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Quality Evolution of Extra Virgin Olive Oils of 'Chemlal' Cultivar during Storage at Environment Temperature: Effect of the Altitude and Age of Olive Trees

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Abstract: This work aimed to study the influence of two factors on oil composition: the altitude of olive groves and the age of olive trees, as well as the duration olive oil was stored in the dark and at room temperature. Firstly, quality parameters (free acidity, peroxide value, K_{232} and K_{270}), minor compounds (pigments, total phenolic compounds, tocopherol fraction and fatty acid profile) and oxidative stability measured at 98.0 °C (by Rancimat) made it possible to evaluate the quality of the samples during one year of storage. A significant difference was reported in pigment contents as a function of altitude. In particular, several changes were observed during storage, which led on the one hand to a continuous increase in free acidity, peroxide value, K_{232}, K_{270} and on the other hand a degradation of natural antioxidants such as phenolic compounds, pigments and tocopherols and consequently to oxidation stability. However, the modification of the fatty acid composition was small and did not show any major changes.

Keywords: extra virgin olive oils; Kabylia; phenolic compounds; fatty acids; storage; oxidative stability; tocopherols

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1. Introduction

Algeria is a Mediterranean country with an olive-growing reputation since antiquity, where the olive tree is of major socio-economic importance. Currently, in Algeria there are two typologies of olive groves: traditional and intensive. The traditional typology, concentrated in the north, is practised in an extensive way with archaic means and intended for family consumption and the local market. On the other hand, the modern typology is practised through the intensive exploitation of new lands and climates, namely in the high plateaus and the south of Algeria, characterised by a semi-arid or even arid climate; this production, often respecting the new practices of high-quality olive oil production, is intended for local and especially international markets. In an approach aiming for the promotion of the local product, especially to evaluate its potential and possibly improve it, the predominant variety 'Chemlal' was chosen for this study. This study, a pioneer in Algeria, consists of a comparison of the quality of extra virgin olive oils in three different regions and follows its qualitative evolution during twelve months of storage at ambient temperature conditions. This study was based on the monitoring of the evolution of quality parameters (free acidity, peroxide value, K_{232} and K_{270}) as well as pigment contents, fatty acid composition, total phenolic compounds, tocopherol fraction and oxidation stability.

The storage of olive oils can be influenced by various factors such as chemical composition including fatty acid profile, the content of minor compounds [1] and the availability

of oxygen in the head of the bottle [2]. Light and storage temperature can accelerate the oxidation phenomenon through the action of photo-sensitizers such as chlorophylls [2–6]. In addition, the packaging material is not only a marketing tool but also prevents the transmission of light, oxygen and interactions with metals that can occur in some materials. Several studies have been devoted to this effect [2,7] proving that glass remains the best packaging to preserve the physicochemical and sensory quality and that terracotta packaging should be avoided.

Generally, it is accepted that opaque glass and tin are the appropriate packaging for the storage of olive oil [8].

Another real factor influencing possible content-content interactions or changes in physicochemical composition [2,5,8–11] is the extraction technology employed [12]. The oxidation of olive oils is a process that can be accentuated during extraction or storage; it is distinguished by auto- or photo-oxidation. Moreover, it can be affected by physicochemical factors such as high temperature, exposure to light or contact with metals [13]. The most important deterioration factor of extra virgin olive oils is oxidation. Highly unstable primary products such as hydroperoxides decompose or interact with other molecules present in the oil and lead to secondary products derived from oxidised fatty acids (OFAs), oxidised polymers and volatile compounds [13]. This mechanism is enhanced at high temperatures [14] and slowed down at low temperatures [15].

The aim of this work was to study the influence of two factors: the altitude of the olive groves and age of olive trees and, in relation to olive oils produced, the length of time olive oil is stored in the dark and at room temperature. This study was focused on three regions of Algeria and on the variety 'Chemlal'.

2. Materials and Methods

2.1. Study Area, Variety and Age of Olive Trees

This study examined extra virgin olive oil (EVOO) of the 'Chemlal' variety of the olive-growing region. The olive oils were obtained from young (5–15 years) and old (centenarians) olive trees, in three localities of Kabylia (Algeria), namely Beni Yenni (wilaya of Tizi Ouzou at an altitude of 820 m), Mekla (wilaya of Tizi-Ouzou at an altitude of 566 m) and Taourga (wilaya of Boumerdes at an altitude of 340 m) (Figure 1). In winter, the average temperature range in Beni Yenni was 2–11 °C, in Taourga 4–13 °C and in Mekla 5–14 °C.

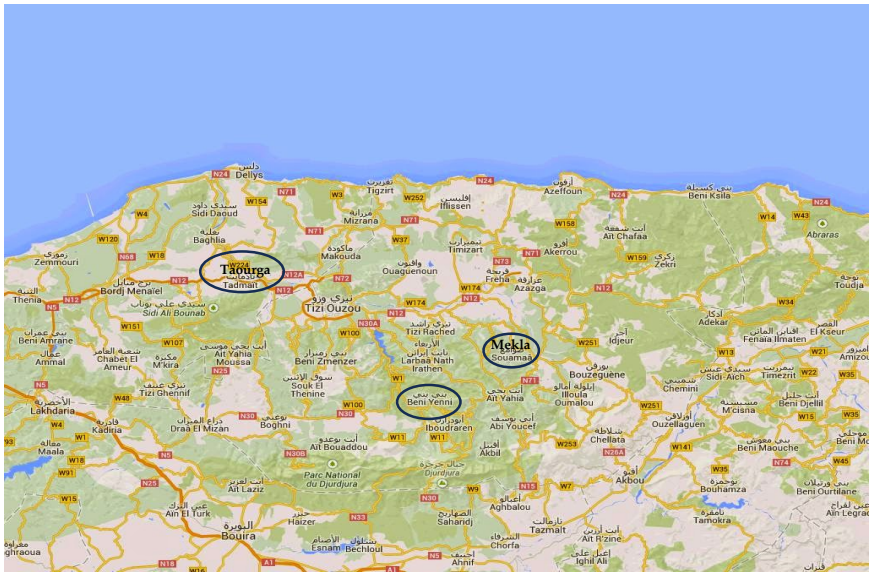


Figure 1. Map of different regions of this study in Kabylia (Algeria).

Sample collection in the three regions took place in the period between 30 November and 15 October.

A quantity of 9 kg of healthy, undamaged olives were harvested and extracted within 24 h at the Technical Institute of Fruit and Vine Growing in Bejaia (ITAFV), using a 2-outlet decanter ('oleodoser') without adding water, in discontinuous operation. The process consisted of crushing by hammer mill, a malaxation stage of 30 min and separation by centrifuge for 1 min at 3500 rpm.

The extracted oil was left to settle naturally for 6 h, after which it was filtered using a paper filter with a porosity of 60 µm. Part of the samples obtained were stored in opaque glass bottles at a temperature not exceeding 4 °C until the beginning of the initial analyses, while another part was stored at room temperature for routine analyses at a frequency of three months (3, 6, 9 and 12).

2.2. Maturity Index (MI)

This parameter was determined using the method of Uceda and Frias [16,17]. A total of 100 healthy, undamaged olives were randomly selected for each sample and classified into the following categories: 0 for olives with intense green or dark green skin, 1 for olives with yellow or yellowish green skin, 2 for olives with yellowish skin but with reddish spots on less than half of the fruit, 3 for olives with reddish or light purple skin on more than half of the fruit, 4 for olives with black skin and totally white pulp, 5 for olives with black skin and violet pulp (less than 50%), 6 for olives with black skin and violet or purple pulp (more than 50%), 7 for olives with black skin and totally dark pulp. The maturity index was calculated using Equation (1).

$$M.I. = (a \times 0 + b \times 1 + c \times 2 + d \times 3 + e \times 4 + f \times 5 + g \times 6 + h \times 7)/100 \quad (1)$$

where '*a*' to '*h*' is the number of fruits in each category.

The samples harvested in each region had the following maturity index: Beni Yenni (Young) 2.93, (Old) 3.01; Taourga (Young) 2.94, (Old) 3.0; Mekla (Young) 2.90, (Old) 2.99.

2.3. Reagents

The characteristics of the reagents/products used in this research were the following: Ethanol absolute, diethyl ether and methanol from Honeywell (Offenbach, Germany) with purity (p) $\geq$ 99.8%; chloroform ($p \geq$ 99.5%) and cyclohexane ($p \geq$ 99.8%) from Sigma Aldrich (Steinheim, Germany); acetic acid glacial with purity $\geq$ 99.7% from J.T. Baker (Phillipsburg, NJ, USA); n-hexane ($p \geq$ 97%), n-heptane ($p \geq$ 99.9%) and 2-propanol ($p \geq$ 99.9%) from Sigma Aldrich (Steinheim, Germany); Folin and Ciocalteu's phenol reagent from Scharlab (Barcelona, Spain); gallic acid ($p \geq$ 98.0%), sodium bicarbonate ($p \geq$ 99.7%) and sodium hydroxide ($p \geq$ 98%) from Sigma Aldrich (Steinheim, Germany).

2.4. Quality Parameters

Different physicochemical determinations were carried out to monitor the quality evolution of the olive oil samples: free acidity expressed as % oleic acid, peroxide value expressed as mEq of active O_2 per kg of olive oil and UV absorption (in parameters K_{232} and K_{270}) by a UV spectrophotometer (Shimadzu, mod. UV1280, Kyoto, Japan). All the analyses were carried out in triplicate following the analytical methods described by the International Olive Council [18].

2.5. Pigment Contents

A quantity of 7.5 g of olive oil in each sample was dissolved in 25 cm^3 of cyclohexane and then measured with a UV spectrophotometer at a wavelength of 670 nm for chlorophylls and 470 nm for carotenoids to determine the pigment contents [19]. Pigment contents were calculated according to Equations (2) and (3).

$$Chlorophylls = \frac{(A_{670} \times 10^6)}{613 \times 100 \times d} \quad (2)$$

$$Carotenoids = \frac{(A_{470} \times 10^6)}{2000 \times 100 \times d} \quad (3)$$

where 'd' is the width of the cuvette (1 cm).

2.6. Oxidative Stability Determination

This parameter, expressed by the induction time in hours, was measured with the Rancimat 679 apparatus (Metrohm Ltd., Herisau, Switzerland). A quantity of 3 g of olive oil was heated to 98.0 °C ± 1.6, at an air flow rate of 10 dm^3/h. The results obtained referring to the oxidative stability were expressed as induction time in hours [20].

2.7. Total Phenolic Compounds

The contents in total phenolic compounds were determined according to the method adopted by Capannesi et al. [21]. A sample of extra virgin olive oil was weighed (2.5 g) and extracted with 2.5 cm^3 of n-hexane after 2.5 cm^3 of MeOH/H_2O (80/20). The mixture was separated using a centrifuge (Hettich Rotofix, 32A, Gforce 4 165, Tuttlingen, Germany) at 5000 rpm for 5 min. A quantity of 2.5 cm^3 of Folin–Ciocalteu reagent and 5 cm^3 of sodium bicarbonate (7.5%, w/v) were completed with distilled water to obtain a final volume of 50 cm^3. The mixture was incubated for 2 h in dark at room temperature.

The absorbance of the mixture was measured at 765 nm in a UV spectrophotometer (Shimadzu, mod. UV1280, Japan). Previously, a calibration line was established between gallic acid concentration (expressed in mg/dm^3) and absorbance measured at 765 nm. The gallic acid concentration interval ranged from 0 to 100 mg/dm^3. Results were expressed in mg gallic acid/kg oil.

2.8. Composition in Fatty Acids

This composition was determined by gas chromatography. The FAMEs were prepared by vigorously stirring the solution of oil in n-heptane (0.1 g in 2 cm^3) with 0.2 cm^3 of a 2 N methanolic potassium hydroxide solution. The analysis of the FAMEs was carried out with an auto system gas chromatograph, equipped with an FID detector (HP 6890 N, Agilent Technologies, Santa Clara, CA, USA). The column used was an Agilent CP-Sil88 capillary (length 50 m, id 0.25 mm and film thickness 0.20 µm), and the analysis conditions were as follows: the column temperature was first programmed at 165 °C for 25 min and then at a gradient of 5 °C/min to 195 °C; the temperature of the injector and detector was set at 250 °C; helium was the carrier gas, with a flow rate through the column of 1 cm^3/min and a division ratio of 1:100; and the injection volume was 1 mm^3 [18]. The Cis FAMEs were identified by a comparison of their retention times against pure standards analysed under the same conditions. They were quantified according to their percentage surface area, obtained by peak integration. The results are expressed as percentages of individual fatty acids in the lipid fraction. The fatty acids considered in this study are as follows: myristic (C 14:0), palmitic (C 16:0), palmitoleic (C 16:1), margaric (C 17:0), margaroleic (C 17:1), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), linolenic (C 18:3), arachidic (C 20:0) and gondoic (C 20:1).

2.9. Tocopherols

Sample preparation and calibration were performed according to the updated IUPAC method 2.432 as ISO 9936:2016. Virgin olive oil samples (0.1 g) were dissolved in n-hexane (10 cm^3).

This solution was analysed by Shimadzu HPLC equipment, using a Silica column (25 cm × 4 mm, 5 µm) and using a fluorescence detector (RF 535), set at 290 nm (Ex) and 330 nm (Em). The mobile phase was n-hexane/Isopropanol (99:1, v/v) at a flow rate of 1 cm^3/min. The run time was 15 min. The sample injection volume was 20 mm^3.

Tocopherol concentrations (α, β, γ) were calculated from the integral areas of the samples and the corresponding calibration curves of external standards (Sigma-Aldrich, St. Louis, MO, USA). The samples were analysed at least by duplicate.

2.10. Statistical Analysis

This study was conducted with three replicates for each analysis. Results are expressed as mean ± standard deviation. The analysis of variance (ANOVA one-way) with 5% was applied to the factors: region and storage time, the Tukey test and the Pearson test. All these analyses were performed with the programmes Statistica v.12 and Minitab v.19 (Algiers, Algeria).

3. Results and Discussions

3.1. Quality Parameters in the Initial State and Evolution during Storage

Table 1 summarises the results of analyses of the physicochemical parameters of olive oils obtained. The low values are in line with the limit values recommended by the International Olive Oil Council (acidity $\leq$ 0.8%, peroxide value $\leq$ 20 mEq O_2/kg oil, $K_{270} \leq 0.22$, $K_{232} \leq 2.5$). The oils analysed are classified as extra virgin olive oil. These values may be explained by the conditions that are met during the technical process of elaboration of the samples from the harvest (where only the healthy olives harvested from the tree are selected for extraction), through the malaxation (the time did not exceed 45 min at T = 27 °C), up to the conservation of the samples (carried out at a temperature not exceeding 4 °C) before proceeding to the initial quality analyses. Nevertheless, the analysis of variance showed the K_{270} parameter had a *p*-value = 0.0004 for the region factor and no explanation could be found apart from the impact of a number of pre- and post-extraction factors [15]. At the end of the extraction process or at the beginning of the storing of oils, it is observed that K_{270} and K_{232} values are lower in higher altitude regions. In general, the oils from old trees present slightly higher values of UV parameters.

At the beginning of storage, the olive oil samples studied showed low acidity, a low peroxide value and tolerated K_{232} and K_{270} values, which allowed them to be classified in the EVOO category. Storage of the oils for a period of 12 months in opaque glass containers, in the dark and at room temperature, induced a significant increase in free acidity (*p* value < 0.05), after the sixth month until exceeding the limit by 0.8% at the ninth month. The highest value was recorded in Region 1 (Beni Yenni) in the elderly category and the lowest in Region 2 (Taourga) in the young category; these results are probably due to the release of free fatty acids from the lipids by hydrolysis [22]. Indeed, the acidity is mainly due to a hydrolysis of triglycerides by action of lipases present in the olive followed by microbial growth on the mesocarp of which 70% have a hydrolytic action, while oxidation is mainly produced during extraction [2].

The post-harvest and storage effects favour the progressive oxidation of lipids with a decrease in oxidative stability due to the continuation of the chemical processes set up by the initial peroxidations [23]. The work carried out by Lanza et al. [22] during 12 months of storage of olive oil cultivar 'Taggiasca' under conditions similar to ours (room temperature and opaque glass) showed similar results. Studies have also confirmed that acidity increased according to the nature of the container, conditions and duration of storage from the sixth month exceeding the barrier of 0.8% in the ninth month [10,24,25]. It has been shown that the acidity value could increase significantly after 12 months of dark storage at room temperature [2,26], and even after 9 months in different containers at room temperature [10].

Table 1. Mean value and standard deviation of the analytical parameters evaluated in olive oils obtained from fruits of young and old olive trees located in different regions of Kabylia (Mean ± SD, $n = 3$) a,A,*. These values correspond to the results of the Tukey test ($p < 0.05$) with respect to storage time, region and age, as mean values of single oil type control samples at same factor labelled by different capital letters, are statistically different (Tukey's test, $p < 0.05$).

Sample	Storage Time (Month)	Acidity (% Oleic Acid)	Peroxide Value (mEq O_2/kg)	K_{232}	K_{270}	Olive Oil Quality IOC
Beni Yenni (Young)	0	$0.14 \pm 0.04^{y,A,*}$	$8.37 \pm 0.60^{a,A,*}$	$0.31 \pm 0.10^{a,A,*}$	$0.11 \pm 0.03^{a,A,*}$	EVOO
	3	$0.27 \pm 0.08^{a,A,*}$	$12.57 \pm 0.90^{b,A,*}$	$0.59 \pm 0.40^{a,A,*}$	$0.13 \pm 0.01^{a,A,*}$	EVOO
	6	$0.28 \pm 0.07^{a,A,*}$	$14.32 \pm 10.40^{b,A,*}$	$2.05 \pm 0.20^{b,A,*}$	$0.18 \pm 0.01^{a,b,A,*}$	EVOO
	9	$1.79 \pm 0.70^{b,A,*}$	$15.25 \pm 10.90^{c,A,*}$	$2.89 \pm 0.10^{c,A,*}$	$0.20 \pm 0.02^{b,c,A,*}$	VOO
	12	$3.83 \pm 0.20^{c,A,*}$	$23.30 \pm 4.90^{d,A,*}$	$3.4 \pm 0.60^{d,A,*}$	$0.32 \pm 0.03^{c,A,*}$	LOO
Beni Yenni (Old)	0	$0.15 \pm 0.04^{y,A,*}$	$9.03 \pm 0.60^{a,A,*}$	$0.56 \pm 0.20^{a,A,*}$	$0.11 \pm 0.01^{a,A,*}$	EVOO
	3	$0.23 \pm 0.01^{a,A,*}$	$13.80 \pm 3.40^{b,A,*}$	$0.67 \pm 0.60^{a,A,*}$	$0.17 \pm 0.01^{y,A,*}$	EVOO
	6	$0.31 \pm 0.10^{a,A,*}$	$22.20 \pm 3.20^{b,A,*}$	$2.31 \pm 0.20^{b,A,*}$	$0.21 \pm 0.01^{a,b,A,*}$	EVOO
	9	$2.31 \pm 0.8^{b,A,*}$	$23.06 \pm 2.15^{c,A,*}$	$2.54 \pm 0.20^{c,A,*}$	$0.21 \pm 0.03^{b,c,A,*}$	VOO
	12	$3.95 \pm 0.30^{c,A,*}$	$31.50 \pm 8.10^{d,A,*}$	$4.02 \pm 0.30^{d,A,*}$	$0.36 \pm 0.02^{c,A,*}$	LOO
Taourga (Young)	0	$0.14 \pm 0.05^{y,A,*}$	$7.70 \pm 1.00^{a,A,*}$	$0.57 \pm 0.20^{a,A,*}$	$0.07 \pm 0.01^{y,A,*}$	EVOO
	3	$0.48 \pm 0.30^{y,A,*}$	$15.80 \pm 1.10^{b,A,*}$	$0.64 \pm 0.09^{a,A,*}$	$0.10 \pm 0.01^{y,A,*}$	EVOO
	6	$0.62 \pm 0.30^{y,A,*}$	$16.93 \pm 3.20^{b,A,*}$	$2.10 \pm 0.10^{b,A,*}$	$0.19 \pm 0.03^{a,b,A,*}$	EVOO
	9	$3.15 \pm 1.50^{b,A,*}$	$24.59 \pm 3.70^{c,A,*}$	$2.90 \pm 0.01^{c,A,*}$	$0.21 \pm 0.03^{b,c,A,*}$	VOO
	12	$3.70 \pm 0.50^{c,A,*}$	$32.70 \pm 4.70^{d,A,*}$	$3.3 \pm 0.20^{d,A,*}$	$0.28 \pm 0.02^{c,A,*}$	LOO
Taourga (Old)	0	$0.13 \pm 0.07^{a,A,*}$	$8.09 \pm 0.90^{a,A,*}$	$0.76 \pm 0.04^{a,A,*}$	$0.09 \pm 0.02^{a,A,*}$	EVOO
	3	$0.16 \pm 0.07^{a,A,*}$	$15.60 \pm 0.50^{b,A,*}$	$0.85 \pm 0.20^{a,A,*}$	$0.13 \pm 0.03^{a,A,*}$	EVOO
	6	$0.27 \pm 0.07^{a,A,*}$	$18.43 \pm 0.90^{b,A,*}$	$2.48 \pm 0.70^{b,A,*}$	$0.18 \pm 0.02^{a,b,A,*}$	EVOO
	9	$1.78 \pm 0.20^{b,A,*}$	$27.14 \pm 5.50^{c,A,*}$	$2.85 \pm 0.10^{c,A,*}$	$0.21 \pm 0.01^{b,c,A,*}$	VOO
	12	$3.90 \pm 0.60^{c,A,*}$	$31.20 \pm 3.10^{d,A,*}$	$3.08 \pm 0.10^{d,A,*}$	$0.26 \pm 0.02^{c,A,*}$	LOO
Mekla (Young)	0	$0.08 \pm 0.01^{y,A,*}$	$8.40 \pm 0.30^{a,A,*}$	$0.58 \pm 0.2^{a,A,*}$	$0.13 \pm 0.05^{a,A,*}$	EVOO
	3	$0.20 \pm 0.01^{y,A,*}$	$14.90 \pm 1.80^{b,A,*}$	$0.84 \pm 0.4^{a,A,*}$	$0.15 \pm 0.02^{a,A,*}$	EVOO
	6	$0.21 \pm 0.03^{a,A,*}$	$15.30 \pm 1.80^{b,A,*}$	$2.41 \pm 0.20^{b,A,*}$	$0.21 \pm 0.01^{a,b,A,*}$	EVOO
	9	$1.11 \pm 0.00^{b,A,*}$	$22.40 \pm 1.70^{c,A,*}$	$2.80 \pm 0.07^{c,A,*}$	$0.89 \pm 0.20^{b,c,A,*}$	VOO
	12	$3.50 \pm 0.20^{c,A,*}$	$26.40 \pm 2.70^{d,A,*}$	$3.08 \pm 0.10^{d,A,*}$	$1.15 \pm 0.10^{c,A,*}$	LOO
Mekla (Old)	0	$0.08 \pm 0.02^{a,A,*}$	$9.00 \pm 0.60^{a,A,*}$	$0.70 \pm 0.03^{a,A,*}$	$0.15 \pm 0.01^{a,A,*}$	EVOO
	3	$0.17 \pm 0.05^{a,A,*}$	$14.50 \pm 2.30^{b,A,*}$	$1.04 \pm 0.10^{a,A,*}$	$0.19 \pm 0.02^{a,A,*}$	EVOO
	6	$0.18 \pm 0.03^{a,A,*}$	$16.10 \pm 1.90^{b,A,*}$	$2.10 \pm 0.50^{b,A,*}$	$0.21 \pm 0.03^{a,b,A,*}$	EVOO
	9	$1.04 \pm 0.10^{b,A,*}$	$24.00 \pm 1.80^{c,A,*}$	$2.70 \pm 0.09^{c,A,*}$	$0.33 \pm 0.20^{b,c,A,*}$	VOO
	12	$3.70 \pm 0.30^{c,A,*}$	$26.60 \pm 3.90^{d,A,*}$	$2.88 \pm 0.10^{d,A,*}$	$0.39 \pm 0.20^{c,A,*}$	LOO

The peroxide value approached the upper limits in less than nine months with the exception of R1 where the limit exceeded 20 mEq O_2/kg oil after the ninth month in the young category and before the sixth month in the aged category. This result is probably due to self-oxidation, as during the first stages of storage in darkness, oxidation is the product of an absorption of O_2 in oil [27] producing hydroperoxides during the primary oxidation step following secondary products [28]. In general, it is detected that the oils from aged trees present peroxide values higher than those from young trees (Table 1).

Ultraviolet measures have shown continuously increasing values during storage. These results indicate the presence of conjugated dienes (K_{232}) and the formation of polyunsaturated fatty acids (primary oxidation) while the K_{270} parameter is an indicator of the second stage of oxidation related to the presence of end products such as conjugated trienes and unsaturated carbonyl compounds [6,29].

Unlike the peroxide value, K_{232} is the first to reach the upper limit of 2.5. These results are consistent with the observations made by Bendini et al. [30]; in this research group, K_{232} was the most useful analytical tool in the analysis and monitoring of quality to determine the category of oils. Considering the results of the quality parameters of the extra virgin

olive oil studied, it should be pointed out that after the ninth month of storage, all the samples moved to the VOO category. Moreover, at the 12th month, all the oils revealed values of free acidity > 3.3, PV > 20 mEq O_2/kg oil, K_{232} > 2.5 and K_{270} > 0.22, downgrading them to the category of lampante olive oils and being unfit for consumption as it is necessary to carry out a refining process. The analysis of variance showed a significant effect on storage duration and no significance with respect to age and region factors.

3.2. Natural Antioxidants and Oxidative Stability in the Initial State and Evolution during Storage

Extra virgin olive oils are one of the few oils obtained by simple extraction without the need for chemical treatments, which has made it possible to safeguard minor compounds with the capacity to confer self-protection thanks to their action as natural antioxidants that resist possible oxidative damage, especially phenolic compounds, tocopherols and carotenoids that delay oxidation and the production of undesirable products. Chlorophylls and carotenoids are the pigments most present in extra virgin olive oils, responsible for its characteristic colour and influence in consumers' opinion. They are considered a marketing factor. The influence of altitude on chlorophyll and carotenoid contents has been demonstrated by the value in the Tukey test of $p < 0.05$. The maximum content for pigments was recorded in Region 3 (Taourga) in the young category (1.33 mg/kg oil for chlorophylls and 1.34 mg/kg oil for carotenoids), Table 2. These variations depend on genetic characteristics and environmental factors, mainly the duration and intensity of exposure to sunlight [31].

Table 2. Evolution of the contents of total phenolic compounds (TPCs), chlorophylls (CHLs), carotenoids (CARs) and oxidative stability (OS) during storage (Mean ± SD, n = 3). a,A,* correspond to the results of the Tukey test ($p < 0.05$) with respect to storage time, region and age, as mean values of single oil type control samples at same factor labelled by different capital letters, are statistically different (Tukey's test, $p < 0.05$).

Sample	Storage Time (Month)	TPCs (mg/kg Oil)	CHLs (mg/kg Oil)	CARs (mg/kg Oil)	OS (h)
Beni Yenni (Young)	0	$79.66 \pm 42.10^{a,A,*}$	$0.92 \pm 0.20^{a,A,*}$	$0.92 \pm 0.20^{a,A,*}$	$21.90 \pm 1.40^{a,A,*}$
	12	$37.07 \pm 4.90^{b,A,*}$	$0.60 \pm 0.08^{b,A,*}$	$0.66 \pm 0.20^{b,A,*}$	$10.90 \pm 2.90^{a,A,*}$
Beni Yenni (Old)	0	$85.25 \pm 12.80^{a,A,*}$	$1.08 \pm 0.20^{a,A,*}$	$1.08 \pm 0.20^{a,A,*}$	$22.56 \pm 1.20^{a,A,*}$
	12	$71.90 \pm 10.32^{b,A,*}$	$0.41 \pm 0.10^{b,A,*}$	$0.49 \pm 0.14^{b,A,*}$	$4.04 \pm 1.70^{b,A,*}$
Taourga (Young)	0	$243.60 \pm 96.50^{a,A,*}$	$1.00 \pm 0.10^{a,B,*}$	$1.00 \pm 0.15^{a,B,*}$	$24.57 \pm 4.70^{a,A,*}$
	12	$43.59 \pm 34.60^{b,A,*}$	$0.17 \pm 0.03^{b,B,*}$	$0.21 \pm 0.07^{b,B,*}$	$14.88 \pm 3.50^{b,A,*}$
Taourga (Old)	0	$113.90 \pm 27.90^{a,A,*}$	$0.91 \pm 0.10^{a,B,*}$	$0.91 \pm 0.19^{a,B,*}$	$19.34 \pm 1.10^{a,A,*}$
	12	$52.58 \pm 15.08^{b,A,*}$	$0.22 \pm 0.10^{b,B,*}$	$0.24 \pm 0.06^{b,B,*}$	$11.46 \pm 0.60^{b,A,*}$
Mekla (Young)	0	$163.20 \pm 93.50^{y,A,*}$	$1.33 \pm 0.10^{a,B,*}$	$1.34 \pm 0.10^{a,B,*}$	$23.86 \pm 0.20^{a,A,*}$
	12	$60.37 \pm 6.90^{b,A,*}$	$0.10 \pm 0.01^{b,B,*}$	$0.10 \pm 0.01^{b,B,*}$	$3.28 \pm 1.50^{b,A,*}$
Mekla (Old)	0	$104.40 \pm 21.24^{a,A,*}$	$0.74 \pm 0.05^{a,B,*}$	$0.74 \pm 0.05^{a,B,*}$	$19.97 \pm 0.12^{a,A,*}$
	12	$29.80 \pm 22.50^{b,A,*}$	$0.21 \pm 0.00^{b,B,*}$	$0.30 \pm 0.01^{b,B,*}$	$4.21 \pm 1.60^{b,A,*}$

The results of this study are in agreement with those obtained by [32–34] who supported in their studies the hypothesis that pigment contents depend on geographical location. The authors argued that soil and climatic conditions can indeed significantly affect pigment contents. According to Criado et al. [35], oils from the same variety can be classified not only on the basis of the variety but also its geographical origin [36–38]. Age has no influence on pigment contents. In relation to phenolic compounds, the highest content in this study was obtained in Taourga, category young, with a value of 243 mg/kg oil and the lowest in Region 1 (Beni Yenni), category young, with 79 mg/kg oil, Table 2. Similar values have been observed in previous studies for the 'Chemlal' variety in Alge-

ria [39–41]. These values show a low content of total phenolic compounds in the 'Chemlal' variety when compared with that of other varieties in the world. In Spain, with the 'Picual' cultivar, in regions with a similar altitude and average temperature, the concentration of total phenolic compounds was higher, reaching values in the range of 600–700 mg/kg [20].

Table 3 shows the tocopherol contents (α, β, γ and total) for the 'Chemlal' variety harvested in different regions of Kabylia in different young and old trees.

Table 3. Evolution of tocopherol (TOC) contents during storage (Mean ± SD, n = 2).

Sample	Time Storage (Month)	α-TOC (mg/kg)	β-TOC (mg/kg)	γ-TOC (mg/kg)	Total TOC (mg/kg)
Beni Yenni (Young)	0	238.59 ± 35.70	16.86 ± 3.85	4.75 ± 0.05	258.16 ± 37.72
	12	160.59 ± 86.06	11.31 ± 0.91	3.93 ± 0.34	175.79 ± 87.39
Beni Yenni (Old)	0	252.23 ± 16.41	16.86 ± 3.85	5.71 ± 1.94	272.63 ± 19.51
	12	249.57 ± 43.22	15.02 ± 1.64	5.12 ± 1.35	271.56 ± 48.43
Taourga (Young)	0	278.34 ± 2.09	12.68 ± 0.07	5.63 ± 0.21	296.65 ± 1.95
	12	215.0 ± 22.28	9.45 ± 0.14	3.91 ± 0.24	228.36 ± 22.37
Taourga (Old)	0	293.91 ± 21.18	9.45 ± 0.72	5.39 ± 0.26	311.20 ± 22.28
	12	213.93 ± 10.54	6.12 ± 0.09	3.91 ± 0.38	225.45 ± 10.71
Mekla (Young)	0	166.51 ± 29.74	7.65 ± 0.26	3.44 ± 0.14	177.61 ± 30.16
	12	138.94 ± 84.84	3.07 ± 0.09	2.43 ± 0.67	144.45 ± 85.43
Mekla (Old)	0	244.85 ± 23.74	8.55 ± 0.21	4.83 ± 0.25	258.23 ± 23.78
	12	145.76 ± 4.07	3.81 ± 0.16	3.37 ± 0.05	152.94 ± 3.84

The main compound quantified was α-tocopherol, which represents more than 94% of the total, ranging between 166.51 and 293.91 mg/kg olive oil. It is followed by β-tocopherol (7.65 to 16.86 mg/kg oil) and smaller amounts of γ- tocopherol (3.44 to 5.71 mg/kg oil).

The analysis of variance showed a significant effect of region or altitude on the content of total (p value = 0.01), alpha (p value = 0.02), beta (p value = 0.000004) and gamma tocopherol (p value = 0.005).

The results obtained show higher contents than those obtained by Bengana et al. [40] for the same variety.

After 12 months of storage, a significant decrease in pigments ($p < 0.05$) was observed in the chlorophyll contents of all olive oil samples analysed. This observation was most noticeable in Region 3 (Mekla) for the young category, where the chlorophyll content decreased in 1.23 mg/kg oil, while the decrease varied between 0.3 and 0.8 mg/kg in the other samples. The evolution of the carotenoid content was similar to that of the chlorophylls as a continuous decrease was recorded and the most marked decrease was in Region 3 in the young category with a value of 1.2 mg/kg oil, while the decrease in the other samples varied between 0.2 and 0.7 mg/kg oil, as shown in Table 2.

Similar results were reported by Criado et al. [35] and Gómez-Alonso et al. [42] where a reduction in pigment was more pronounced in samples with higher initial carotenoid content. According to Ayton et al. [11], Baiano et al. [25], Gallardo-Guerrero et al. [31] and Hornero-Méndez et al. [43], the pigment profile of olive oils is affected by the extraction of the olive oil and during storage even under controlled temperature and dark conditions; they deduced a conversion of chlorophylls to pheophytins and then to pyropheophytins. Psomiadou et al. [44] claimed that the decrease in carotenoid contents is due to their sensitivity to auto-oxidation and therefore their vulnerability to the oxygen that can be dissolved in the bottle. The storage of the oils at room temperature caused a considerable decrease in total phenolic compounds after 12 months of storage. The largest decrease was recorded in Region 2 (Taourga), young category, with 82.11% loss, even if it showed the highest content in the initial state. These results are consistent with previous studies that have estimated considerable losses in the concentrations of total phenolic compounds in extra virgin olive oils [45–47].

Indeed, Criado et al. [35] noted a significant loss in extra virgin olive oils of the 'Arbequina' variety after 12 months of storage under the same conditions as ours. Bendini et al. [30] and Gómez-Alonso et al. [42] also observed a decrease in total phenolic compounds after 21 months of storage at room temperature. According to Criado et al. [35], this result is related to the decomposition process occurring in the most complex forms by the oxidation process. Table 2 lists the results of oxidative stability obtained by the Rancimat method. A decrease was perceptible in all categories after 12 months of storage especially in Mekla, young category, where the decrease was 86% in relation to the initial value. While the smallest decrease was recorded in the young and old categories in Taourga with 39.4% and 40.7%, respectively, it also corresponds to the region with the highest total phenolic compounds and pigment contents.

In relation to tocopherol fraction, after twelve months of storage of olive oil samples at room temperature, losses were observed compared to the initial contents (Table 3).

The storage time factor influences total tocopherol content in the three types of tocopherols of alpha, beta and gamma. The olive oils preserved their tocopherol contents well despite the losses recorded, which do not exceed 45%. In fact, all tocopherols decreased by 30 to 41%.

The tocopherol most present in olive oils (alpha tocopherol) decreased by an average of 24% out of all the oils studied, while beta tocopherol decreased by 30%, and gamma tocopherol decreased by only 23%.

Previous studies have shown much higher levels of degradation compared to those of our results [35], and after 12 months of storage at room temperature in the dark, an almost total loss was found in alpha tocopherol.

Tocopherols play an important role as antioxidants in oxidation processes, a relevant role during the oxidation induction period. Tocopherols have a strong influence on shelf life, preserving oils from rancidity by interrupting the chain reactions involved in hydroperoxide formation [48].

In general, the reduction in α-tocopherol suggests a protective effect of hydrophilic phenols, probably based on their ability to reduce its oxidised forms.

According to Okogeri and Tasioula-Margari [46], the factor that decreased the impact of storage and was able to preserve high levels of tocopherols was the absence of the luminous factor.

According to Deiana et al. [49], the initial content of alpha tocopherol is a determinant of the stability of olive oil versus oxidation, its shelf life and therefore its quality; this fact is variable according to the variety of olives, extraction process used and storage conditions of the oil, in particular temperature and exposure to light [50].

3.3. Fatty Acid Profile and Evolution during Storage

The profile of the fatty acid composition detected in the olive oils studied was extensive, in both young and old categories in the different regions. The predominant fatty acids are as follows: oleic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:2), palmitoleic acid (C16:1) and stearic acid (C18:0), as shown in Table 4. Their values correspond to those required by the European Commission [20]. However, fatty acids such as linolenic acid (C18:3), arachidic acid (C20:0), gondoic acid (C20:1) and behenic acid (C22:0) were detected in small quantities. Margaric acid (17:0) was also detected in low concentration. The contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and the ratio of oleic acid to linoleic acid (C18:1/C18:2) were also calculated. The highest polyunsaturated fatty acid content corresponds to the lowest C18:1/C18:2 ratio, and the highest C18:1/C18:2 relation corresponds to the highest content of total phenolic compounds (Figure 2). Statistical analyses (ANOVA) did not show significant differences for either the age or region factor.

Table 4. Evolution of fatty acid composition (%) in different regions of Kabylia (Algeria) during storage.

Sample	Time Storage (Month)	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
Beni Yenni (Old)	0	16.39 ± 0.10	2.07 ± 0.01	0.09 ± 0.01	2.04 ± 0.01	67.20 ± 0.10	10.58 ± 0.05	0.50 ± 0.10	0.40 ± 0.08	0.35 ± 0.02	0.17 ± 0.05
	12	16.50 ± 0.50	1.80 ± 0.20	0.04 ± 0.06	1.80 ± 0.10	68.29 ± 1.47	9.9 ± 0.35	0.51 ± 0.06	0.30 ± 0.02	0.28 ± 0.03	0.37 ± 0.03
Beni Yenni (Young)	0	16.24 ± 0.50	1.90 ± 0.02	0.08 ± 0.01	2.06 ± 0.05	67.72 ± 0.27	10.28 ± 0.41	0.50 ± 0.02	0.40 ± 0.01	0.49 ± 0.20	0.13 ± 0.01
	12	16.37 ± 0.01	1.78 ± 0.02	0.07 ± 0.01	1.91 ± 0.01	68.34 ± 0.10	9.80 ± 0.20	0.50 ± 0.01	0.30 ± 0.01	0.30 ± 0.02	0.39 ± 0.06
Taourga (Old)	0	17.5 ± 0.08	3.06 ± 0.10	0.11 ± 0.01	1.71 ± 0.04	63.15 ± 0.70	12.60 ± 0.60	0.75 ± 0.01	0.40 ± 0.01	0.38 ± 0.01	0.13 ± 0.01
	12	18.6 ± 0.04	2.90 ± 0.04	0.08 ± 0.01	1.72 ± 0.01	62.65 ± 0.14	12.23 ± 0.10	0.60 ± 0.03	0.34 ± 0.01	0.32 ± 0.10	0.34 ± 0.03
Taourga (Young)	0	15.82 ± 0.50	1.90 ± 0.10	0.06 ± 0.05	2.02 ± 0.01	68.48 ± 1.40	9.96 ± 0.80	0.50 ± 0.08	0.50 ± 0.10	0.37 ± 0.03	0.14 ± 0.01
	12	16.13 ± 0.30	2.01 ± 0.10	0.09 ± 0.20	2.04 ± 0.01	68.23 ± 1.50	9.80 ± 1.10	0.60 ± 0.01	0.38 ± 0.02	0.35 ± 0.03	0.28 ± 0.02
Mekla (Old)	0	13.20 ± 1.40	1.36 ± 0.60	0.05 ± 0.04	2.25 ± 0.20	70.03 ± 3.20	10.88 ± 1.19	0.60 ± 0.04	0.53 ± 0.07	0.54 ± 0.05	0.29 ± 0.10
	12	14.16 ± 3.00	1.31 ± 0.60	0.03 ± 0.04	2.12 ± 0.30	70.8 ± 3.13	9.78 ± 0.30	0.59 ± 0.09	0.39 ± 0.09	0.37 ± 0.19	0.39 ± 0.03
Mekla (Young)	0	16.45 ± 0.40	2.02 ± 0.02	0.10 ± 0.08	1.80 ± 0.03	66.57 ± 1.13	11.48 ± 0.90	0.50 ± 0.03	0.45 ± 0.08	0.37 ± 0.03	0.09 ± 0.07
	12	16.04 ± 0.60	1.93 ± 0.08	0.08 ± 0.01	1.80 ± 0.01	67.02 ± 1.80	11.50 ± 1.11	0.51 ± 0.10	0.35 ± 0.01	0.35 ± 0.02	0.30 ± 0.03

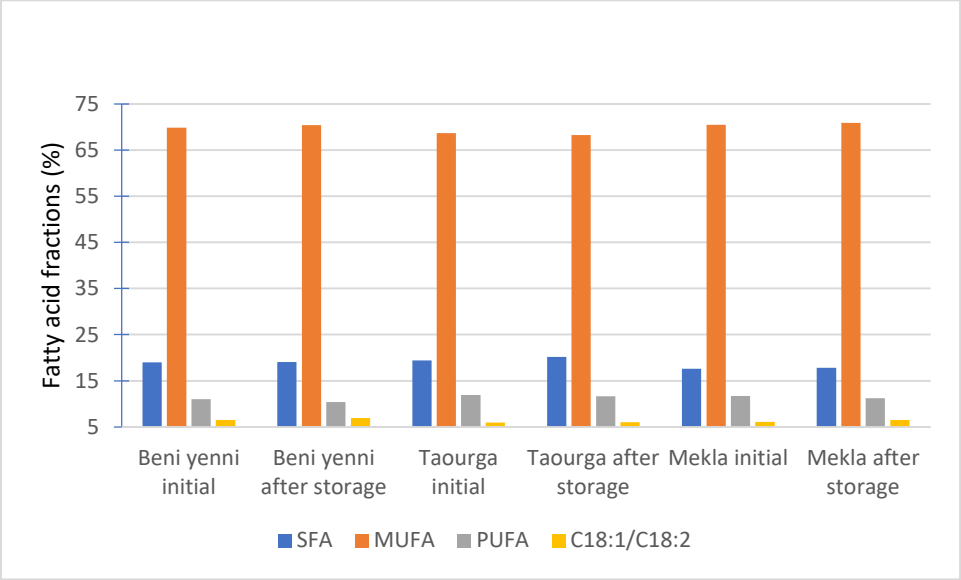


Figure 2. Evolution of fatty acid fractions (%) in different regions during storage (at 12 months).

The fatty acid composition is an important aspect allowing for the quantitative analysis of extra virgin olive oils. It represents a relevant tool in the detection of fraud (adulteration) and the authentication of olive oils.

Lipids undergo widespread deterioration due to the unsaturations present in the fat compounds, which are sensitive to auto- or photo-oxidation during storage [51]. The fatty acid profile of the different samples after storage showed that the fatty acid values remain within the levels recommended for extra virgin olive oils, and only behenic acid (C22:0) increased above 0.2%. The region and age factor did not show a particularly significant effect. It is noteworthy that the level of monounsaturated fatty acids, particularly oleic acid, increased. A slow decrease was observed in the level of polyunsaturated fatty acids, which could be attributed to the degradation of linoleic acid. Some authors report no or very little change during storage [6,10,52].

The data obtained show on the one hand that the highest level of polyunsaturated fatty acids corresponds to the highest level of the peroxide value, which seems obvious since the hydroperoxides detected are formed from polyunsaturated fatty acids [53], and on the other hand, it corresponds to the lowest ratio of C18:1/C18:2. Also, the highest value recorded in the peroxide value corresponds to lower in monounsaturated fatty acids (oleic acid C18:1 = 63.15%, which is the lowest), and this can be explained by the fact that olive oils with multiple unsaturations oxidise faster than those with fewer double bonds [35]. A negative correlation was observed between oxidative stability and the oleic acid/linoleic acid ratio.

3.4. Correlations between Quality Parameters and Natural Antioxidants

During storage at room temperature, Pearson correlations were used to better explain the evolution of quality parameters and natural antioxidants (Table 5). A positive correlation was deduced between the peroxide value and the UV absorption at 232 nm (R^2 = 0.913). This is due to the common information provided by these two parameters, which is the degree of primary oxidation of the lipids. Based on similar studies, it can be noted that it is more interesting to use the UV absorption at 232 nm as a routine analytical tool than the peroxide value. A positive correlation links the UV absorption at 232 nm and 270 nm (R^2 = 0.510). The K_{232} value informs us of primary oxidation and thus of conjugated dienes absorbed at this wavelength, while the K_{270} value informs us of the presence of conjugated trienes and thus of a secondary oxidation. This being said, when the rate of primary products increases, the rate of secondary oxidation increases in parallel, following the decomposition of primary oxidation products to give other products called secondary oxidation products such as ketones and aldehydes and thus implying negative attributes to the olive oil, which are detected during sensory characterization.

Table 5. Pearson Coefficients between quality parameters and oxidative stability.

	Acidity	Peroxide Value (PV)	K_{232}	K_{270}	Oxidative Stability
Chlorophylls	−0.54	−0.53	−0.50	−0.44	0.48
Carotenoids	−0.50	−0.51	−0.46	−0.44	0.44
Total phenolic compounds	−0.60	−0.57	−0.54	−0.32	−0.22
Acidity	*	0.93	0.95	0.55	−0.58
PV	0.93	*	0.91	0.53	−0.51
K_{232}	0.95	0.91	*	0.51	−0.60
K_{270}	0.55	0.53	0.51	*	−0.61
Oxidative stability	−0.58	−0.51	−0.60	−0.61	*
C18:1/C18:2	ND	−0.67	ND	ND	−0.50
α Tocopherol	−0.47	−0.47	−0.50	−0.47	0.30

In addition, enzymatic activity may take place in the stored fruit through lipases or lipoxidases. It is well known that the two stages of auto-oxidation are the formation of free radicals and their reaction with hydroperoxides of oxygenated origin. These molecules are not stable and their degradation produces a variety of volatile compounds, such as alcohols or other oxidation products (e.g., aldehydes and ketones) [13].

A negative correlation between the peroxide value, total phenolic compounds (R^2 = −0.569) and alpha tocopherol (R^2 = −0.47) has been demonstrated.

Indeed, phenolic compounds act as a barrier protecting lipids from oxidation, thus acting as natural antioxidants. The content of these compounds and the degree of oxidation are inversely proportional. In this sense, different authors demonstrated the existence of an antioxidant activity of alpha tocopherol, especially at low concentrations (of the order of 100 mg/kg oil) [54,55].

Tocopherols can compete with lipids and unsaturated oils for peroxy lipid radicals. Lipid peroxy radicals react with tocopherols much faster than with lipids. In general, it is also known that one molecule of tocopherol can protect about 105 molecules of polyunsaturated fatty acids with low peroxide values [56].

The same observation was made for pigments and acidity as well as the peroxide value and K_{232}. It is known that pigments act as antioxidants in the dark and pro-oxidants in the presence of light. The reduction in these compounds found in trace amounts in olive oils allows acidity, the peroxide value and K_{232} to increase during the oxidation process of the olive oils. In this sense, there is a negative relationship between acidity, peroxide value, K_{232}, K_{270} and oxidative stability.

4. Conclusions

The literature data concerning the storage of Algerian olive oils are non-existent. To this end, this study aimed to enrich the database, particularly for the 'Chemlal' variety. In addition, the investigation focused on determining the physicochemical characteristics of the 'Chemlal' variety, its oxidative stability as well as its shelf life. According to the results obtained, it can be deduced that altitude or geographical location has an impact on the pigment contents, making them a tool for characterisation and traceability of the varietal origin.

After the storage of olive oils in an opaque glass container at room temperature, several significant changes were perceptible. The samples studied were downgraded from the extra virgin olive oil category after 9 months, and at 12 months of storage they were unfit for consumption.

Thus, it can be concluded that the optimal storage period for extra virgin olive oils, under the same conditions as those in this study, is about 9 months, but the oil remains edible up to 12 months after production. The overall lipid composition after storage changed slightly, resulting in an increase in the oleic acid concentration and a degradation without any particular distinction for age or region, followed by a decrease in polyunsaturated fatty acids (linoleic and linolenic acid) and natural antioxidants (pigments, total phenols and tocopherols) resulting in an alteration of the oxidative stability.

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