



Multivariate stability monitoring and shelf life models of deterioration of vegetable oils under real time ageing conditions – Extra virgin olive oil as a main case of study

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ABSTRACT

Modelling quality depletion of oils and establishing their shelf life (or ageing rate) remains a challenge for food industry. A persistent issue in the control of lipid oxidation is deciding which oxidation products are the most suitable to be monitored. Several attempts have been done in this regard, however, the number of parameters that can be considered to assess oil shelf life is excessively wide and the proposed univariate models have proven to be extremely dependent on experimental conditions. For this reason, the methodology for carrying out a multivariate kinetic model is presented, combining physico-chemical and sensory parameters from experimental analytical determinations, and chemometric data processing tools. The main objective is to develop a multivariate shelf life model that allow predicting the number of months in which an extra virgin olive oil complies with the requirements of its commercial category when ageing takes place under standard trading conditions.

1. Introduction

Edible olive oil is a food commodity marketed under different designations referring to different categories, being the extra virgin olive oil (EVOO) the highest quality which is determined by threshold values of physico-chemical and sensory characteristics which in the latter case requires that must be free of defective organoleptic attributes (European Commission, 2019). In addition, EVOO, defined as a juice obtained from the fruit of the olive tree (*Olea europaea L.*) which has not undergone any treatment other than milling, washing, decanting, centrifuging and filtering, is found at the base of the 'Mediterranean Diet' pyramid and has proven considerable beneficial effects on human health (Sánchez Quesada et al., 2020).

However, sensory characteristics and the most of physico-chemical properties of EVOO are not stable through the time. Among the deterioration processes that EVOO could undergo from the moment it is produced, they can be grouped into three general mechanisms: (i) enzymatic oxidation, (ii) photo-oxidation, (iii) autoxidation; favoured by the incidence of various factors, all of which oxygen, temperature and light are the most important (Morales & Przybylski, 2013; Talbot, 2011).

Once EVOO is extracted, filtered and packaged, or stored in suitable containers, and provided that it is kept away from direct sunlight and heat, the main alteration is due to autoxidation, also known as rancidity. During the autoxidation reaction, a number of new compounds are formed, while minor components are degraded, causing off-flavours, loss of nutritional value and finally consumer rejection.

The concept of stability of vegetable edible oils is generally accepted as the shelf life of the product until rancidity becomes evident (Moschopoulou, Moatsou, & Drosinos, 2019). Current legislation demands that producers must indicate a best-before date on the label of EVOO, which is usually set at around 18–24 months by agreement (Government of Spain, 2021). After this period, an EVOO could be consumed, but its sensory attributes and healthy attributes are diluted over time and there may be an associated risk with human health and safety concerns. Therefore, it is easy to find olive oils in the market labelled as a higher-quality product than it really is. These edible oils would be engaging in non-conformity food fraud. Consumers increasingly appreciate food quality and, consequently, it is necessary to provide advance analytical information, with scientific support, for the quality characterisation of oils and other high fat food products.

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One question that persists in lipid oxidation control is deciding which oxidation products are the most suitable to be monitored. There are a lot of advances and research papers in this topic (Pignitter & Somoza, 2012), however, the number of parameters that could be considered for assessing the shelf life of EVOO is excessively wide: total phenol content (Psomiadou & Tsimidou, 2002), pyropheophytins trend (Aparicio, Roca, & Gandul Rojas, 2012), changes and evolving of the fatty acid composition, volatile compounds, ultraviolet absorbance or peroxide value (Guillaume & Ravetti, 2016), among others; so that, in practice, it is not feasible to acquire a clear idea of their behaviour by examining separately the evolution of each one.

In recent years, relying on multiparametric kinetic studies and modelling approaches by applying specific data mining methods, which formally referred to as 'chemofoodmetrics', progress has been made in the understanding of complex food quality-related reactions such as EVOO. Formally, 'chemofoodmetrics' could be defined as the science that relates measurements of chemical or physico-chemical parameters to the quality features of a food by applying mathematical or statistical methods. Multiparametric kinetic and shelf life models are often more generically applicable than single-parameter models and could be more easily applied to other products or processes (Grauwet, Vervoort, Colle, Van Loey, & Hendrickx, 2014). A detailed review of multivariate approaches applied on vegetable oil stability studies monitoring wide selection of parameters have been recently reported (Cui et al., 2023; Martín Torres, Ruiz Castro, Jiménez Carvelo, & Cuadros Rodríguez, 2022). Although a wide number of studies dealing with EVOO stability are available in the literature, both single-parameter monitoring models and multiparametric models, they often do not allow shelf life data to be obtained due to lack of a clearly defined acceptability limit.

For this reason, this paper deals with explanation and discussion of fundamental and framework for carrying out a multivariate shelf life model that allows an estimation of the shelf life of the vegetable oil under consideration. EVOO oxidation kinetics and shelf life results, when oxidised under standard storage conditions simulating supermarket shelves, are reported.

2. Methodology

2.1. EVOO samples

83 freshly produced and filtered EVOO samples were supplied by different producers during 2019/2020 harvest season just after milling, so that its ageing time could be initially considered as zero. Several olive varieties, degree of ripeness and geographical regions (mainly from Spain, but also Morocco and Portugal) were considered in the aim of being representative of the product variability found on the market.

2.2. Storage

As mentioned above, oxygen, temperature and exposure to light are the main causes of the oxidation of vegetable oils. So that, the control of storage conditions for shelf life studies is a critical step. Two different strategies can be pursued: (i) actual shelf life testing, that mimics the expected real storage conditions carefully chosen to realistically simulate storage and which must be kept constant until the end of the study; (ii) accelerated shelf life testing, under forced light and temperature conditions capable of increasing the rate at which food quality is lost.

In this study, seven batches were considered, consisting of the 83 different EVOOs, which were packaged in transparent food-grade PET 60 mL bottles (labelled from A to H) and were stored in a temperature-controlled room (20 ± 5 °C) exposed to 12 h of cold white LED light (6200 K), simulating standard supermarket store conditions during 14 months. The first lot (A), it was analysed at the beginning of the study, consider as zero month of ageing. After 2 months of storage, lot (B) was analysed. Consecutively, every two months, an aliquot of each oil sample was analysed (up to lot (H) after 14 months of ageing) for

physico-chemical and sensory parameters.

2.3. Analytical equipment

Refractive indexes were obtained using an Abbe refractometer ORT1RS. Spectroscopic UV absorption values were measured on Genesis 10SUV-Vis while anisidine value was determined using Agilent 8453 spectrophotometers. Oxidative stability was performed on Methrom 892 Professional Rancimat. Varian 3800 gas chromatographer equipped with a flame ionisation detector (Varian 450 GC) and an RXI-5HT capillary column was used to the quantification of the relative amounts of 1,2- and 1,3-diglycerides. Same equipment with Agilent HP-INNOWAX column was used to acquire the volatile compound fingerprints. The analytical determination of tocopherols was performed on Agilent 1100 Series liquid chromatograph equipped with a G1321A fluorescence detector using Ultrabase Sil column. Zorbax eclipse plus C18 and Agilent 1260 multiple wavelength detector were used for pyropheophytin a content measurement. Polar (phenolic) compounds quantification was performed on Agilent 1260 equipped with G7111B diode array detector.

2.4. Testing

Once samples and storage conditions are selected, it is then necessary to carefully chosen quality indicators to monitor the changes as a function of time. It is advisable to consider primary and secondary oxidation analytical parameters to have a clearer picture of the on-going oxidation situation together with sensory evaluation in order to identify off-flavours that are easily appreciable by the consumer's senses. In order to carry out a multivariate-based estimation of the EVOO shelf life, the following parameters were monitored.

2.4.1. Physico-chemical analyses

Refractive index was determined according to ISO 280 standard (ISO 280, 1998). Spectroscopic UV absorptivity values, K_{232} , K_{270} and ΔK were determined in compliance with COI/T.20/Doc. No 19 standard (COI/T.20/Doc. No 19/ Rev.5, 2019). The oxidative stability was measured by using the Rancimat method at 120 °C as described in ISO 6886 standard (ISO 6886, 2016). Both peroxide and anisidine values were determined according to the recognised methods described in COI/T.20/Doc. No 35 (COI/T.20/Doc. No 35/ Rev.1, 2017) and ISO 6885 standards (ISO 6885, 2016) respectively.

2.4.1.1. Chromatographic profiling. The quantification of the relative amounts of 1,2- and 1,3-diglycerides was performed by gas chromatography as described in the ISO 29822 standard (ISO 29822, 2009). Total, α -, β -, γ - and δ -tocopherols were quantified after high performance liquid chromatography (HPLC) and subsequent fluorescence detection according to ISO 9936 standard (ISO 9936, 2016). 'Pyropheophytin a' content was determined in accordance with ISO 29841 standard (ISO 29841, 2009).

The extraction, separation and subsequent detection of polar (phenolic) compounds by HPLC-DAD were carried out following the principles outlined in COI/T.20/Doc No29 method (COI/T.20/Doc. No 29, 2009) with slight modifications (previously published) (Cuadros Rodríguez et al., 2021). Ratio of areas of phenolic alcohols, phenolic acids, secoiridoids, lignans and flavonoids, referred to the total biophenols area, was expressed as a percentage.

2.4.1.2. Chromatographic fingerprinting. Volatile compounds were characterised by headspace solid-phase microextraction followed by GC-FID analysis. A more detailed explanation of both sample preparation and chromatographic conditions are described in a previous paper (Ortega Gavilán, Valverde Son, Rodríguez García, Cuadros Rodríguez, & Bagur González, 2020). After volatile fingerprints acquisition,

chromatograms were exported from the instrument software and embedded in a vector of data, and further pre-processed: (i) selection of region of interest; (ii) alignment using an average chromatogram as reference; and (iii) normalisation of intensities with respect to the internal standard. Each data vector containing the related chromatographic fingerprint of an EVOO sample consisted of 8391 elements, each of which is a single variable indicating a normalised signal intensity. When fingerprinting methodology is applied, the compounds referred to are neither identified nor quantified in the conventional way. It is therefore necessary to apply a process of selection of the variables that best define the evolution of the oil. This issue will be suitably explained later.

2.4.2. Sensory analyses

EVOO samples were sensory evaluated of rancidity. Rancidity value was agreed by a panel of 2–3 trained and experienced tasters in 'open-tasting' sessions, following the procedure described in COI/T.20/Doc No15 (COI/T.20/Doc. No 15/Rev. 10, 2018). It should be noted that the tasters are part of a sensory panel authorised by the IOC and accredited by the ISO 17025 standard.

2.5. Kinetic modelling and shelf life estimation

The availability of large data set does not mean at the immediate time availability of information promptly accessible. A properly use of chemometric tools that allow the interpretation of the hidden and non-evident information embodied within the data is necessary. All chemometric treatments were carried out using MATLAB (R2017b version, The Mathworks Inc. Natick, MA) and PLS_Toolbox (version 8.7, Eigen-vector Research Inc., Wenatchee, WA).

Two approaches could mainly be differentiated regarding multivariate data analysis of foodstuff stability data: unsupervised and supervised multivariate pattern recognition methods.

Principal component analysis (PCA) is aimed at finding the simplest mathematical model able to describe the data set satisfactorily. PCA looks for a smaller number of underlying factors, named principal components (PCs), that explain most of the variability exhibited by the larger number of measurements made on the objects/samples. It is an unsupervised method because it does not require training input to find the output: no additional knowledge (e.g., y-variable) besides raw data (x-variable) is needed to describe the data set. Models work on its own to improve understanding and accessibility of the intrinsic features of the data. PCA model was built in order to detect the presence or absence of outlier samples and to evaluate relationships between samples and variables, and between variable themselves. After that, PCs scores were used to model kinetic of EVOO oxidation.

Partial least-squares regression analysis (PLS) is aimed at detecting cause-effect relationships. PLS is a supervised multivariate method because, apart from the information on the x-variables measured, the available knowledge on a dependent response y-variable (usually, storage time) is applied to obtain a latent variable (LV) model that optimally describes the response variable. PLS computes a partial least-squares regression model to predict a dependent y-variable from a set of independent x-variables. PLS regression was used to evaluate importance of individual variables in the quality depletion of EVOO using loading plot of the variable's importance in projection (VIP) scores in the PLS model. The VIP formulation as originally proposed is a parameter varying in a fixed range since the sum of squared VIP for all variables sum to the number of variables. A variable with a VIP value close to or greater than one can be considered important in a given model. Variables with VIP values significantly less than one are less important and might be good candidates for exclusion from the model. Note that this criterion is suitable for discarding non-relevant variables, but may have drawbacks if used to assess the feature importance (Cocchi, Biancolillo, & Marini, 2018). After that, LV scores were used to obtain a multivariate equation of EVOO quality depletion and, therefore, to

evaluate shelf life time.

PCA may be more responsive to other sources of variation whereas a PLS prediction model based on ageing months will in general behave more robustly as PLS only takes into account the covariance between spectral profiles and predicted values and which may provide the advantage of being more flexible for future validations.

2.5.1. Acceptability limit

Shelf-life studies require the identification of an acceptability limit; this is what makes it different from a stability study. This is a very hard decision to make when selecting the cut-off value of a critical attribute, i.e., the attribute that has the highest impact on the quality depletion of the oil, or shows the most change over the shortest time period. Normally it is referred to legal or regulatory requirements. When multivariate shelf life model is conducted, acceptability limit vector includes all the attributes that show change over time and gives rise to a single scalar coefficient, Q_C , which is traduced to shelf life time.

Once the PLS regression model of shelf life is built, loading matrix is necessary to calculate Q_C , defined as follows:

$$Q_C = \max(Q_A \cdot L)$$

where Q_A is the autoscaled acceptability limit vector and L the loading matrix of the time related latent variable of the model. Q_C is then interpolated into shelf life model equation to obtain cut off criteria, t_C , or shelf life time.

3. Result and discussion

3.1. Experimental data matrix of ageing

Firstly, data were arranged in a 664×30 matrix: each row corresponds to each sample at specific storage time batch (0, 2, 4, ..., 14 months). Each column corresponds to the value of a single determined parameter. Table S1 (supplementary material) shows all parameters or variables, and a brief description of them, which have been monitored over 14 months.

In order to carry out the previous selection of the volatile variables, all volatile fingerprints were integrated into a data matrix. A table showing all the parameter results at the beginning of the study (month zero of storing) has been included in the supplementary material (Table S2).

Then, a preliminary PLS regression model against storage time was built and the VIP coefficients were calculated. Variables (linked to retention times) showing an absolute VIP coefficient exceeding the threshold value of 1.00 were selected as relevant influential ones and the corresponding chromatographic intensity (height) was considered. Table 1 shows the nine selected volatile variables and the corresponding retention times for each one.

3.2. Modelling of EVOO ageing

Experimental data were processed by multivariate analysis

Table 1

Influential volatile variables, related to the volatile chromatographic fingerprint, on the storage time.

Name	Variable number	VIP scores for Y1	Retention time interval (min)
Volat1	19	1.34	1.89–2.36
Volat2	36	1.04	2.39–2.42
Volat3	55	1.01	2.50–2.51
Volat4	83	1.04	2.71–2.75
Volat5	142	1.22	2.95–3.06
Volat6	178	1.04	4.86–5.11
Volat7	203	1.02	5.33–5.34
Volat8	254	1.06	7.27–7.30
Volat9	280	1.09	8.25–8.37

procedures to select significant parameters on stability of our EVOO lots. All data were mean-centred and the variables were weighed by their standard deviation to give them equal variance (autoscaled).

A multidimensional map of the 83 EVOO samples in relation to the 30 physicochemical parameters and sensory characteristics was obtained by PCA. 6 PCs were selected that explain 75% of the total data variability. The PC1 (40.6% of variance) seemed to group and order the samples according to the ageing of storing (Fig. 1) showing the higher scores a greater number of months stored oils. In order to inspect for 'outlier' samples by checking the residual values and the leverage effect they generate in the model, a Q-T² screening plot is shown on figure S1 (supplementary material). The particular data for three possible outliers are reviewed. No reason for outliers was detected. It was concluded that these must be measurement experimental errors and it was decided to continue without excluding any sample.

3.2.1. Selection of significant variables

A preliminary PLS model, considering all the variables, was then applied to evidence possible relationships between storage time (y-variable) and the potentially significant dependent variables (x-variables). 4 LV were selected; LV1 explained 91% of the cumulative variance of the y-variable which stated a high correlation between among influential analytical parameters and storage time. Fig. 2 shows that samples were linearly distributed with a correlation coefficient of 0.95. This leads to the option of using PLS (instead of PCA) to estimate the life time. In addition, figure S2 (supplementary material) shows VIP coefficients for the PLS model.

Clearly γ -tocopherol and δ -tocopherol, total biophenols, phenolic acids, secoridoids, lignans+flavonoids, and anisidine value variable do not contribute with relevant information to the model and were not further considered (VIP coefficient <0.5). K₂₃₂, ΔK , oxidative stability, phenolic alcohols and refractive index are questionable. On the contrary, pyropheophytin a, 1,2-diglycerides, 1,3-diglycerides, volat1, volat2, volat3, volat4, volat5 and volat8 are the variables with the highest correlation with ageing.

Pearson's correlations between pair of variables included in the model were evaluated. Highly correlated variables (Pearson correlation coefficient >0.70) provide similar information and there is a risk of overfitting the model. Table 2 shows a summary of excluded and selected variable on the multivariate models for the rest of the study.

3.2.2. Kinetic parameters

A new PCA model was built but considering only selected relevant variables (664 × 17 data matrix). On this occasion, 6 PCs were selected increasing both the total cumulative variance explained (>80%) and the variance explained by PC1 (>50%) with respect to the former model. It clearly shows that PC1 is time-structured, making it suitable for estimating the kinetic parameters.

Typically, degradation reactions could follow a zeroth-order, first-order or second-order kinetics, as well as the cumulative form of the Weibull model which has proven useful in numerous studies that improved the fit of experimental observations to various quality indices as a function of time due to its extreme flexibility (Amodio, Derossi, Mastrandrea, & Colelli, 2015).

Zeroth order kinetics

$$\frac{-dQ}{dt} = k; Q = Q_0 - k \cdot t$$

First order kinetics

$$\frac{-dQ}{dt} = k \cdot Q; \ln Q = \ln Q_0 - k \cdot t$$

Second order kinetics

$$\frac{-dQ}{dt} = k \cdot [Q]^2; \frac{1}{Q} = \frac{1}{Q_0} + 2k \cdot t$$

Weibull kinetics

$$Q(t) = Q_0 \cdot e^{-b_m (T)^{n_m (T)}}$$

where Q (or Q(t)) is a variable which collects the PC1 scores of the samples as a function of time, and Q₀ the score of the fresh sample, i.e., a parameter representative of initial conditions at time zero. In all cases, k is the oxidation kinetic constant.

The best model fitting was obtained when the logarithm of PC1 scores is plotted vs. the storage time (coefficient of determination, R² = 0.894; root mean square error, RMSE = 0.0489) so we can stay that the data follow pseudo-first order degradation kinetics with Q₀ = 1.165 and k = 0.031.

3.3. Shelf life modelling

A subsequent PLS model, considering just the selected variables, was set up with 3 LVs (the first variable explains more than 90% of the

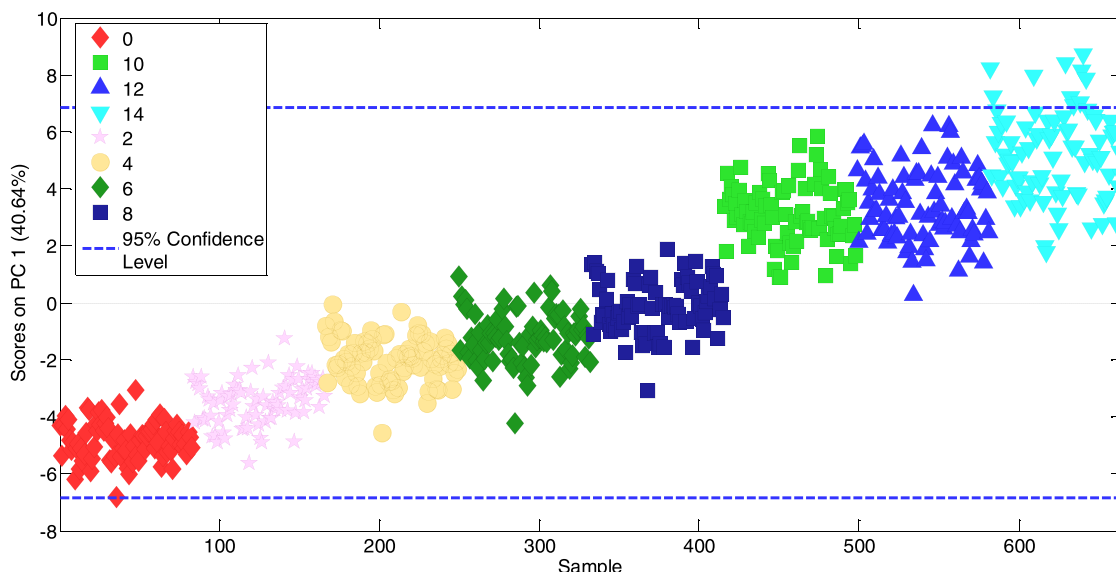


Fig. 1. PC1 scores for the 83 EVOO samples: storage time is used only as class marker.

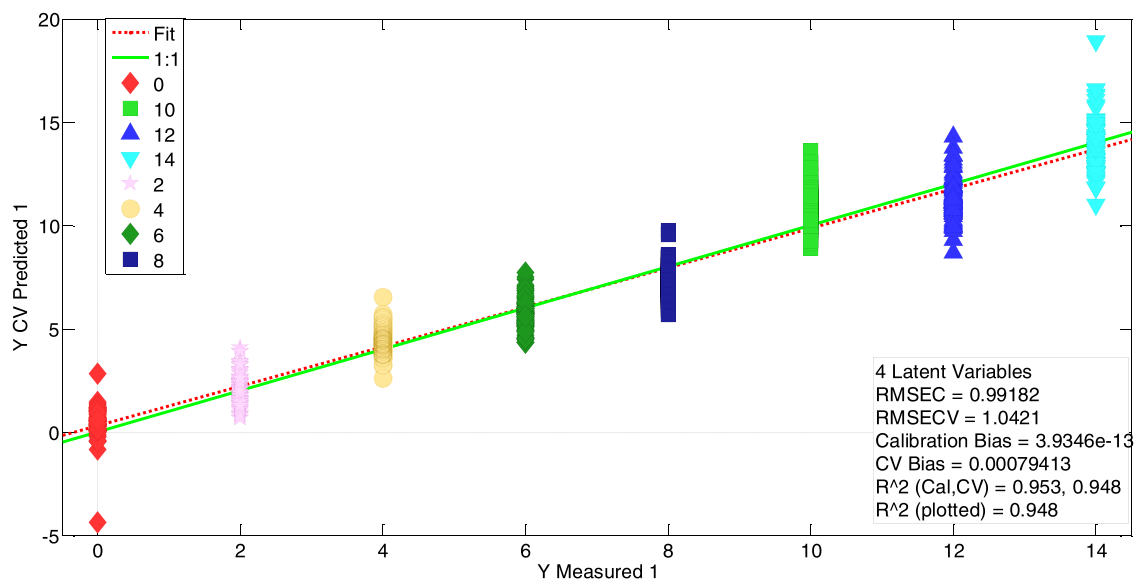


Fig. 2. Relationship between predicted and known values of the independent y-variable (ageing time) obtained from the PLS model considering all variables.

Table 2

Summary of both selected and excluded variables on the EVOO multivariate shelf life model.

Selected variables		Excluded variables	
Peroxide value	1,2-diglycerides	Refractive index ^a	1,3-diglycerides ^a
K ₂₇₀	Volat1	Anisidine value	Volat3 ^a
K ₂₃₂	Volat2	Total biophenols	Volat4 ^a
ΔK	Volat5	Secoridoides	Volat6 ^a
Oxidative stability	Volat7	Phenolic acids	
Rancidity	Volat8	Lignans + flavonoids ^a	
Phenolic alcohols	Volat9	Total tocopherols ^a	
α-Tocopherol	Pyropheophytin a	γ-Tocopherol	
β-Tocopherol		δ-Tocopherol	

^a Excluded variables for having correlation coefficients greater than 0.7 with any of the selected variables.

cumulative variance of the y-variable, *i.e.*, storage time) considering only relevant x-variables. The LV1-LV2 scores plot was evaluated. It can be perceived that the samples were ordered from negative scores for LV1 in the youngest oils, to positive values when storage time increases. It can be also seen how the biggest differences were found after month 10 of ageing, showing a wider dispersion of the samples, with most of the samples being outside the confidence region (95%) of the LV1-LV2 plot (see figure S3a, supplementary material). Moreover, within the samples of the same class, a greater data scatter is found as ageing time progresses, *i.e.*, the samples degraded during 10 months show a greater variability between them than the samples aged 2 months; this suggests unequal ageing rates of the different samples under study. The greatest differences in the score values appear between months 8 and 10; where the slope of growth of LV1 scores changes (see figure S3b, supplementary material).

Finally, a linear regression model is established between predicted and known values of the storage time, obtaining a good fit $R^2 = 0.94$, $RMSE = 0.004$; (as it is shown on figure S4, supplementary material).

LV1 is time-structured (as it can be seen on figure S3b) and accordingly, LV1 scores were used to establish a linear model ($Q(t) = a + b \cdot t$) of the autoscaled values of the experimental data *vs.* the storage time ($R^2 = 0.91$; $RMSE = 0.89$). The estimated coefficients (95% confidence interval) were: $a = -4.267 (-4.393, -4.141)$ and $b = 0.6096 (0.5945, 0.6247)$.

In order to estimate the shelf-life or 'critical time', t_C , of EVOO, is necessary to define the acceptability limit vector collecting the regulatory threshold values of the monitored physico-chemical or sensory characteristics under study (AS 5264, 2011; European Commission, 2019) beyond an olive oil can no longer be considered as EVOO: peroxide value ≤ 20 ; $K_{232} \leq 2.5$; $K_{270} \leq 0.22$; $\Delta K \leq 0.01$; median of rancid defect = 0; pyropheophytin a $\leq 17\%$; 1,2-diacylglycerols $\geq 35\%$.

At this point the question that emerges was: *what limit values should be set for those variables that have significance for the shelf life model but they are not covered by regulations?* To solve that question, we decided to inspect for those scalars in 'unacceptable oils', *i.e.*, olive oils that has exceeded the compulsory limits in one or more physico-chemical or sensory analyses. Due to the unequal ageing (as expected) of the 83 EVOO samples in this study, it was decided to average the data for each ageing time. The robust median statistics was applied to find a representative value for all samples for each storage time. As a result, 14 vectors were obtained providing the median of the 17 selected analytical parameters of the 83 samples in months 0, 2, 4, up to 14 months. When those vectors were compared with the regulatory limits (where applicable), it should note that the median intensity of the rancid attribute exceeds the regulatory parameter from month 10 onwards and consequently may be considered that the half of the olive oils have lost EVOO category.

The acceptability limit vector, Q_A , is then defined from the limit values for all the regulated parameters and the values obtained in the median vector of 10-month aged samples for the non-regulatory parameters. To this regard, the criteria for the definition of the shelf life acceptability limits for EVOO undergoing oxidation are based on (1) compliance to legal requirements, and (2) robust statistics mean values. Table 3 shows acceptability limit vector values.

For estimating a descriptive value of the Q_C scalar, Q_A vector was autoscaled and multiplied by L , the loading vector of LV1, which explains the largest variance of the y-variable, *i.e.*, ageing time, regarding the PLS shelf life model. A value of 3.92 was obtained for Q_C .

Finally, Q_C was interpolated in the regression equation of multivariate shelf life model to obtain a t_C value, *i.e.*, the maximum ageing time at which an olive oil, provided it has been stored and analysed under the same experimental conditions, cannot be classified as EVOO anymore. 13 months were finally estimated to be a representative shelf life time for EVOO stored under the experimental conditions described in this study. Notice that a two-month uncertainty in the predictions is taken into account; this corresponds to the time that elapses from two

Table 3

Acceptability limit values used for building the scores vector to be applied to the EVOO shelf life model.

Variable	Acceptability limit	Variable	Acceptability limit
Peroxide value	20.0	1,2-diglycerides	35.00
K ₂₇₀	0.22	Volat1	0.870
K ₂₃₂	2.50	Volat2	0.382
ΔK	0.01	Volat7	0.045
Oxidative stability	11.10	Volat5	0.002
Rancidity	1.0 ^a	Volat8	0.025
Phenolic alcohols	4.90	Volat9	0.003
α-Tocopherol	165.06	Pyropheophytin a	17.0
β-Tocopherol	2.47		

^a lowest rancid organoleptic intensity being considered perceptible and non-zero.

consecutive analytical controls (experimental work frequency). Therefore, the shelf life time, given in months, should be rounded down to the nearest whole number to give each EVOO the benefit of the doubt and to ensure it complies with requirements.

3.4. Shelf-life index and ageing rate

Once the model is established, and t_C is estimated, the equivalent ageing time, t_i , of every single sample could be calculated as:

$$Q_i = \mathbf{X}_i \cdot \mathbf{L} \Rightarrow Q_i = -4.267 + 0.6096 \cdot t_i$$

where \mathbf{X}_i symbolises the auto-scaled vector of the experimental data of each sample at a specific time, and \mathbf{L} is the vector of loadings of the first latent variable, LV1, from the regression PLS model. Q_i is interpolated in the shelf life model to obtain t_i .

From this values, a 'Shelf Life Index', I_{SL} , is then defined as a cardinal reflecting the number of months in which an EVOO continues in compliance with the requirements of its category. It is calculated from:

$$I_{SL} = t_C - t_i$$

where the value of t_C is set to 13 months.

Likewise, an 'Ageing Rate', %Age, of the EVOO is calculated as follows:

$$\%Age = \frac{t_i}{t_C} \cdot 100$$

3.5. Verifying the shelf life prediction ability

In order to test the reliability of the shelf life model and verify the reliability of the predicted critical times, 5 EVOO samples were randomly selected from among those included in the study:

- **VE003.** 'Arbequina' monovarietal EVOO from Granada (Andalusia, South of Spain). It lost the EVOO category after 8 months of storing, exceeding the regulatory value of K272 and rancid defect.
- **VE038.** 'Hojiblanca' monovarietal EVOO from Granada (Andalusia, South of Spain). It remained as EVOO throughout the study (14 months), *i.e.*, it did not exceed any of the regulated limit values.
- **VE040.** 'Picual' monovarietal EVOO from Jaen (Andalusia, South of Spain). It remained as EVOO until the 14th month, where it presence a rancid defect of 1.
- **VE053.** 'Coupage' blend EVOO from Seville and Jaen (Andalusia, South of Spain). It lost the EVOO category in 12 months, as from which the value of rancid organoleptic intensity is higher than threshold value.
- **VE075.** 'Hojiblanca' monovarietal EVOO from Seville (Andalusia, South of Spain). It reached a rancid intensity value higher than zero from the month 8 of ageing.

For these, Q_i , t_i , I_{SL} and %Age parameters were calculated at each ageing time (see Table 4).

The predicted lifetime from the model, taking as a reference the state of each of the olive oils at months 2 and 6, is then compared with the actual date on which the olive oil loses EVOO category. The objective was to check whether the prediction ability about the predicted time of each EVOOs to meet the requirements when no oxidation (or minimal, *i.e.*, 2 months of storage), and for the same EVOO when ageing is more evident (6 months of storage), was fulfilled.

At month 2 of storage, VE003 is predicted to keep its quality parameters within accepted limits for 10 months. Provided that oil is kept under described ambient condition and analyses are conducted after 10 months of storage, VE003 have just exceeded the acceptability limit and sample is considered non-conforming to the category. In a similar way, VE038, VE053 and VE075 are predicted to have almost 12 to be in compliance with requirements. From month 14 of ageing onwards, they could either remain acceptable for a few months or have just failed to meet the standard. VE040 is predicted to be suitable up to month 16 and at month 14 of storing, it is predicted to be within two months until reaching the end of its shelf life.

Considering predictions at month 6 of storage, VE003 showed a shelf life index of 5 while it is non-compliant with the category in month 12 of storage. VE053 and VE075 are predicted to have shelf life indexes of 7

Table 4

Estimated shelf life index, I_{SL} , and ageing rate, %Age, values for 5 randomly selected EVOO to be used as examples on the reliability of the predictions.

Sample	Ageing time	Q_i	t_i	I_{SL}	%Age
VE003 ^a (Month 8)	0	-3.98	0.47	12.5	4
	2	-2.61	2.72	10.3	21
	4	-0.89	5.54	7.5	43
	6	0.69	8.13	4.9	63
	8	1.97	10.24	2.8	79
	10	3.97	13.51	-0.5	104
	12	4.83	14.93	-1.9	115
VE038 ^a (Stable)	0	-4.33	-0.10	13.1	-1
	2	-3.45	1.33	11.7	10
	4	-1.94	3.82	9.2	29
	6	-1.42	4.68	8.3	36
	8	-0.42	6.32	6.7	49
	10	0.44	7.72	5.3	59
	12	1.08	8.77	4.2	67
VE040 ^a (Month 14)	0	-5.84	-2.59	15.6	-20
	2	5.00	-1.20	14.2	-9
	4	-3.61	1.07	11.9	8
	6	-3.10	1.92	11.1	15
	8	-2.04	3.66	9.3	28
	10	0.74	8.22	4.8	63
	12	1.03	8.70	4.3	67
VE053 ^a (Month 12)	0	-4.99	-1.18	14.2	-9
	2	-3.72	0.90	12.1	7
	4	-1.82	4.01	9.0	31
	6	-0.83	5.64	7.4	43
	8	0.30	7.49	5.5	58
	10	2.37	10.89	2.1	84
	12	2.58	11.24	1.8	86
VE075 ^a (Month 8)	0	-4.58	-0.51	13.5	-4
	2	-3.35	1.51	11.5	12
	4	-2.42	3.04	10.0	23
	6	-1.70	4.21	8.8	32
	8	0.28	7.46	5.5	57
	10	2.16	10.55	2.5	81
	12	2.76	11.52	1.5	89
14	4.06	13.66	-0.7	105	

^a The month in which the olive oil loses EVOO category when the regulatory parameters are monitored independently (univariate approach), is indicated in parentheses; "stable" specifies that the olive oil still retains the EVOO category at the end of 14 months.

and 8 months respectively. Measurements carried out in month 14 predicted that both of them are already oxidised and have just exceeded the limit of acceptability fixed for its commercial category (note that these are true predictions taking into account the uncertainty). Finally, VE038 and VE040 showed indexes of 8 and 11 months to be in compliance with the legal requirements, and, at month 14 of storage (end of the study), they continue in accordance with the EVOO category.

4. Conclusions

The multivariate shelf life test was successfully applied to EVOOs. The results of the present study indicate that the actual shelf life of EVOO could be accurately predicted by measuring some key quality parameters, evaluating their significance and using multivariate data analysis tools to model real time EVOO degradation. Multivariate analysis is based in observation and analysis of more than one experimental variable at a time and taking into account the effects of all variables on the modelling of the feature of interest. The nature of multivariate approach is to reveal the inherent structure and meaning embedded within original set of variables through the application and interpretation of a variety of statistical methods. Conducting a multivariate study is not synonymous to applying multiple univariate approaches simultaneously, as people might think. Therefore, the shelf life of EVOO is predicted in 13 months by considering the changes it undergoes as a whole, just as the value of a work of art (a painting) is measured not only by looking at each parameter (composition, colours, perspective,...) but also by looking at the sense of harmony of the whole artwork.

Research is currently being carried out to verify the application of our multivariate modelling of kinetic and shelf life to directly predict the rate of oil degradation and remaining shelf life of any EVOO when stored under the experimental conditions described in this study. In our opinion, under the same storage conditions the same combination of stability/instability indices may result in an equal constant rate of kinetic degradation parameters. Under other storage conditions, such as different material bottling, the methodology for estimating a finite shelf life would be exactly the same, the same is not true for the results. Several studies focus on oil shelf life comparisons by changing external conditions (Caipo et al., 2021), which is outside the scope of this research.

CRedit authorship contribution statement

Sandra Martín-Torres: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **Juan Antonio Tello-Jiménez:** Conceptualization, Validation, Resources, Funding acquisition. **Rafael López-Blanco:** Conceptualization, Validation, Formal analysis, Investigation. **Antonio González-Casado:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Luis Cuadros-Rodríguez:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

raw data included on supplementary material.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2023.101070.

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