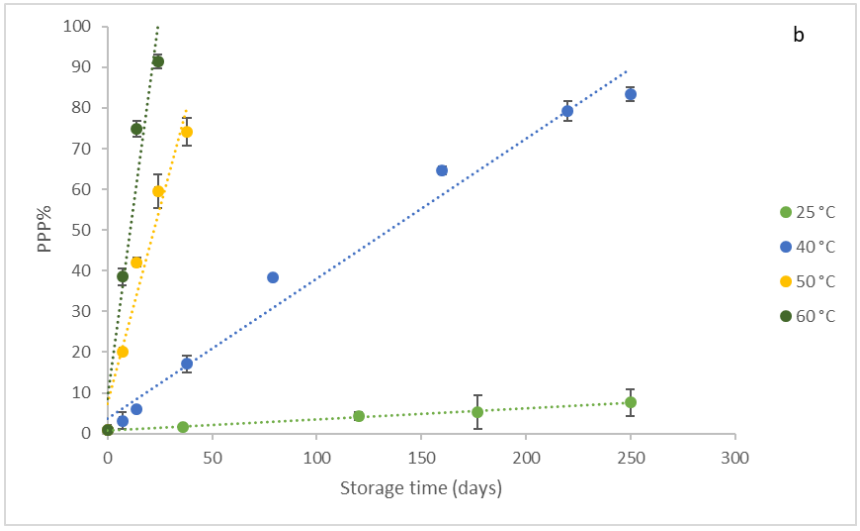

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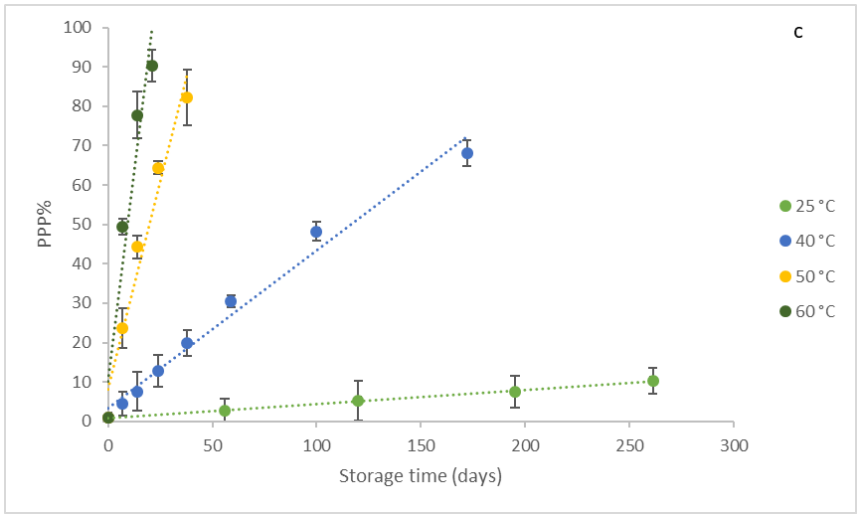

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