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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 fresh made extra virgin olive oils having increased total polyphenol content (156, 273 and 507 mg/Kg) were stored in the dark in glass containers at increasing temperatures (25, 40, 50 and 60 ° C). The changes of K270 and % of pyropheophytin A (PPP) resulted the best indicators to monitor product behaviour during storage. The rate constants of the changes of K270 and %PPP over time showed a temperature dependence describable with the Arrhenius model with activation energies (Ea) in the range of 49-65 kJ/mol and 115-122 kJ/mol for K270 and %PPP, respectively. These values confirmed the significant 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 an early indicator of product performances on the market.
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July 18th 2022

RE: Manuscript submission

Dear Editor,

On behalf of my other colleagues, it is my pleasure to submit the article entitled: "**Application of accelerated shelf-life test (ASLT) procedure for the estimation of the shelf-life of extra virgin olive oils: a validation study**" for publication in *Food Packaging and Shelf Life.*

The shelf-life 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. For these reasons, the possibility to estimate the product shelf-life and the relevant confidential interval appears particularly interesting in the attempt to develop predictive tools for food companies. The application of accelerated shelf-life testing (ASLT) procedures could allow to speed up the process. Within this context, the aim of this work was to validate the previously developed accelerated shelf-life test (ASLT) methodology for the prediction of extra-virgin olive oil shelf-life by considering three freshly made extra virgin olive oils with different compositional characteristics and phenolic content (156, 273 and 507 mg/Kg). Both K270 and %PPP were considered as indicators of quality changes during storage in the dark at temperatures at 25. 40, 50 and 60 °C. After conventional kinetic modelling, a statistical bootstrap procedure was applied, for the first time, to estimate the shelf-life uncertainties. As results, the changes of K_{270} and % of pyropheophytin A (PPP) resulted the best indicators to monitor product behavior during storage. In fact, the rate constants of the changes of K_{270} and %PPP over time showed a temperature dependence describable 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 significant 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 can be concluded that %PPP could be used as an early indicator of product performances on the market.
This study reveals the possibility to estimate olive oil shelf life thus allowing companies to predict in short times the end of life of their products and take decisions on date marking.

I confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.

Feeling confident that the manuscript can be suitable for publication in *Food Packaging and Shelf Life.*

Thanks in advances.
Your sincerely,
Prof. Paolo Lucci

Highlights

- Accelerated shelf life testing procedure resulted profitable to define EVOO shelf life
- K_{270} and %PPP were good indicators of EVOO stability changes during storage
- %PPP had a higher susceptibility that K_{270} to temperature increase
- Shelf life predictive models for EVOO were developed

Graphical Abstract

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Extra virgin olive oils

Polyphenols content:
156 mg/Kg

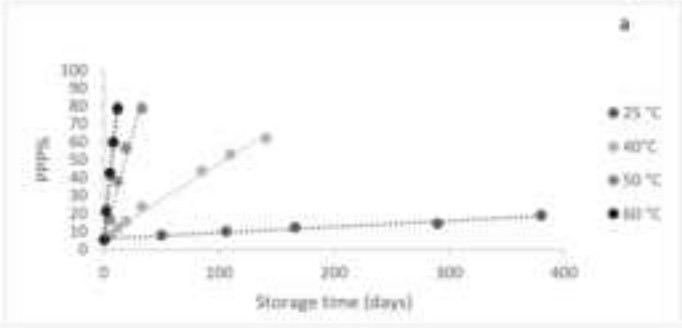
Polyphenols content:
273 mg/Kg

Polyphenols content:
507 mg/Kg

Accelerated shelf-life test

Storage: 25, 40, 50 and 60 ° C

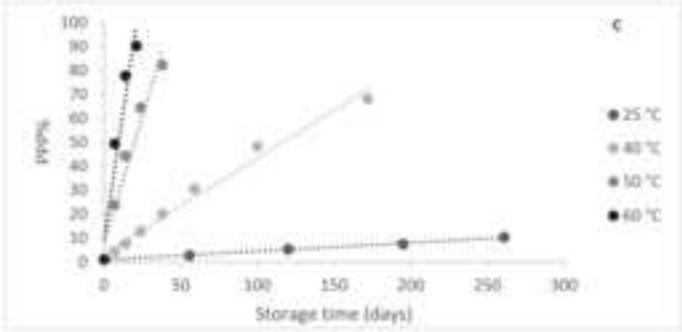
In the dark



Best/earlier quality indicator:

% of pyropheophytin A

No impact of polyphenols content



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 fresh made extra virgin olive oils having increased total polyphenol content (156, 273 and 507 mg/Kg) were stored in the dark in glass containers at increasing temperatures (25, 40, 50 and 60 ° C). The changes of K_{270} and % of pyropheophytin A (PPP) resulted 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 describable 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 significant 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 an early indicator of product performances on the market.

Key words

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

1. Introduction

European law 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 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 shelf-stable products with a long life.

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). Company managers dealing with shelf-life of shelf stable foods are looking for accurate and easily applicable tools allowing to predict in short times the end of life of their products and take decisions on date marking. The application of an *accelerated shelf-life testing* (ASLT) procedure is frequently applied to speed up the shelf-life assessment process of microbiologically stable foods (Nicoli, 2020, Calligaris et al., 2019). Temperature is the most common accelerating factor used in ASLT and the shelf life at the interested temperature is predicted by using the Arrhenius equation generated under accelerated storage conditions (Calligaris et al., 2019, Labuza & Schmidl, 1985).

One high-value shelf stable commodity for which the availability of predictive tools allowing to define in short time the "best before date" 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 232and 270 nm (K_{232} and K_{270}) and the sensory profile. Beside these compulsory indexes, other indicators, mainly related to oil freshness profile, such as phenols, tocopherols and pigments content, could markedly change during product storage. For these parameters mandatory limits to be fulfilled have not been established.

Different literature studies focussed to the identification of prediction models applicable for EVOO shelf-life, as reviewed by Li and Wang (2018). It should be pointed out that not all the reported studies can be listed as shelf-life studies but only as stability tests. The discriminant between these two types of storage test is the knowledge of the acceptability limit value, discriminating products still acceptable for the consumption from those unacceptable (Manzocco, 2016). In the case of EVOO, the acceptability limit is compulsory defined and it would be the quality index firstly reaching the value reported in the Regulation 2568/91 (1991). Examples are peroxide value, ultraviolet absorbance or sensory profile. The use as shelf-life indicator of other chemical parameters (e.g. polyphenols, volatiles, fatty acids profile, 1,2-diacylglycerols, pyropheophytins) important to define EVOO quality and freshness can be potentially considered only if an acceptability limit is defined and internationally recognised. This is the case of pyropheophytin a (%PPP) proposed as freshness indicator in the Australian trade standard (Standards Australia, 2011), in which an acceptability limit of 17% is proposed. Pyropheophytins in olive oil are formed due to degradations of chlorophyll pigments and this reaction begins soon after the oil is extracted. The pigments break down due to a process that involves the decarbomethoxylation of chlorophyll and pheophytins to form pyropheophytins (Gertz & Fiebig, 2006).

Recently, we studied the changes of many quality indicators at temperatures moving from 25 to 60 °C (Conte et al., 2020). It was demonstrated that the K270 can be considered the best early shelf-life indicator among compulsory quality indexes applicable in ASLT. In fact, this was the only compulsory quality index showing a good temperature dependence of its changes during storage describable by the well-known Arrhenius equation (Eq. 2). Interestingly, also the pyropheophytin a content (%PPP) resulted to be an additional good index of oil quality decay versus time.

The main difference among these two indexes is their activation energy (E_a). Since the activation energy of PPP% resulted about two times higher than that of K270, a dramatic acceleration of pheophytins degradation as compared to K270 may occur in case of temperature shifts during storage. This means that the shelf-life calculated considering %PPP (and 17 % as acceptability limit) is shorten than that calculated by using K270 at all the temperatures above 25 °C. Thus, the changes of %PPP was proposed as "a rapid alert" that EVOO is approaching to the end of compulsory shelf-life.

The present study aimed at validating the previously developed ASLT methodology to predict EVOO shelf-life by considering three freshly made extra virgin olive oils with different compositional characteristics. Both K270 and %PPP were considered as indicators of quality changes during storage at temperatures from 25 to 60 °C. 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 argument. The possibility to estimate the product shelf life and the relevant confidential interval appears particularly interesting in the 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 sample derived from a homogeneous batch produced in 2019 just after harvesting and packed within one month after EVOO production.

166 2.2 Storage conditions

167

168 Aliquots of 250 mL of the EVOO samples were packed into clean glass bottles with metal cap, made
169 of polytetrafluoroethylene (PTFE) as internal septum, and with 2 cm of headspace, mimicking the
170 commercial conditions. A total of 40 bottles for each type of EVOO was stored at 25, 40, 50 and 60
171 °C under dark in incubators (FTC 90I Refrigerated Incubator, Monza, Italy) for up to 300 days. At
172 different lengths of time during storage, one bottle of each oil was sampled and subjected to analytical
173 determinations.

174

175 2.3 Analytical determinations

176

177 2.3.1 Fatty acids composition

178

179 In order to determine fatty acid composition (%), the methyl-esters were prepared according to the
180 IOC method (International Olive Council, 2017) and analysed by Thermo Trace 1300 gas
181 chromatograph equipped with a FID detector and an auto-sampler. A fused silica column, SP-2330
182 (60 m length × 0.32 mm i.d. × 0.20 μm film thickness), was used. Helium was employed as carrier
183 gas, with a flow through the column of 1 ml/min. The temperatures of the injector (split) and detector
184 (FID) were both set at 250 °C. An injection volume of 1 μl was used. The operating conditions were
185 as follows: oven temperature was held at 165°C for 5 min, then increased by 3°C min^{-1} to 210°C and
186 held for 10 min. Split ratio was 1:50. Results were expressed as percentage of relative area.

187

188 2.3.2 Total phenolic compounds

189

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

192

193 2.3.3 Tocopherols

194

195 UHPLC analysis was realized using a Shimadzu Nexera (Shimadzu, Kyoto, Japan) coupled with the
196 same components used for polyphenols analysis and a fluorescence detector RF-20Axs with double
197 acquisition channels and a 12 μL cell. The detector was set at 296 nm and 325 nm for exciting and
198 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). To isolate pigments was used an SPE SiOH column 6 mL/1 g (Chromabond Macherey-Nagel GmbH & Co, Düren, Germany) using the first 10mL of a petroleum ether/ethyl ether solution in the ratio 90:10 (v/v) for the elution of non-polar compounds than 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.

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

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

where T_{ref} is a reference temperature (318°K in our case). The reparameterization is recommended because it enhances the statistical properties of the estimates of the unknown coefficients.

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

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

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. Where standard deviation is not shown, the method standard deviation has to be considered. 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 (Vienna, 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, 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 of the different compounds belonging to these component families. Observing the indexes referring to the oxidative status of the samples, in all cases these parameters are well below the Regulation limits with a limited variability among samples.

3.2 *Changes of the quality indicators during storage*

The changes of some selected quality indicators (peroxide value, K_{232}, K_{270}, polyphenols, tocopherols, conjugated trienes, hexanal and pyropheophytins) were monitored during storage at 25, 40, 50 and 60 °C for increasing time.

In agreement with our previous results (Conte et al., 2020), PV, K_{232}, polyphenols and tocopherol content did not significantly change during storage at any considered temperatures (data not shown). These results confirm that primary oxidation products did not further develop during storage under

reduced oxygen content in the headspace of the bottles. Similarly, both polyphenols and tocopherols did not decrease during storage even at the highest temperatures, confirming once again previous reported data.

On the other hand, the K_{270}, which is the indicator of the formation of secondary oxidation products, showed a progressive increase during the storage (**Figure 1a, b, c**). As expected, as storage temperature increased, a concomitant increase of the changes of these indexes also increased.

The changes of pyropheophytin a content during storage at 25, 40, 50, and 60 °C were also monitored (**Figure 2 a, b, c**). In agreement with previous results,[15] a linear increase of this index was observed as a function of time.

3.3 Data modelling

The kinetics of the changes of K_{270} and %PPP were modelled by using a pseudo zero reaction order and apparent zero-order rate constants were computed by linear regression analysis. Results of the kinetic analysis were reported in **Table 2** along with the relevant standard error and the coefficient of determination. In all cases, the selected reaction order well described the evolution of the selected indexes ($R^2 > 0.80$; $p < 0.05$).

To study the temperature dependence of K_{270} and %PPP, the values of *k* reported in **Table 2** were analysed according to the reparametrized Arrhenius model (eq.4). **Table 3** shows the acquired results. In all cases, the Arrhenius behaviour was fulfilled in the entire range of temperatures considered ($R^2 > 0.97$, $p < 0.05$) and the relevant E_a values were calculated (**Table 3**). It can be noted that the E_a relevant to %PPP resulted significantly higher and almost double than those obtained for K_{270}, 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) 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 K270 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 the initial content of polyphenols and other quality indexes cannot be considered as a predictive indication of the storage stability of the oil and the susceptibility of the product to temperature changes. Surprisingly, the temperature dependence of the rate constant resulted independent to the chemical composition of the oil.

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 SL:

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

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

where I_0 is the initial value of the selected index, $I_{\lim}$ is the value of the index defined as acceptability limit and k_T the rate constant at the temperature at which the SL would be defined. Considering I_0, it should be noted that in the further calculations it was used the experimental value (data reported in **Table 1**) instead of the calculated value of the intercept of Eq (1). It should be noted that the final SL values resulted in any case strictly dependent on the initial quality of the product. Regarding the *k* values in Eq. (6) and (7), they were computed by using the Arrhenius equations reported in **Table 3** inserting the value of the temperature of interest.

The estimated shelf lives, together to the bootstrap confidence intervals, for the three EVOOs were computed at different temperatures from 20 to 60 °C (**Table 4**). As it can be seen from the size of the confidence intervals, the variability is generally quite high at the actual storage conditions, notwithstanding it is possible to evaluate what could happen during the commercial storage of the product and if the product would comply or not with the best before day reported on the label.

The estimated shelf lives of the three EVOOs were computed at different temperatures from 20 to 60 °C (**Table 4**). In this contest, the quantification of the uncertainty of the estimate is quite complex for two reasons: i) uncertainty from the first linear regression (Eq 1) does not propagate to the second regression that is the reparametrized Arrhenius model (Eq 3) (indeed predicted values from the first linear regression are used as fixed quantities in the second linear regression); ii) the relationship between shelf life and the parameters of the second linear regression is highly non-linear, and only approximate results can be obtained with error propagation formulas. For these reasons we decided to apply a bootstrap procedure. By applying this procedure, it is possible to obtain a mean value of SL and an estimation of the variability of the SL value. This variability is generally quite high at the actual storage conditions but allows to obtain an estimation of what could happen during the

commercial storage of the product with a good estimation if the product would comply or not with the best before date reported on the label.

The shelf-life estimates at 25 °C moved from around 686 days to 870 days considering K_{270} and from 341 to 432 days considering %PPP. Considering EU regulation (1991) and K_{270} as SL index, it is expected that all the selected oil will not overcome the acceptability limit before the expected shelf life of 18 months (540 days) at 25 °C. However, possible temperature fluctuation over 30 °C during storage could significantly impact product shelf life, reducing shelf-life value below the 18 months "best before" date. Considering the parameter % PPP, in agreement with previous reported data (Gertz & Fiebig, 2006), the shelf life predicted by using %PPP resulted significantly shorted than that calculated by considering K_{270} and in all cases lower than 18 months. It should be remembered in this case that we used 17 %PPP as acceptability limit reported on the Australian trade standard (Standards Australia, 2011), but this value is not mandatory and universally accepted and other limits might be applied.

Interestingly, independently on the selected index, the content of total polyphenols did not affect the predicted shelf life in a positive way (high polyphenols content = high shelf-life). It should be remembered that no significant changes during storage was observed for this index in all the considered conditions (data not shown). Since polyphenols are expected to act as antioxidants in the initial phases of the reaction acting as chain-breakers, they probably did not intervene during the formation of secondary oxidation products or the degradation of pheophytins. Thus, this parameter cannot be considered as predictive index of the product behaviour during storage.

4. Conclusions

This validation study confirmed the feasibility of the ASLT methodology to predict the shelf-life of EVOOs. It was also confirmed that K_{270} and % PPP are the parameters allowing to monitor EVOO quality changes during storage at different temperatures, considering that neither primary oxidation products nor antioxidant content significantly changed during storage. Interestingly, the chemical characteristics of the fresh produced oils resulted not critical in determining the temperature dependence of the changes of both the selected indexes. The activation energies obtained by considering the different oils were comparable despite their different initial polyphenol content and ranging in small intervals that were 49-65 kJ/mol for K_{270} and 115-122 kJ/mol for % PPP. These values clearly highlighted the different effect of temperature on the kinetics of the two indexes: the increase of 10 °C of temperature caused the approximate halving of the shelf life for the first index, while the same temperature increases led to a four times reduction of the shelf life for the second one. It can be concluded that % PPP resulted the most sensitive to temperature changes resulting in an

early indicator of product performances on the market. It can be used by producers as a "red flag" of quality changes during storage. At this point it should be opened a point of discussion on the value of the acceptability limit of % PPP to be use in SL tests.

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 K270 of oils containing increasing polyphenol content (a:156, b: 273 and c: 507 mg/Kg).

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

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	5.3	4.0
K_{232} (e_x, 1%, 1cm)	1.82	1.74	1.78
K_{270} (ex, 1%, 1cm)	0.11	0.11	0.15
Total Polyphenols (mg/kg)	156	273	507
Total Tocopherols (mg/kg)	284	225	268
Fatty Acids %			
Palmitic acid (16:0)	12.79	11.37	9.30
Stearic acid (18:0)	1.67	1.75	2.37
Oleic acid (18:1 w9)	72.14	77.08	79.21
Vaccenic acid (C18:1 w7)	2.70	1.78	1.02
Linoleic acid (18:2)	8.04	5.96	6.14
Linolenic acid (18:3)	0.49	0.52	0.61
Others	2.20	1.50	1.30

510 **Table 2.** Apparent zero-order reaction rate (estimate ± SE) of K_{270} and %PPP of EVOO stored at 25,
511 40, 50 and 60 °C having increasing polyphenol content (150, 273 and 507 mg/Kg)
512

Polyphenol content	**Temperature (°C)**	**K270**		**%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

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515 **Table 3.** Activation energy (E_a) and pre-exponential factor (estimate ± SE) of K270 and %PPP in the
516 three EVOO samples analysed.

Polyphenols content mg/Kg	**Index**	E_a**(kJ/mol)**	**log**k_{ref}	**R²**
156	K_{270}	66.07±5.38	-7.30±0.09	0.99
	%PPP	121.6±11.47	-0.31±0.18	0.98
273	K_{270}	49.29±4.87	-7.57±0.08	0.98
	%PPP	120.9±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

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519 **Table 4.** Shelf-life estimated data in days considering K270 as SL index.

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	Polyphenols content in oil								
	156 mg/Kg			273 mg/Kg			507 mg/Kg		
	Predicted shelf life (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

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529 **Table 5.** Shelf-life estimated data in days considering %PPP as SL index.

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	Polyphenols content in oil								
	156 mg/Kg			273 mg/Kg			507 mg/Kg		
	Predicted shelf-life (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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Figure 1

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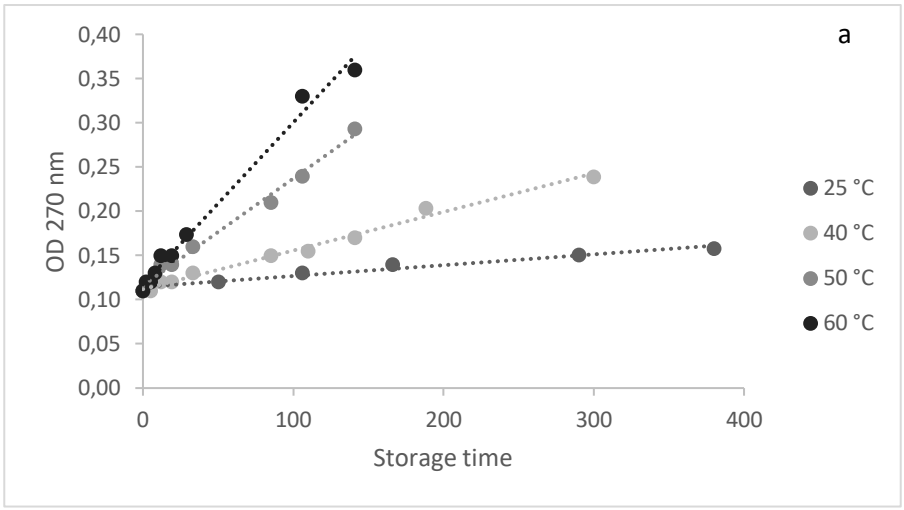

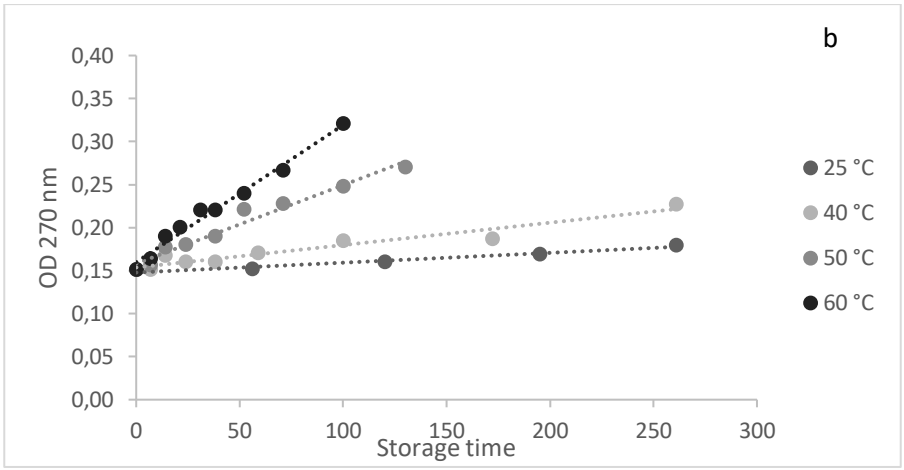

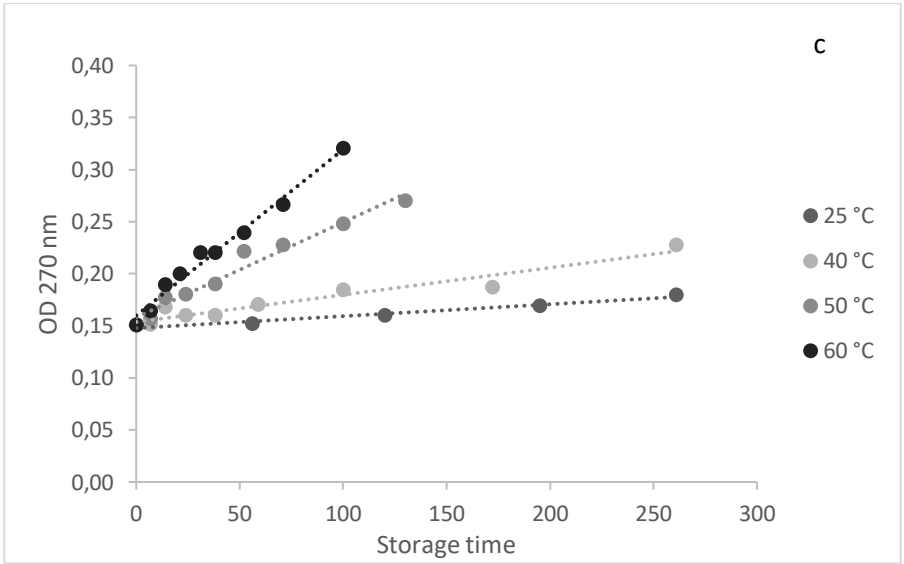

Figure 2

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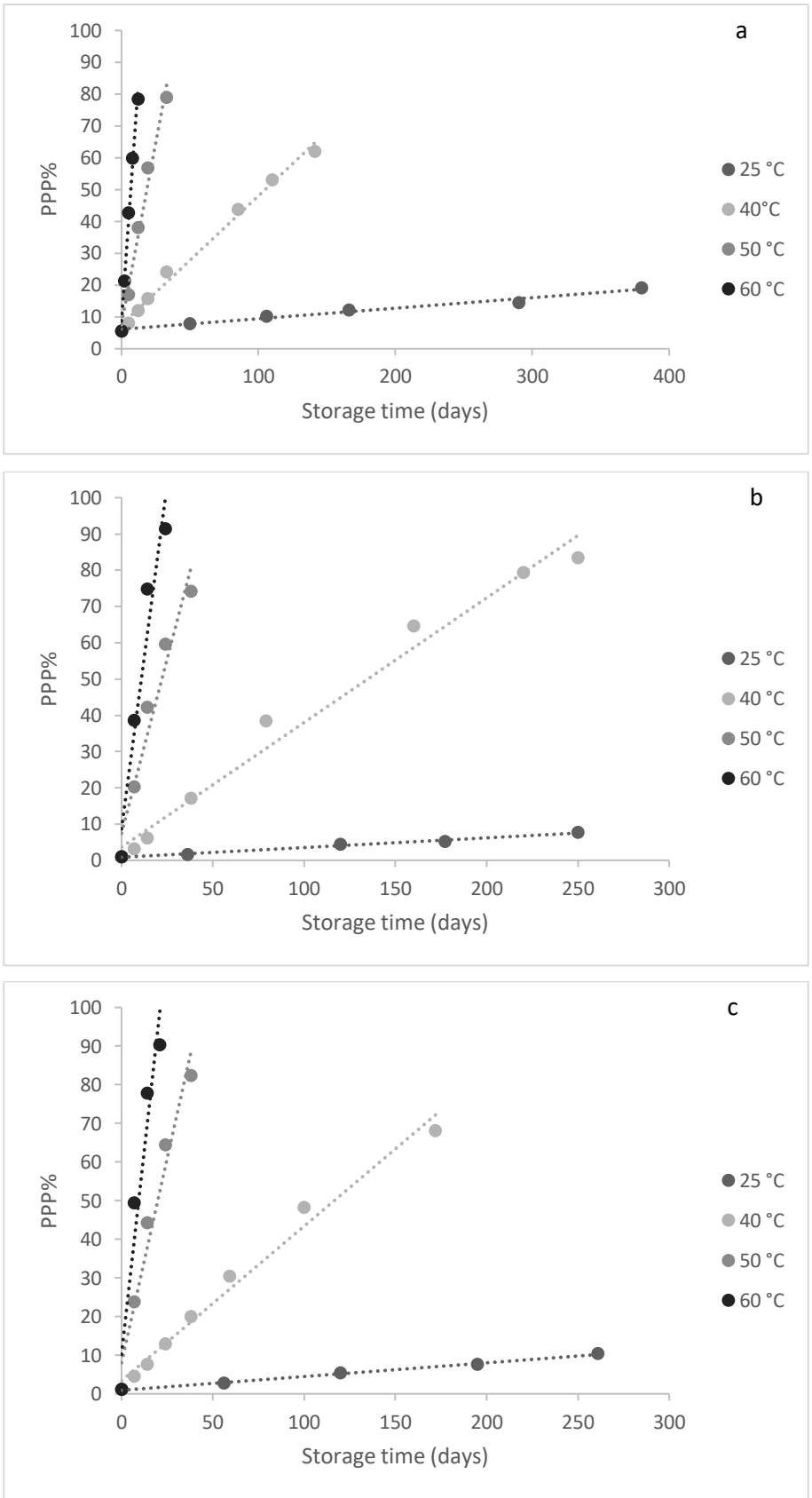

Figure 1. Changes of K270 of oils containing increasing polyphenol content (a:156, b: 273 and c: 507 mg/Kg).

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

July 11th 2022

Declaration of Competing Interest

Dear Editor,

on behalf of my other colleagues, I declare the authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Thanks in advances.

Your sincerely,
Prof. Paolo Lucci