Predictive Study on Tuscan Extra Virgin Olive Oil Stability under Several Commercial Conditions

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Industries aim to ensure extra virgin olive oil (EVOO) stability especially during commercial activities up to use by end consumers. The objective of this work was to set up predictive models of EVOO stability during commercial activities. Stability was studied on five lots of a batch of Tuscan virgin olive oil to simulate different commercial activities. Chemical, physical, and sensory analyses were carried out on EVOO samples. Experimental data were processed by multivariate analyses to select significant parameters and by regression analyses to set up kinetic models. A few parameters were found to be significant: hydroxytyrosol and tyrosol contents, carotenoid absorbance at 475 and 448 nm, α-tocopherol content, Rancimat induction time, and K_{232}. It was also shown that the stability of this EVOO was not significantly influenced by different uncontrolled bottling, transport, and storage conditions in supermarkets. Empirical models were set up to predict the time to reach a reference value for K_{232}.

Keywords: *Extra virgin olive oil; stability; prediction*

INTRODUCTION

Extra virgin olive oil (EVOO) industries run the risk of both legal and customer expectation nonconformities when they produce and commercialize this product. EVOO oxidation may result in the exceeding of legal limits, such as the spectroscopic index K_{232} and some unpleasant odors and flavors, and also decrease the nutritional quality of product, due to a loss of antioxidants such as polyphenols and tocopherols (Robards et al., 1988; Montedoro et al., 1992, 1993; Akasbi et al., 1993; Ciappellano et al., 1994; Tsimidou, 1998).

EVOO oxidation is mainly caused by exposure to oxygen, raw materials, that is, olives and virgin oils, at high oxidation levels or with a low concentration of antioxidants, long times and high temperatures of storage, exposure to light, and the presence of metal traces (Capella et al., 1981; Miyashita and Takagi, 1986; Kiritsakis and Tsipeli, 1992; Frankel, 1996; Tsimidou, 1998; Frega et al., 1999). Process critical control points of EVOO stability include quality of olive and virgin oil supplies, blending of different virgin oils, industrial storage of both loose and bottled oil, commercial activities such as transport, and shelf life.

Industries aim to ensure EVOO stability, especially during commercial activities up to use by end consumers, when the product can be subjected to uncontrollable oxidation-producing factors such as long time and high temperature of storage and exposure to light. Because it is extremely difficult to monitor product stability on the market, it may be useful to set up a predictive model of stability to control the product during commercial activities.

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Two types of predictive models can be set up to simulate EVOO oxidation: (i) kinetic models and (ii) empirical models.

Kinetic models are able to predict the kinetics of oxidation parameters as a function of different variables, that is, time, temperature, oxygen pressure, etc. Typical EVOO oxidation parameters are acidity, peroxide value, spectroscopic indices in the UV and visible regions, hydroperoxides, and secondary oxidation products. Many data on variations in the above-mentioned parameters under several operating conditions are available (Gasparoli et al., 1987, 1990; Gasparoli and Fedeli, 1991), but no kinetic models are reported.

Empirical models are able to predict maximum shelf life as a function of the values for stability parameters. Typical stability parameters are polyphenol and tocopherol contents and Rancimat induction time. Some empirical models are reported in the literature (Gutfinger, 1981; Tsimidou et al., 1992; Gordon and Mursi, 1994), but no examples of their applications are shown.

The aim of this work was to set up predictive models of EVOO stability during commercial activities on a reference sample from Tuscany, to show their practical application and to provide a methodological approach to their setting up.

MATERIALS AND METHODS

EVOO stability was studied on a batch of Tuscan virgin olive oil during the 1995−1996 olive harvest. At the end of November 1995 virgin olive oil was produced by OL.MA. (Grosseto, Italy) through centrifugation after milling and malaxing of olives (Canina, Frantoiano, Moraiolo, and Leccino varieties) harvested manually or by mechanical means. The EVOO batch was stored in a 35000 L stainless steel tank under nitrogen at 20 °C. Physicochemical composition and sensory characteristics of the freshly made oil are reported in Table 1.

The following five lots were taken from the EVOO batch to simulate different commercial activities.

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Table 1. Physicochemical Composition and Sensory Characteristics of Freshly Made EVOO Batch (Time = 0 Shelf Life)

parameter	mean ± SD
chemical	
acidity (oleic acid %)	0.34 ± 0.01
peroxide value (mequiv of O_2/kg)	7.45 ± 0.04
α-tocopherol (ppm)	216 ± 1
tyrosol (ppm)	5.67 ± 0.03
hydroxytyrosol (ppm)	3.1 ± 0.2
deacetoxyoleuropein aglycon (ppm)	12.4 ± 0.2
deacetoxyligstroside aglycon (ppm)	12.55 ± 0.01
tyrosolelenolic acid aglycon (ppm)	13.7 ± 0.1
oleuropein aglycon (ppm)	10.90 ± 0.09
physical	
spectroscopic index K_{232}	1.70 ± 0.03
spectroscopic index K_{270}	0.15 ± 0.01
chlorophyll absorbance at 670 nm (OD)	0.413 ± 0.007
chlorophyll absorbance at 610 nm (OD)	0.419 ± 0.004
chlorophyll absorbance at 560 nm (OD)	0.077 ± 0.005
chlorophyll absorbance at 535 nm (OD)	nd[a]
carotenoid absorbance at 475 nm (OD)	0.72 ± 0.02
carotenoid absorbance at 448 nm (OD)	0.94 ± 0.01
carotenoid absorbance at 414 nm (OD)	1.37 ± 0.02
Rancimat induction time (h)	9.72 ± 0.01
sensory characteristics	
bitter taste	2.6 ± 0.8
astringency	3.0 ± 1.1

[a] nd, not determined.

Lot A was taken from the freshly made batch and stored in 100 mL dark glass bottles, closed with screw caps, in the dark at 20 °C for ∼21 months. This was the reference lot; it was stored under controlled, optimal shelf life conditions.

Lots B_1 and B_2 were taken from the EVOO batch after 77 and 188 days of storage, respectively. They were bottled in 500 mL dark glass bottles, closed with screw caps, shipped to Australia, and stored in a supermarket (The Essential Ingredients Ltd., Camperdown, Australia) under uncontrolled light and temperature conditions for ∼16 (lot B_1) and ∼14 months (lot B_2).

Lots C_1 and C_2 were taken from the EVOO batch after 98 and 188 days of storage, respectively. They were bottled in 500 mL dark glass bottles, closed with screw caps, and stored in a supermarket (Cantina Cooperativa Grossetana, Grosseto, Italy) close to OL.MA. under uncontrolled light and temperature conditions for ∼17 (lot C_1) and ∼14 months (lot C_2).

Variations in temperature of lots B and C were monitored by temperature loggers (Smart-Reader SR-001, ACR, Surrey, Canada).

The EVOO lots were sampled approximately every 2 months. Samples from Australian and Italian supermarkets were rapidly sent to the laboratory and stored at 10 °C until analysis.

Chemical Analyses. Acidity was determined according to the EU official method (Anonymous, 1991). Oil samples were dissolved in 50 mL of a diethyl ether/ethanol (1:1 v/v) mixture prior to titration with a sodium hydroxide solution (0.1 N). Analyses were carried out in triplicate, and acidity was calculated as percent oleic acid.

The peroxide value was determined according to the EU official method (Anonymous, 1991). Oil samples were dissolved in 25 mL of an acetic acid/chloroform (3:2 v/v) mixture, and 0.5 mL of a saturated potassium iodure solution was added prior to titration with a sodium thiosulfate solution (0.01 N). Analyses were carried out in triplicate, and the peroxide value was calculated as milliequivalents per kilogram.

The polyphenol content was determined by HPLC according to the method of Cortesi et al. (1995). A tetrahydrofuran/water (4:1 v/v) mixture was used as an extraction solvent. Each sample extraction was carried out in duplicate. The HPLC apparatus consisted of an L-7100 Merck pump (Hitachi Co., Tokyo, Japan), an L-7400 UV Merck Hitachi detector at 280 nm, and a D-7500 Merck Hitachi integrator. Operating conditions were as follows: RP-18 Spherisorb ODS-2 column (25 cm × 0.46 cm, 5 μm) equipped with an RP-18 Waters precolumn; injection volume, 20 μL; mobile phase, elution with a binary gradient of methanol/acetonitrile (50:50 v/v) and 0.5% H_3PO_4 in water, at 1 mL/min flux.

Tyrosol and hydroxytyrosol contents (parts per million) were calculated using a standard curve of tyrosol (Merck) at a concentration of 2−10 ppm. The deacetoxyoleuropein aglycon, deacetoxyligstroside aglycon, tyrosolelenolic acid aglycon, and oleuropein aglycon contents (parts per million) were calculated using a standard curve of oleuropein (Merck) at a concentration of 10−50 ppm.

α-Tocopherol was determined by HPLC according to the method of Tonolo and Marzo (1989). Ethyl acetate was used as an extraction solvent. Each sample extraction was carried out in duplicate. The HPLC apparatus described previously was used, and the detector was set at 290 nm. Operating conditions were as follows: Waters Symmetry C-18 column (25 cm × 0.46 cm, 5 μm) equipped with a Waters Symmetry C-18 precolumn; injection volume, 20 μL; mobile phase, isocratic elution 90:10 with methanol/water (96:4 v/v), followed by rinsing with ethyl acetate, at 1.4 mL/min flux.

The α-tocopherol content (parts per million) was calculated using a standard curve of α-tocopherol (Merck) at a concentration of 5.6−56 ppm.

Physical Analyses. Spectroscopic indices, K_{232} and K_{270}, in the UV region were determined in triplicate according to the EU official method (Anonymous, 1991). Spectroscopic indices in the visible region were absorbances at 670, 610, 560, and 535 nm, related to chlorophylls, and absorbances at 475, 448, and 414 nm, related to carotenoids. Analyses were carried out in triplicate.

The induction time was measured by using a Metrohm Rancimat 679 (Metrohm Ltd., Herisau, Switzerland) in triplicate. Operating conditions were as follows: 5 g of sample, air flow rate of 20 L/h, and reaction temperature of 120 °C.

Sensory Analyses. A panel consisting of 12 trained judges selected from OL.MA. staff (6 men and 6 women), aged between 30 and 60 years, was used.

Three training sessions were carried out using a scoring method on three EVOO reference samples (one from OL.MA. and two from a local supermarket) and one olive oil sample. After training, all judges were qualified.

The EVOO samples (25 mL) were served in plastic glasses in random order under fluorescent light at ∼28 °C. Four EVOO samples were evaluated during each session. Within each session the design was balanced for order and carry-over effect (MacFie et al., 1989). Judges were requested to evaluate the intensity of bitter taste and astringency by assigning a score between 0 (i.e., absence of sensation) and 5 (i.e., extremely intense) according to the EU official method, called the COI test (Anonymous, 1991).

Data Processing. Principal component analysis (PCA) and partial least-squares analysis (PLS) were used to classify samples by Unscrambler 6.0 software package (Camo As., Trondheim, Norway).

PCA is aimed at finding the simplest mathematical model able to describe the data set satisfactorily. In the most appropriate statistical approach, the problem is to detect the relative importance of individual variables for determining the data structure.

PLS is aimed at detecting cause−effect relationships.

Comparison of regression lines was carried out by applying the least significant difference test by Statgraphics Plus 1.0 software package (Manugest KS Inc., Rockville, MD).

RESULTS AND DISCUSSION

Selection of Significant Parameters. All experimental data (Roberti, 1997) were processed by multivariate analysis procedures to select significant parameters on stability of our EVOO lots. A multidimensional map of all the EVOO samples (i.e., 37) in relation to the 21 physicochemical parameters and sensory characteristics was obtained by PCA. This statistical pro-

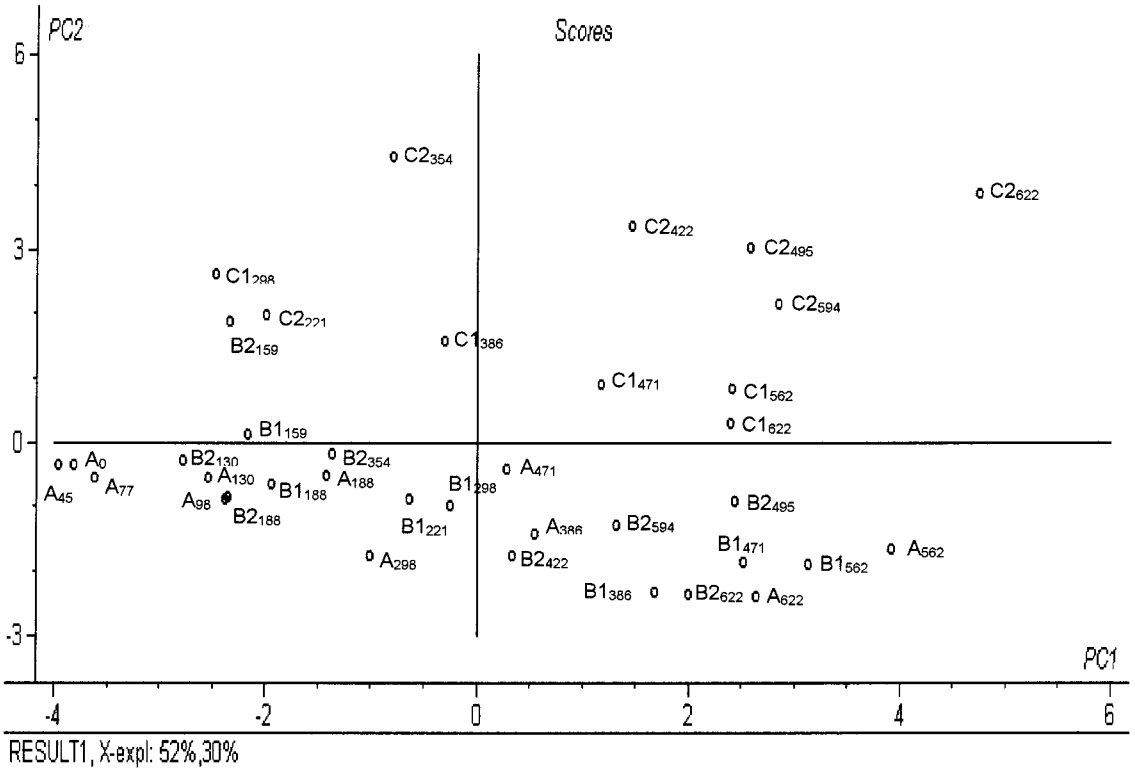

Figure 1. Sample score plot by PCA method. Letters represent the lot, and numbers represent the days of shelf life.

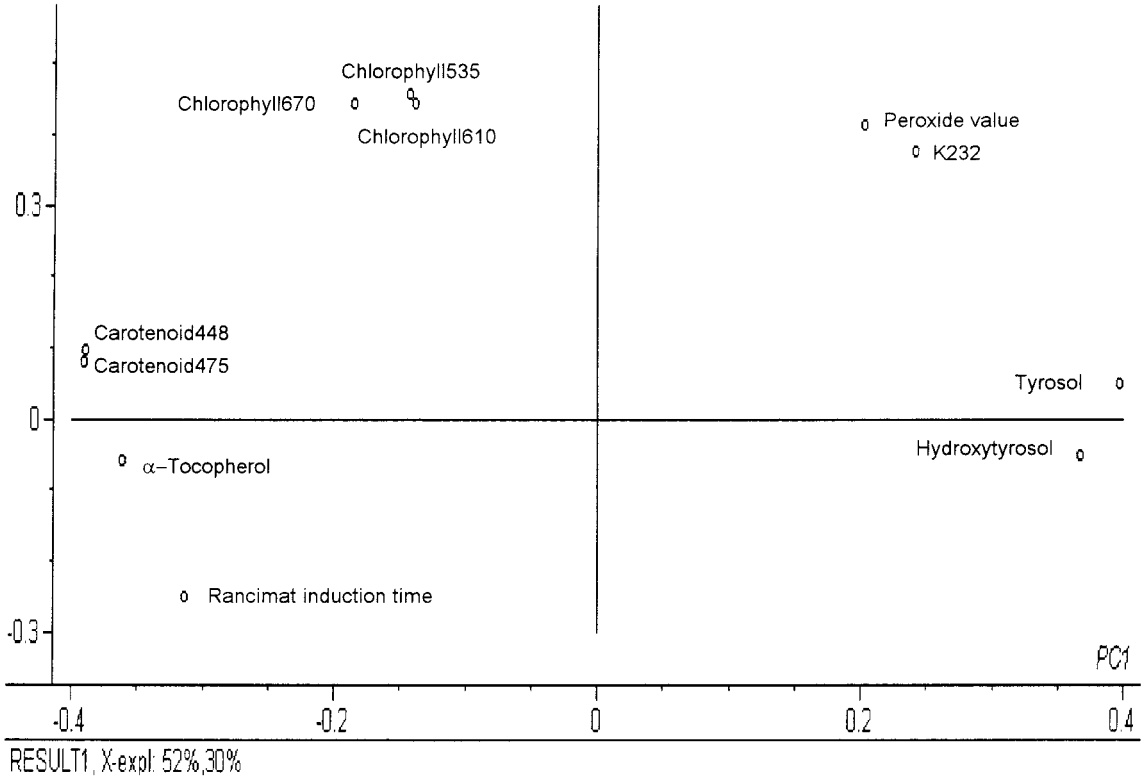

Figure 2. Sample loading plot by PCA method.

cessing allowed us to exclude the least significant parameters, which were positioned near the axes origin. The variables that participated to a minor extent in the total variance explanation were acidity, polyphenolic aglycon content, K_{270}, chlorophyll absorbance at 560 nm, carotenoid absorbance at 414 nm, bitter taste, and astringency. PCA was then repeated after exclusion of these parameters. The relevant score plot and loading plot are reported in Figures 1 and 2, respectively. The samples in these graphs were labeled by both lot initials and label numbers representing the total days of shelf life.

A comparison between the score plot and the loading plot showed that the younger samples were positioned along the first principal component (PC1) on the left side of the graph (Figure 1), which explains 52% of data

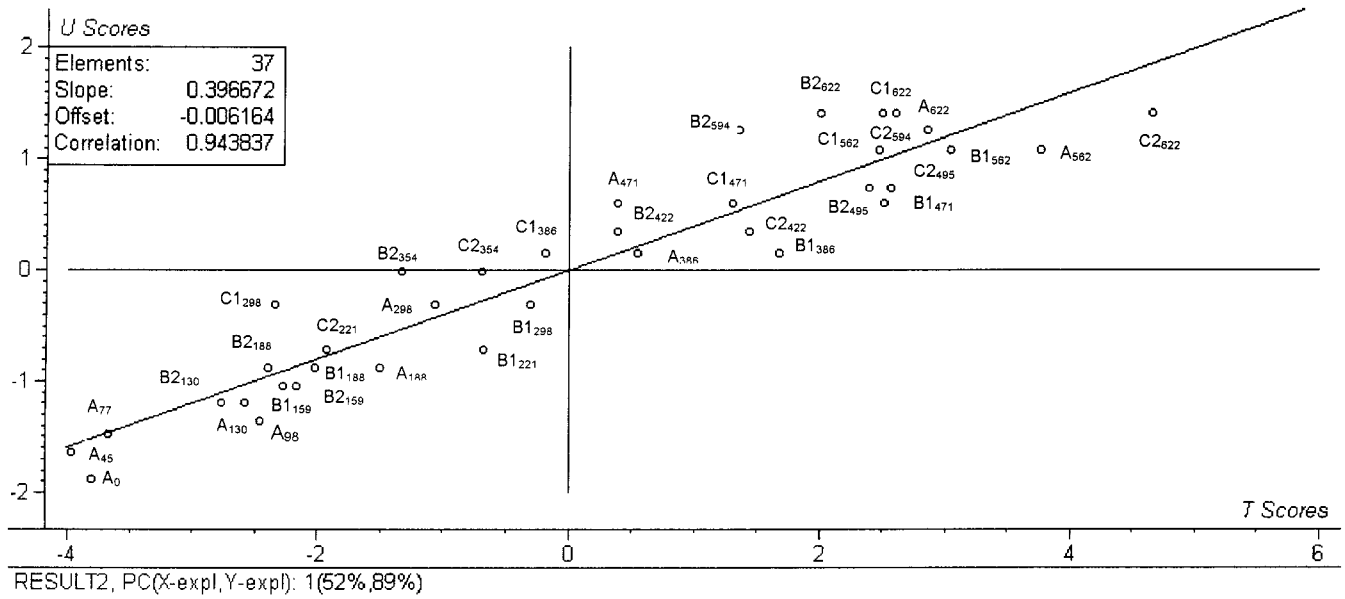

Figure 3. Relationship between dependent variable *X* and independent variable *Y* (time) by PLS method.

variability. They were characterized by higher values for Rancimat induction time, α-tocopherol content, and carotenoid absorbances (Figure 2). The older samples were located on the right side of the graph (Figure 1). They were characterized by high tyrosol and hydroxytyrosol contents (Figure 2).

PLS modeling with latent variables was then applied to evidence possible relationships between storage time and analytical parameters. PLS, after data set standardization, provided the best regression model between the independent variable (*Y*) (i.e., time) and the potentially significant dependent variables (*X*). Figure 3 shows that samples were linearly distributed with a correlation coefficient of 0.94.

The *Y* variance rate, explained by the model (89%), indicated a high correlation between analytical parameters and storage time. Loading of variables is reported in Table 2.

It shows that the most significant parameters were hydroxytyrosol and tyrosol contents, carotenoid absorbance at 475 and 448 nm, α-tocopherol content, Rancimat induction time, and K_{232}.

At the end of the multivariate analysis procedure we were able to select only 7 of the 21 analytical parameters measured as significant to model the stability of our EVOO lots. Among them the only legal parameter was K_{232}.

Kinetic Models. Kinetic models of the significant parameters were set up on the EVOO reference lot (i.e., lot A) stored in the dark at 20 °C for up to 622 days, a time representing the usual maximum commercial shelf life of EVOO.

EVOO oxidation in our oil samples resulted in tyrosol and hydroxytyrosol formation. Figure 4 shows that their contents linearly increased with time following pseudo-zero-order kinetics

$$T = T_0 + k_T \cdot t$$
($r = 0.99$; F ratio $= 877.46$; P value $= 0.0001$)

$$\mathrm{HT} = \mathrm{HT}_0 + k_{HT} \cdot t$$
($r = 0.99$; F ratio $= 75.47$; P value $= 0.0001$)

where t is the storage time (days) and T and HT are the tyrosol and hydroxytyrosol contents (ppm) at time t, respectively. Their initial amounts were $T_0 = 5.68$ ppm and $\mathrm{HT}_0 = 3.42$ ppm, which were similar to the experimental values (Table 1). Their apparent reaction rate constants were $k_T = 0.012$ ppm/day and $k_{HT} = 0.022$ ppm/day.

Table 2. Relationship between Response Variables and Storage Time by PLS Method

variable	loading
peroxide value	0.177
K_{232}	0.212
Rancimat induction time	−0.295
chlorophyll absorbance at 670 nm	−0.157
chlorophyll absorbance at 610 nm	−0.109
chlorophyll absorbance at 535 nm	−0.108
carotenoid absorbance at 448 nm	−0.377
carotenoid absorbance at 475 nm	−0.401
α-tocopherol (ppm)	−0.377
tyrosol (ppm)	0.409
hydroxytyrosol (ppm)	0.419

EVOO oxidation resulted in color variation. Carotenoid absorbances at 475 and 448 nm decreased with time following pseudo-first-order kinetics. For example, the kinetics of carotenoids at 475 nm was

$$\mathrm{Car} = \mathrm{Car}_0 \exp(-k_{\mathrm{Car}} t)$$
($r = 0.91$; F ratio $= 154.36$; P value $= 0.0001$)

where Car is the carotenoid absorbance at 475 nm (OD) at storage time t. Its initial value was $\mathrm{Car}_0 = 0.80$ OD, which was similar to the experimental value (Table 1). Its apparent reaction rate constant was $k_{\mathrm{Car}} = 3.4 \times 10^{-4}$ day^{-1}.

EVOO oxidation resulted in a nutritional quality decrease. The α-tocopherol content decreased with time following pseudo-first-order kinetics

$$\mathrm{Toc} = \mathrm{Toc}_0 \exp(-k_{\mathrm{Toc}} t)$$
($r = 0.90$; F ratio $= 87.51$; P value $= 0.0001$)

where Toc is the α-tocopherol content (ppm) at storage time t. Its initial value was $\mathrm{Toc}_0 = 217$ ppm, which was similar to the experimental value (Table 1). Its apparent reaction rate constant was $k_{\mathrm{Toc}} = 3.2 \times 10^{-4}$ day^{-1}.

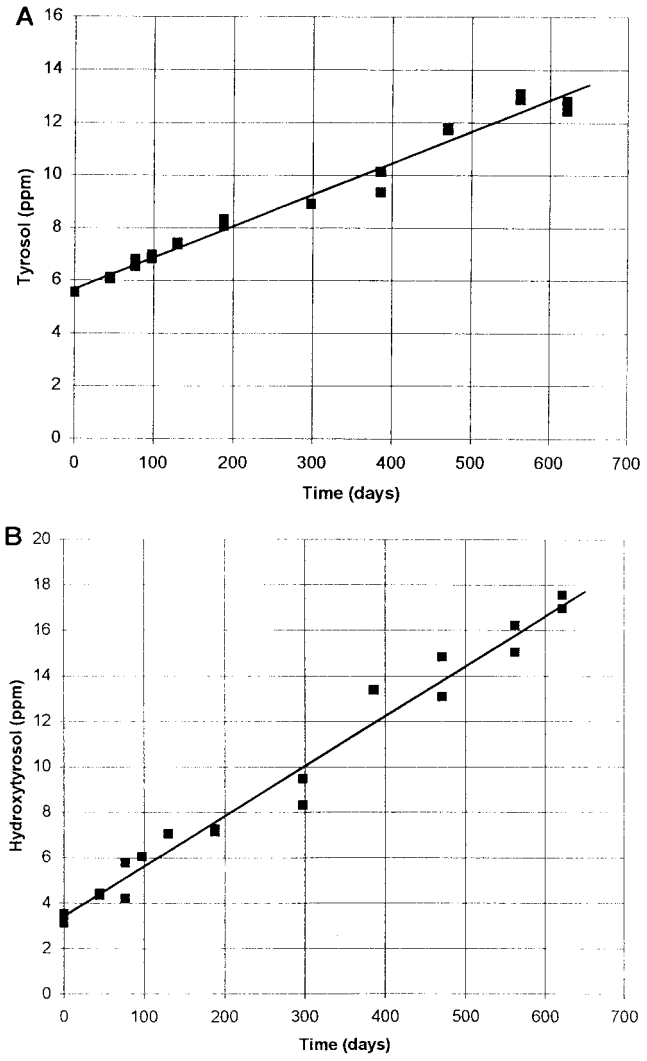

Figure 4. Kinetics of tyrosol (A) and hydroxytyrosol (B) formation in lot A in the dark at 20 °C: (■) experimental data; (−) calculated data.

EVOO oxidation resulted in a stability decrease. The Rancimat induction time decreased with time following pseudo-first-order kinetics

$$IT = IT_0 \exp(-k_{IT}t)$$
$$(r = 0.92;\ F \text{ ratio} = 157.31;\ P \text{ value} = 0.0001)$$

where IT is the Rancimat induction time (h) at storage time t. Its initial value was $IT_0 = 9.68$ h, which was similar to the experimental value (Table 1). Its apparent reaction rate constant was $k_{IT} = 5.0 \times 10^{-4}$ day^{-1}.

Figure 5 shows the kinetics of K_{232}, which followed two-step kinetics. During the first step it increased up to 2.1 after ∼200 days, due to the formation of peroxides (Gasparoli et al., 1990; Gasparoli and Fedeli, 1991), following pseudo-zero-order kinetics:

$$K_{232} = K_{232_0} + k_{K_{232}}t$$
$$(r = 0.95;\ F \text{ ratio} = 144.69;\ P \text{ value} = 0.0001)$$

K_{232} initial value was $K_{232_0} = 1.72$, which was similar to the experimental value (Table 1). Its apparent reaction rate constant was $k_{K_{232}} = 1.8 \times 10^{-3}$ K_{232}/days. During the second step K_{232} remained almost constant

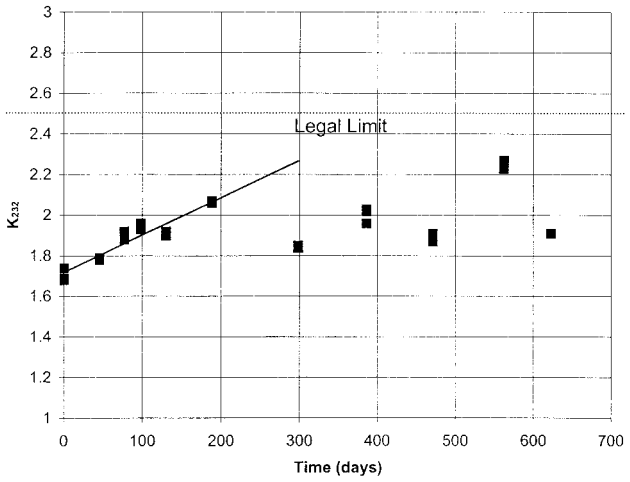

Figure 5. Kinetics of K_{232} increase in lot A in the dark at 20 °C: (■) experimental data; (−) calculated data.

and did not show EVOO legal nonconformity (i.e., $K_{232} > 2.5$) up to 622 days; peroxides decreased as a result of their degradation, due to the absence of oxygen in the headspace of bottles (Gasparoli et al., 1990; Gasparoli and Fedeli, 1991).

Use of Kinetic Models. Kinetic models were applied for the following two objectives: comparing stability of EVOO lots and setting up empirical models to predict stability.

EVOO lots B and C differed from lot A in terms of time bottling, travel stress, and storage conditions.

Lots B_1 and B_2 were bottled after approximately 2.5 and 6 months of storage in an industrial tank under nitrogen at 20 °C. They were shipped to Australia in ∼40 days at a maximum monitored temperature of 20−30 °C during summer and a minimum monitored temperature of 15 °C during winter. They were stored in a supermarket for >1 year at 25−30 °C during summer and 15−20 °C during winter.

Lots C_1 and C_2 were bottled after approximately 3 and 6 months of storage in an industrial tank. They were not subjected to travel stress because the factory and supermarket were quite close. The lots were stored in the supermarket for >1 year in a range of temperatures similar to that of the Australian lots but in opposite seasons.

The tyrosol and hydroxytyrosol variation kinetics was used to compare lot A with lots B and C, which also showed pseudo-zero-order kinetics (Roberti, 1997). Figure 6 shows a comparison between kinetics of lots A and B_2.

No statistically significant differences were found between the kinetics. The same results were also obtained for the other lots. For the EVOO batch studied, bottling, travel stress, and storage conditions had no significant effects on oil stability.

Empirical models were set up to predict the time to reach a given value for the legal parameter K_{232} as a function of the value for significant stability parameters. The maximum K_{232} value achieved by lot A (i.e., 2.1) was chosen as a reference value.

Table 3 shows linear correlation coefficients between the parameters. The correlations between tyrosol content, hydroxytyrosol content, Rancimat induction time,

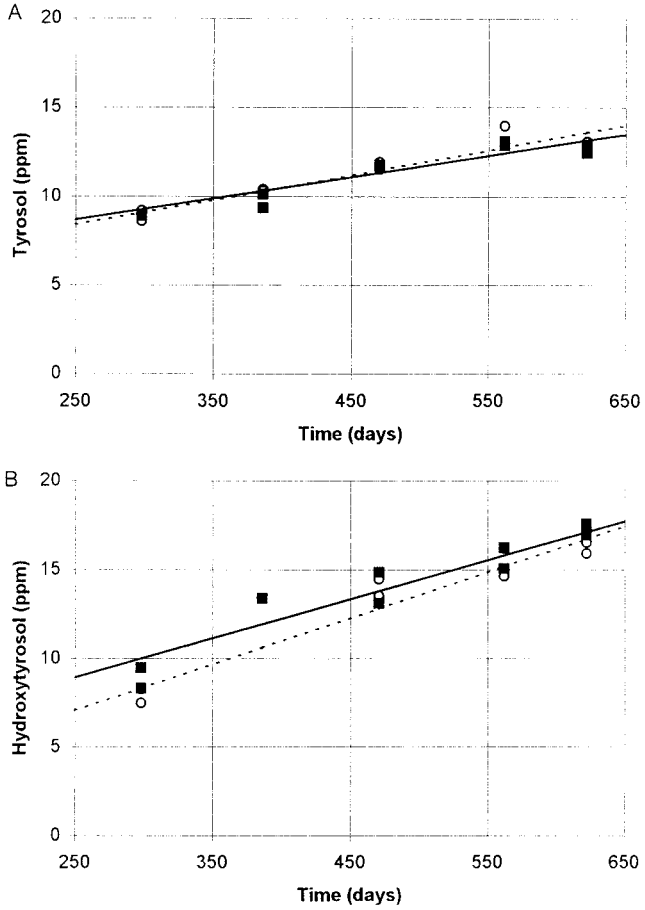

Figure 6. Comparison between kinetics of tyrosol (A) and hydroxytyrosol (B) variation in lots A [(■) experimental data; (−) calculated data] and B_2 [(O) experimental data; (- - -) calculated data].

Table 3. Linear Correlation Coefficients between the Significant Parameters on EVOO Stability and K_{232}

parameter	linear correl coeff (r)
Rancimat induction time	−0.90
carotenoid absorbance at 475 nm	0.09
α-tocopherol	−0.82
tyrosol	0.95
hydroxytyrosol	0.89

and K_{232} were statistically significant (P value < 0.01). The following empirical models were derived:

$$t = 1130.84 \ln(\mathrm{IT}) - 2388.13$$

$$t = 329.02 - 38.11(\mathrm{HT})$$

$$t = 580.34 - 68.11T$$

t is the storage time (days) to reach $K_{232} = 2.1$.

Empirical models underestimated experimental storage time by 20 days for Rancimat induction time, 10 days for hydroxytyrosol content, and 5 days for tyrosol content.

CONCLUSIONS

The main aim of this work on oil oxidation was to show a method to predict EVOO stability. The method was as follows:

1. Kinetics studies were carried out of usual oxidation and stability parameters on EVOO lots representative of different operating conditions (i.e., storage, transport conditions, etc.).

2. Significant parameters on stability of the lots from our Tuscan sample were selected by multivariate analysis procedures. A few parameters were shown to be significant: hydroxytyrosol and tyrosol contents, carotenoid absorbance at 475 and 448 nm, α-tocopherol content, Rancimat induction time, and K_{232}.

3. Simple kinetics models of the significant parameters were set up to compare the stabilities of the different lots. It was shown that EVOO stability in our oil samples was not significantly influenced by different uncontrolled bottling, transport, and storage conditions in a supermarket.

4. Empirical models were set up to predict stability by correlation between parameters. In this work empirical models were able to predict the time to reach a reference value for K_{232}.

This work was not intended to find results able to be extended to all EVOO types. The stability of EVOO depends on several variables: initial oxidation degree, type of cultivar, ripening degree, and processing conditions. To find such results, many analytical data for many raw materials and process conditions should obviously be available.

This work may have a practical application interest for industries because it shows a method to collect and process all analytical data on EVOO stability. For example, it may be useful for planning activities such as selection of new olive/oil suppliers and composition of a new blend. It may also be useful for control activities such as comparison between olive/oil suppliers, olive harvest year, and storage conditions. Results from this approach may be improved by applying stability studies to specific industrial EVOO typologies for several years.

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