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REGULAR ARTICLE

# Improved stability to auto-oxidation of the olive oil by addition of citric acid

**Khaoula Bekkar, Rabah Oumeddour*, Soraya Nigri, Radia Selaimia**

*Industrial Analysis Laboratory and Materials Engineering, University 8 May 1945, Guelma 24000, Algeria*

## ABSTRACT

The effect of addition of citric acid on the oxidation stability of olive oil produced in North-East of Algeria was assessed using UV- Visible spectrophotometry ($K_{232\ nm}$, $K_{270\ nm}$, carotenoids (470 nm), chlorophylls (670 nm)), free acidity, peroxide value, Fourier Transform Infrared spectroscopy (FTIR) and chemometric methods. Five olive oils (Chemlal variety) were collected from five geographical areas. Two preparations were made for each geographical area: the first one by adding a small amount of citric acid aqueous solution 1/1000 in volume and the second preparation without any additive. All analyses were carried out every 30 days during one year. A higher oxidative stability of olive oil samples was reached when using 1/1000 of citric acid in comparison with samples without any additive through the values of quality analytical indices. FTIR has been used to evaluate auto-oxidation of olive oils. The frequency regions of 3474-2679 $cm^{-1}$ and 1746-1032 $cm^{-1}$ were picked up for olive oil storage time quantification. A close relationship between actual and predicted storage time shows a good correlation with $R^2$ of 0.999, 0.989, 0.980, 0.984 and 0.983 for all samples. Thus, citric acid can enhance oxidative stability and improve the shelf life of olive oil.

**Keywords:** Auto-oxidation; Chemometric methods; Citric acid; Olive oil; Peroxide value

## INTRODUCTION

The nutritional qualities of virgin olive oil (VOO) are attributed to its differential composition with respect to other vegetable oils, which may be divided into two fractions: major and minor components (Ballus et al., 2015). Olive oil is mainly composed of glycerides (98-99 %) (Giuffrè, 2014a), and minor components such as sterols (Giuffrè and Louadj, 2013), waxes (Giuffrè, 2014b), sesquiterpene hydrocarbons, phenols and squalene. Some minor components are responsible of the flavor and aroma of the oil such as volatile compounds (aldehydes, alcohols, ketones and esters) which are influenced by the extraction conditions and the planting density of the olive trees (Giuffrè, 2014c). The human body does not produce fatty acids but rather takes them from the food it receives (Choi et al., 2014). The composition of some Algerian olive oil has previously been studied by Louadj and Giuffrè (2010) and the findings show that the oleic acid was present in its highest concentration; the values ranged between 61% and 67%. Compared to other vegetable oils as peanut oil (Giuffrè et al., 2016) and tomato seed oil (Giuffrè and Capocasale, 2016), olive oil has low levels of saturated (~16%) and high levels of monounsaturated (~70%) fatty acid.

Aroma is the result of the volatile compounds, whereas the phenolic compounds are associated with a set of elements. One: taste, two: anti-oxidant properties, three: anti-inflammatory and anti-microbial activities (Nigri et al., 2012; Gouvinhas et al., 2015). VOO's stability and shelf-life is determined by the both volatile and phenolic compounds (Luz Pizarro et al., 2013). Several illnesses such as cancer (Babbs, 1990), atherosclerosis (Covas, 2007), and cellular damage associated with aging are thought to be caused by the free radicals (Ashok and Ali, 1999); whereas the consumption of dietary antioxidants seems to play an important role in protecting the human body against these degenerative events (Silva et al., 2010). Because of their bioavailability importance and sensory contribution, the hydroxytyrosol and tyrosol have been the central concern of extensive studies (Mulinacci et al., 2013). The food samples' concentration of antioxidants can also be used as origin and freshness indicators. Many

***Corresponding author:**
Rabah Oumeddour, Industrial Analysis Laboratory and Materials Engineering, University 8 May 1945, B.P. 401, Guelma 24000, Algeria.
**E-mail:** oumeddour.rabah@univ-guelma.dz

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methods have been developed for the estimation of the antioxidant properties of natural products which can be classified into two main categories: those based on the evaluation of the radical scavenging activity (RSA) and those based on the evaluation of reducing natural samples activity (Christodouleas et al., 2015; Özcan and AL Juhaimi, 2015). Lipids that contain unsaturated fatty acids are prone to oxidation. The preservation of food quality is determined by their protection against oxidation rancidity by the use of antioxidants (Samaram et al., 2015). The postharvest and the storage effects promote a gradual lipid oxidation with a decrease in the shelf-life stability due to the continuous organic chemical processes set up by the initial peroxidations (Aparicio and Harwood, 2013). Oils oxidation is the result of different reactions conducing to the formation of the alkyl and the alkylperoxyl radicals in addition to the decomposition of hydroperoxide (Ontanón et al., 2013). The shelf- life of food products can be reduced by lipid oxidation as well as by the unfavorable reactions in edible oils. The antioxidants addition is effective in delaying the oxidation of lipids and lipid containing foods. A large number of investigations are devoted to study the oxidation stability and the anti-oxidative properties of different olive oil species (Tavakoli et al., 2013). A decline in phenolic compounds and green pigmentation occurs with the ripening, which results in a decrease in olive oil stability as the fruit ripens. Many catalytic systems can oxidize lipids. Among these are light, temperature, enzymes, metals, metallo-proteins, and microorganisms. Most of these reactions involve some type of free radical or oxygen species. The oxidation may be produced either in the dark (auto-oxidation) or in the presence of light (photo-oxidation), which differ in their oxidation pathway due to the action of external variables (Selaimia et al, 2017; Mishra et al, 2017; Aparicio and Harwood, 2013; Li et al., 2014).

VOOs are graded according to the sensory assessment and the three chemical parameters (free acidity, peroxide value and UV absorbance) (Velasco et al., 2014; Ciemniewska-Żytkiewicz et al., 2014; Consleg, 2015; IOC, 2015). The variations in the composition and quality of the olive as well as the corresponding olive oil along the ripening process have been studied by several researchers (Kesen et al., 2014). Free acidity generally increases due to the activity of lipolytic enzymes while the other basic quality parameters like peroxide value (PV) and ultraviolet spectrophotometric indices ($K_{232}$, $K_{270}$) either decrease, increase or remain constant for various cultivars in the literature (Kesen et al., 2014). With the formation of hydro-peroxides, conjugated dienes are simultaneously produced as a result of double bond displacements in polyunsaturated fatty acids. This leads to an absorption increase of 232 nm in oxidized fats and oils. These factors stimulate lipid per-oxidation (LPO) reactions. LPO refers to the oxidative degradation of lipids and the formation of lipid free radicals (Janakat et al., 2015). The European Commission Regulation, No 2568/1991 and subsequent amendments No 1830/2015, impose a maximum acidity of 0.8 g oleic acid/100 g oil and a maximum peroxide value of 20 meq $O_2$/kg oil, for extra virgin olive oil (Consleg, 2015).

Oils fortification with antioxidants has been adopted as an approach to address this issue (Alavi and Golmakani, 2017). Citric acid (CA), an important organic acid with a wide range of applications, was crystallized from lemon juice by Scheele (Scheele, 1784). It is a natural component of many citrus fruits. CA, a potent antioxidant among the hydroxycinnamic acids, is widespread in the plant kingdom. The use of CA may provide dual benefits through inhibiting lipid oxidation since it is a free radical scavenger; it may also increase the nutritional values of the final product (Kiliç et al., 2002).

FTIR spectroscopy is an excellent tool for the quantitative analysis since the intensities of spectral bands are proportional to concentration. In addition to other methods, FTIR is used to distinguish olive oils according to their different geographical regions (Janin et al., 2014). As it is described by several authors, the FTIR is also used to evaluate fatty acid composition (Inarejos-Carcía et al., 2013), oxidized fatty acids (Lerma-García et al., 2011), peroxide value (Bendini et al., 2007), acidity and sensory characteristics, phenolic and volatile compounds (Lerma-García et al., 2011).

FTIR–PLS is a tool to determine some analytical parameters (water content, phenolic content and antioxidant activity) in olive oils (Cerretani et al., 2010). This technique plays a very important role in the study of edible fats and oils, especially for the authentication study (Nigri and Oumeddour, 2013). The elaboration of the relationship between the concentration of analysts and the response of instrumental assay like FTIR spectra combined with fluorescence spectroscopy and using multivariate analysis permit to fight adulteration of olive oil (Nigri and Oumeddour, 2016).

The aim of the present study is to access the oxidative stabilities of different samples, of virgin olive oil of the same variety, harvested in the North-East of Algeria based on their structural changes in the constituents as monitored by changes in their FTIR spectra and physical chemical parameters. The analyses are regularly carried out every 30 days during one year through $K_{232}$, $K_{270}$, carotenoid and chlorophyll contents, free acidity and peroxide value, FTIR spectra for virgin olive oil samples and for those containing a small amount of citric acid in water 1/1000 as an antioxidant. It is known that the quality of olive oil is considered regarding values without any additive. Even

if the International Olive Council forbids the inclusion of additives in olive oil; the presence of a diluted solution (up to 1 % in volume) of citric acid, improves the stability of olive oil against the destructive effects of peroxides. FTIR spectroscopy combined with multivariate calibration was used for the auto-oxidation evaluation of virgin olive oil. PLS model correlates the actual and FTIR estimated values of storage time with a high coefficient of determination.

## MATERIALS AND METHODS

### Samples collection

Five olive oils, of Chemlal variety, were obtained from units of extraction located in five olive growing areas of different North-East Algerian locations (Tebessa, Souk Ahras, Bouati Mahmoud, Hamam N'bail, Bouchougouf) harvested in the fall of 2013/2014. These oils are classified according to their source and the process of extraction is a continuous system without heating. For each olive oil, two preparations are made; the first one with acid citric additive and the second one without any additive. The two preparations were prepared as follows:

Samples 1a, 1b, 1c, 1d and 1e: the preparations are made for testing the effect of the citric acid. The preparation is composed of 1 ml of aqueous solution of citric acid (10% in volume) which is added to 99% of each olive oil.

Samples 2a, 2b, 2c, 2d and 2e: with 100% of each olive oil; to determine the olive oil comportment without any additive.

### UV-Visible method

All values were obtained by the use of Shimadzu spectrophotometer UV-1800 working with a software program—UV probe version 2.4

### Determination of $K_{232}$ and $K_{270}$

The UV spectrophotometric indexes ($K_{232}$ and $K_{270}$) were determined according to the European Communities official methods. To calculate the $K_{232}$ and $K_{270}$ values, the oil samples were diluted in hexane (1:100 v/v), placed into a 1 cm quartz bowl, and analyzed at the wavelengths of 232 and 270 nm, against a blank of hexane. Hexane (UV–Vis Spectroscopy) was purchased from Sigma Aldrich Germany. Three replicates were prepared and analyzed for each sample.

### Determination of carotenoid, chlorophyll and pigments

Carotenoid and chlorophyll contents of the samples were determined using a spectrophotometric method (Minguez-Mosquera et al. 1991). The samples absorbance was recorded at 470 nm. and 670 nm. Then, calculations were performed using the formulas below: the amount of carotenoid was determined at 470 nm using the specific coefficient of lutein by equation (1)

$$C_1=(Abs\_470\times10^6)/(E_0\times100\times d) \quad (1)$$

Where:

$c_1$ represents the contents of carotenoid pigments expressed in mg/kg of lutein

d represents the thickness of the spectrophotometer cell (1 cm)

$E_0$ = 2000 for lutein as the major component of the carotenoid fraction

Three grams of olive oil were exactly weighed and dissolved in hexane up to a final volume of 10 ml. The amount of chlorophyll was evaluated from the absorption value of the olive oil solution at 670 nm and specific coefficient for pheophytin using equation (2)

$$C_2=(Abs\_670\times10^6)/(E_0\times100\times d) \quad (2)$$

Where:

$c_2$ represents the amount of chlorophyl expressed in mg/kg of pheophytin

E = 613 for pheophytin as the major component of the chlorophyll fraction

d represents the thickness of the spectrophotometer cell (1 cm)

### Chemical analyses

The determination of free acidity (given as % of oleic acid) and peroxide value (PV) ($meqO_2$/kg of oil) were carried out following the analytical methods described by the International Olive Council (IOC., 2015).

The determinations were made in triplicate following the analytical methods described in the EC Regulation (Consleg, 2015)

### FTIR spectra acquisition

A Perkin-Elmer Spectrum, one FTIR spectrophotometer equipped with a deuterated triglycerine sulphate (DTGS) detector, was used to collect FTIR spectra. This method is mechanized by a data acquisition spectrum software which permits the saving of the spectra in the infrared mean of the used combination function source/separatist/detector. The data interval is provided by the instrument for a resolution of 4 $cm^{-1}$ at 20 scans.

A small quantity (2µL) of the sample was deposited by using Pasteur pipette creating a thin film between two well-polished KBr disks. Duplicate spectra were collected for each sample. All spectra were recorded from 4000 to 450 $cm^{-1}$ and processed with the computer software program Spectrum for Windows (Perkin-Elmer).

### Chemometric analysis

The chemometric analysis, including quantification using partial least square regression PLS, was carried out using the MINITAB 16 2010 software. The optimum number of PLS factors was determined using cross validation by plotting the number of factors against the root mean square error of cross validation (RMSECV) and determining the minimum factors.

## RESULTS AND DISCUSSION

To determine the effect of storage time in the dark at room temperature on the olive oil quality, the results of each parameter were compared to the values obtained for the samples analyzed immediately after an extraction (controls: t=0 month).

The quality of the selected olive oil was evaluated by spectroscopic indices $K_{232}$, $K_{270}$; free acidity and peroxide values and also by spectroscopic data of FTIR.

### Changes in $K_{232}$, $K_{270}$ parameters

Ultraviolet absorption, a more delicate indicator of oxidation, is related to the presence of conjugate diene and triene systems (ultraviolet absorbance at 232 and 270 nm. respectively).

$K_{232}$ is a measure of the primary oxidations products, the formation of hydroperoxide and conjugated dienes. $K_{270}$ is associated to the secondary phase of oxidation because it is related to the final products presence such as trienes or unsaturated carbonyl compounds; which is characteristic of an oxidized oil.

The maximum permitted values of $K_{232}$ and $K_{270}$ for extra virgin olive oils are 2.50 and 0.20 respectively (Consleg, 2015; IOC, 2015).

The initial values of the $K_{232}$ coefficient are between 0.272 and 1.863. Whereas the initial values of the $K_{270}$ are between 0.104 and 0.182. These values are within the limits permitted by the legislation. In Table 1 after 6 months of storage it can be seen that primary and secondary oxidations increase in all the samples. For the olive oil with citric acid the $K_{232}$ exceeded the limit after 6 months whereas it exceeded the limit before 6 months for olive oil without any additive. Also, the $K_{270}$ were within the limit before 12 months of storage for the olive oil with citric acid whereas it is within limit in a period less than 6 months of storage for the olive oil without any additive.

The observed efficiency acted upon the olive oil can be due to the progressive release of antioxidant activity compounds from citric acid into the olive oil.

### Variation of carotenoids and chlorophylls

The presence of carotenoids and chlorophylls in olive oil is essential in the oxidative stability because of two main properties: the antioxidant nature in the dark and prooxidant activity in the light. They are for the most part responsible for the color of virgin olive oil from the yellow-green to greenish golg (Criado et al., 2008).

Changes in the amounts of carotenoids and chlorophylls in the extra virgin olive oil samples during storage for 12 months in the dark at room temperature are shown in Table 2.

During 12 months of storage; the values of the carotenoids and chlorophylls concentrations ranged from 0.250 mg/kg to 0.010 mg/kg for carotenoids and between 0.636 mg/kg to 0.016 mg/kg for chlorophylls. Carotenoids and chlorphylls levels of extra olive oil declined during storage.

Carotenoids are present in olive oils and are responsible for its yellow coloration. Chlorophylls are present too in

**Table 1: Evolution of the $K_{232}$, $K_{270}$ in the studied samples during storage time**

| Samples | Storage time (month) | (a) Tebessa | (b) Souk Ahras | (c) Bouati Mahmoud | (d) Hammam N'bail | (e) Bouchegouf |
|---|---|---|---|---|---|---|
| | | | $K_{232}$ | | | |
| | 0 | 0.475±0.014 | 0.647±0.019 | 0.272±0.008 | 1.863±0.060 | 0.446±0.013 |
| 1 | 6 | 1.791±0.054 | 2.012±0.060 | 1.480±0.044 | 2.311±0.069 | 2.008±0.060 |
| | 12 | 3.366±0.100 | 3.385±0.101 | 3.398±0.102 | 3.282±0.098 | 3.398±0.102 |
| 2 | 6 | 1.925±0.057 | 2.345±0.070 | 1.488±0.044 | 2.331±0.069 | 2.312±0.069 |
| | 12 | 3.452±0.103 | 3.486±0.104 | 3.481±0.104 | 3.486±0.104 | 3.484±0.104 |
| | | | $K_{270}$ | | | |
| | 0 | 0.104±0.003 | 0.182±0.005 | 0.153±0.004 | 0.107±0.003 | 0.109±0.003 |
| 1 | 6 | 0.256±0.008 | 0.226±0.006 | 0.281±0.008 | 0.275±0.008 | 0.257±0.007 |
| | 12 | 0.300±0.009 | 0.446±0.013 | 0.510±0.015 | 0.576±0.017 | 0.585±0.015 |
| 2 | 6 | 0.270±0.008 | 0.280±0.008 | 0.282±0.008 | 0.275±0.008 | 0.275±0.008 |
| | 12 | 0.330±0.009 | 0.613±0.018 | 0.867±0.026 | 0.646±0.019 | 0.699±0.21 |

1: Olive oil with citric acid; 2: Olive oil without any additive Mean±SD (n=3); in each column and for each part

**Table 2: Evolution of the carotenoids and chlorophylls in the samples studied during storage time**

| Samples | Storage time (month) | (a) Tebessa | (b) Souk Ahras | (c) Bouati Mahmoud | (d) Hammam N'bail | (e) Bouchegouf |
|---|---|---|---|---|---|---|
| | | | **Carotenoids** | | | |
| | 0 | 0.165±0.005 | 0.150±0.004 | 0.150±0.004 | 0.250±0.007 | 0.165±0.005 |
| 1 | 6 | 0.125±0.003 | 0.125±0.003 | 0.105±0.003 | 0.200±0.006 | 0.100±0.003 |
| | 12 | 0.055±0.001 | 0.010±0 | 0.060±0.002 | 0.060±0.002 | 0.020±0 |
| 2 | 6 | 0.135±0.004 | 0.130±0.004 | 0.105±0.003 | 0.150±0.004 | 0.100±0.003 |
| | 12 | 0.065±0.002 | 0.015±0 | 0.050±0.001 | 0.045±0.001 | 0.050±0.001 |
| | | | **Chlorophylls** | | | |
| | 0 | 0.277±0.008 | 0.326±0.009 | 0.620±0.018 | 0.636±0.020 | 0.554±0.016 |
| 1 | 6 | 0.228±0.006 | 0.103±0.003 | 0.461±0.013 | 0.571±0.020 | 0.278±0.008 |
| | 12 | 0.049±0.001 | 0.081±0.002 | 0.163±0.005 | 0.160±0.005 | 0.016±0 |
| 2 | 6 | 0.261±0.007 | 0.161±0.005 | 0.457±0.014 | 0.571±0.017 | 0.251±0.007 |
| | 12 | 0.016±0 | 0.049±0.001 | 0.114±0.003 | 0.196±0.006 | 0.016±0 |

1: Olive oil with citric acid; 2: Olive oil without any additive Mean±SD (n=3); in each column and for each part

olive oils and are the responsible for its greenish coloration. Those pigments are also important in olive oil stability.

### Free acidity (FA)

Virgin olive oil contains about 98% neutral lipids, mainly triglycerides (96–97%) followed by small quantity of diglycerides (1–2%) and a variable quantity of free acidity which are used as a marker of oil quality (Olias and Garcia, 1997).

The quantity of free acidity measured as acidity (% oleic acid) is very important to determine the olive oil's quality. This value allows a classification for the olive oils. All olive oils were extra virgin at the starting time of this experience. Normally, the free acidity value remained relatively stable during storage in the dark at room temperature. In this work, no significant differences were observed between the five samples initially used, but a great evolution was observed after one year. Olive oils harvested in Tebessa, Souk Ahras remained extra virgin but olive oils collected from Bouati Mahmoud, Hammam N'bails and Bouchegouf became virgin. Fig. 1 illustrates this evolution.

### Peroxide value (PV)

Hydroperoxides were measured to determine the oxidation initial rate because they are generally accepted as the first products formed by oxidation (Hamilton and Rossell, 1986). Hydroperoxide formation in a crude olive oil can serve as an indicator of both; the oxidative processes and the oil's quality.

Thus, a rapid hydroperoxide formation demonstrates the initiation of the oxidative reactions that precede rancidity.

The peroxide value, being a crude indicator of the amount of primary oxidation product, is a measure of the active oxygen content. It is expressed as meq $O_2$/kg oil.

The effect of storage conditions on the formation of primary oxidation products, expressed as PV, versus time of storage is shown in Fig. 2.

The changes in the peroxide value during storage period, up to twelve months, may be due to vicinity of the double bound that is attached by oxygen and variation in proportion of unsaturated bonds of triglycerides that are more prone to auto oxidation.

The final peroxide values PVs of all the samples were more than their initial values after every month of storage in the dark at room temperature. It is clear that the olive oil with the citric acid gave the lowest PV. For the first month, the rise rate in the PV is approximately the same for the olive oil samples with and without additive. After three, six and twelve months of storage, the addition's effect of the citric acid is very remarkable. For example in Fig. 2a, the percentages of increasing of PVs are respectively 15.45, 32.58 and 102.25% for the olive oil with the citric acid and 19.10, 34.69, and 107.87% for the olive oil without any additive. For Fig. 2e, the percentages of increasing PVs values are respectively 15.09, 31.58 and 53.94 for the olive oil with the citric acid and 17.79, 43.75 and 74.96% for the olive oil without any additive. The evolution during twelve months of storage of the peroxide values shows significantly higher values in all samples of olive oil without any additive. Then, the introduction of a tiny quantity 1/1000 of citric acid contributes to slowing down the phenomenon of ageing through peroxide braking in the medium.

### Chemometric analysis

#### *Partial Least Squares (PLS) model*

The analysis of the variations in FTIR spectra is not very easy because these changes are very weak. PLS is one of the most useful methods for the study of the oxidation of olive oil.

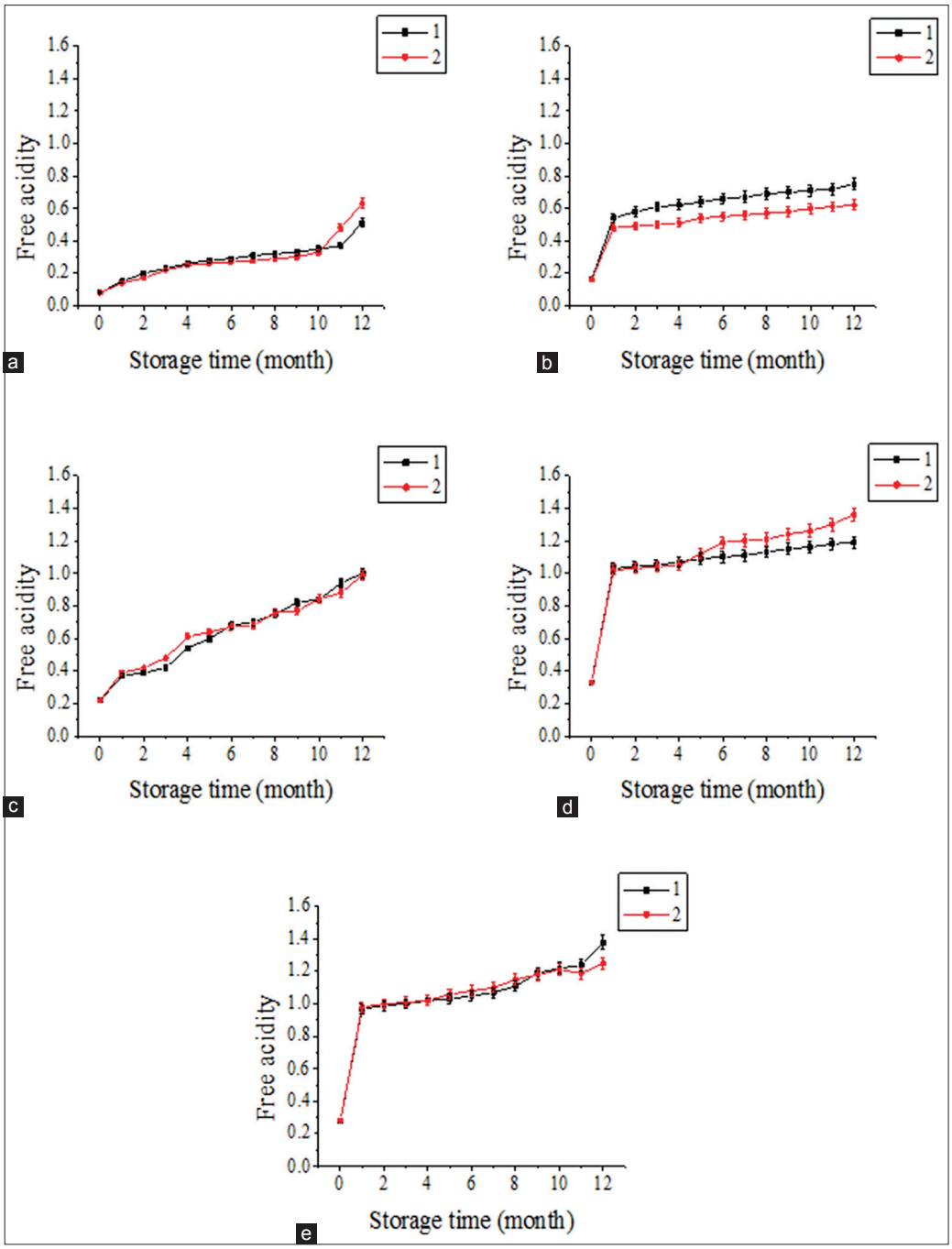


**Fig 1.** Variation in free acidity (%) during storage time of olive oil produced in (a) Tebessa, (b) Souk Ahras, (c) Bouati Mahmoud, (d) Hammam N'bail, (e) Bouchegouf.
Olive oil with citric acid; Olive oil without any additive

In the PLS calibration models, the evaluation of the method linearity was carried out in order to show a proportional relationship between responses versus storage time of olive oil. The frequency regions of 3474-2679 $cm^{-1}$ and 1746-1032 $cm^{-1}$ were picked up for olive oil's storage time quantification.

The appropriate number of PLS-factors is determined by application of Haaland and Thomas (1988) criterion based on the minimal stable predicted residual sum of squares PRESS. The performance of the model was evaluated by the coefficient of determination $R^2$ and the root mean square error of cross validation RMSECV.

In order to validate the developed model, cross validation using leave one out technique was used. Using PLS, the excellent model is obtained for BM sample; the $R^2$ value obtained is 0.985 (in calibration) and 0.969 (in prediction) with lowest RMECV (0.804). Three latent factors were

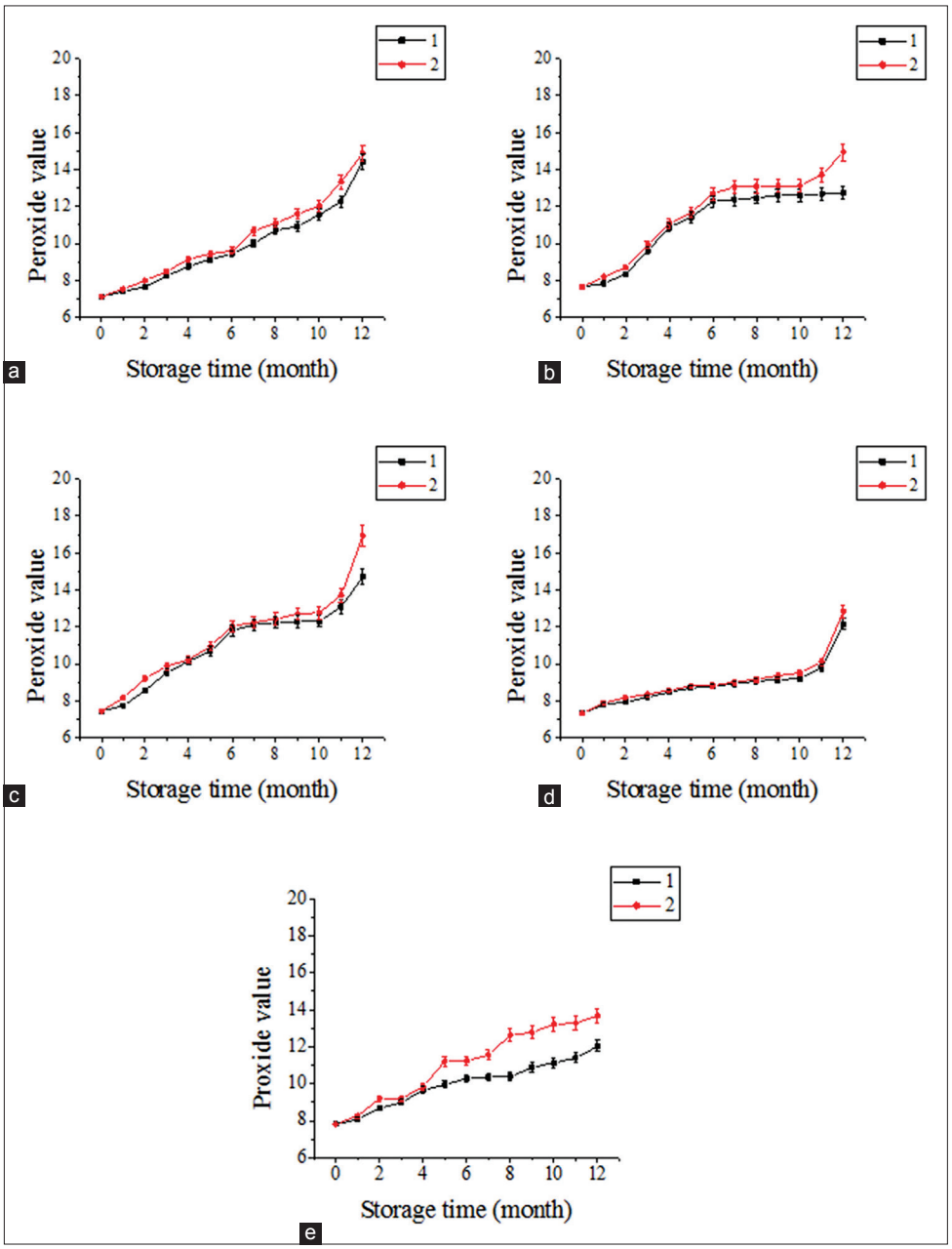


**Fig 2.** Variation in peroxide value (meq O2 /kg) during storage time of olive oil produced in (a) Tebessa, (b) Souk Ahras, (c) Bouati Mahmoud, (d) Hammam N'bail, (e) Bouchegouf.
Olive oil with citric acid; Olive oil without any additive

selected for building PLS models. PLS offers a better calibration model for T than SA. Inversely, it gives better prediction model for SA. The high value of RMECV (1.558) was obtained for B.

The chemometric analysis demonstrates that the main wavelength regions selected to construct the PLS model of T and BM samples are corresponded to: first, peroxide value and second, acidity and symmetric stretching vibration of the aliphatic $CH_2$ group at 2925 $cm^{-1}$. For SA sample model corresponded to peroxide value, acidity and -C=O (ester) at 3474 $cm^{-1}$. The model of HM sample corresponded to: first peroxide values, second at 1656 $cm^{-1}$ indicate the presence of grouping C=C. Third, bending vibration of the $CH_2$ and $CH_3$ aliphatic groups. Finally =C-H (cis) at 1402 $cm^{-1}$.

Figure 3 exhibits the scatter plot for the relationship between actual and predicted storage time for all samples. It indicates a close relationship between two variables assessed using FTIR-PLS. The relationship between actual and predicted storage time show a good correlation with $R^2$ of 0.999, 0.989, 0.980, 0.984 and 0.983 for all samples respectively.

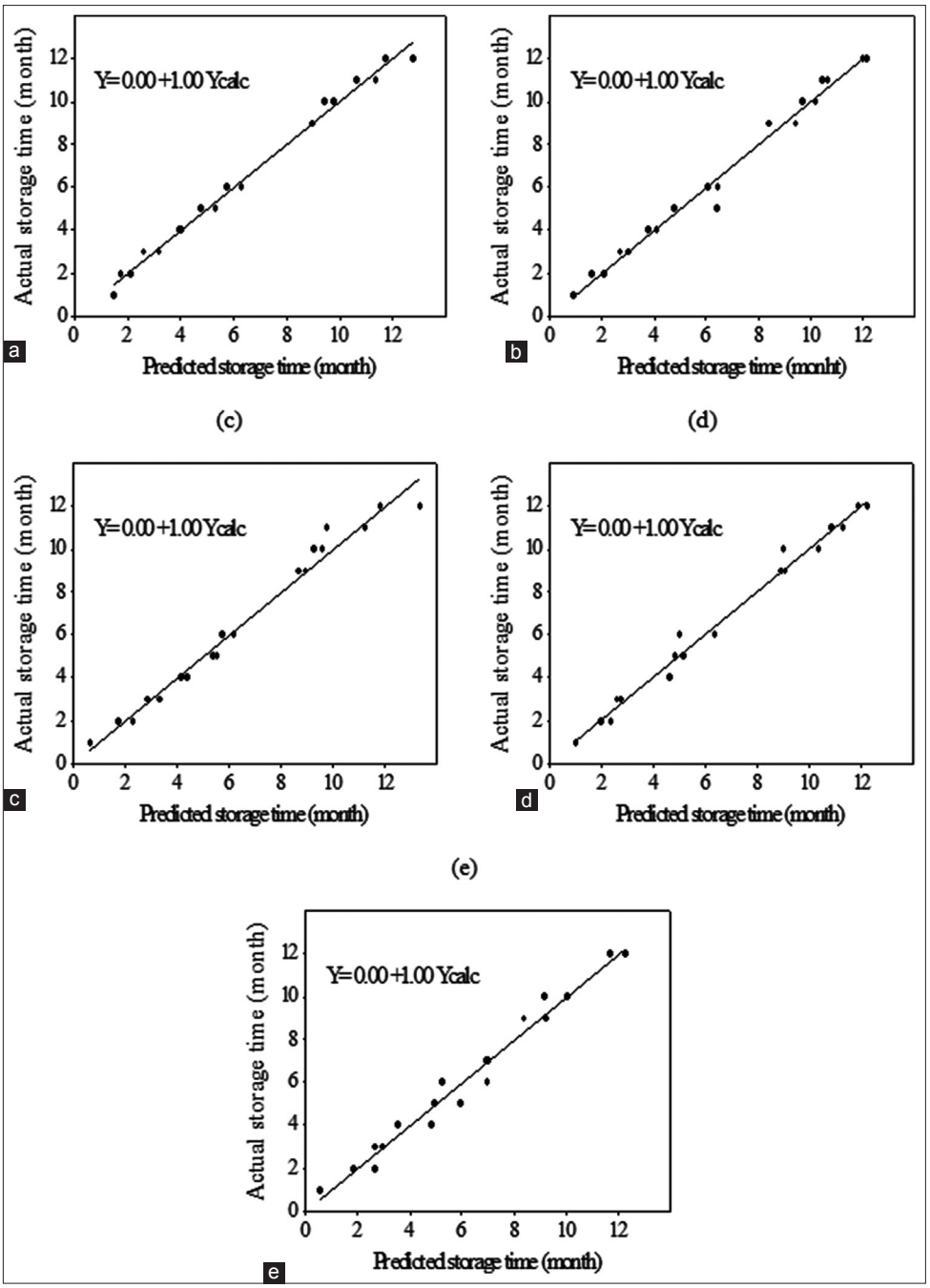


**Fig 3.** Correlation plot of the actual versus the predicted storage time of olive oil produced in (a) Tebessa, (b) Souk Ahras, (c) Bouati Mahmoud, (d) Hammam N'bail, (e) Bouchegouf.

## CONCLUSION

To determine the effect of storage time of olive oil, in dark at room temperature, the results were compared to the values obtained for the samples analyzed immediately after extraction. The results were taken each month up to twelve months.

The quality of the selected olive oil was evaluated by spectrophotometric indices $K_{232}$, $K_{270}$; free acidity and peroxide values and also by spectroscopic data of FTIR.

The initial values of the $K_{232}$ coefficient were between 0.272 and 1.863. Whereas the initial values of the $K_{270}$ were between 0.104 and 0.182. On above we observe significant changes in the concentrations of carotenoids at 470 nm. and chlorophylls at 670 nm.

Olive oil with the citric acid indicates, after one month that the effect is not significant, but after two months of storage, the addition`s effects is very marked; the average of percentage of increase in the PV is respectively 4.71%

and 76.13 % for all the olive oils with and without any additive. The evolution during twelve months of storage of the peroxide values shows significantly higher values in all samples of olive oil without any additive.

FTIR–PLS is a tool to determine some analytical parameters in olive oils. The frequency regions of 3474-2679 $cm^{-1}$ and 1746-1032 $cm^{-1}$ were picked up for olive oil's storage time quantification. The relationship between actual and predicted storage time shows a good correlation with $R^2$ of 0.999, 0.989, 0.980, 0.984 and 0.983 for all samples respectively.

It is known that quality of olive oil is measured for samples without any additive; but the introduction of a tiny quantity 1/1000 of citric acid contributes to slow down the phenomenon of ageing through peroxide braking in the medium and enhance the stability of olive oils against auto-oxidation. We strongly recommend tolerating such additions in the same way as others like aromatic plants and garlic or spiruline which are likely to contribute more and consequently improve the quality of olive oil. Then, the consumers have to consider this argument to choose the appropriate olive oil.

### Authors' contributions

K. Bekkar conducted the experimental work and literature research, analyzed the results and drafted the manuscript. R. Oumeddour supervised the research, interpreted the spectroscopic data, discussed all the results and revised the manuscript. S. Nigri discussed the chemometric methods, corrected the manuscript. R. Selaimia contributed in the correction of the manuscript.

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