Volume 82(6). Published April, 01, 2021
www.jnsciences.org
E-ISSN 2286-5314
BEJAOUI et al (2021)

Virgin olive oil quality in relation to olive ripening stage and malaxation temperature

K. BCHIR BEN HASSINE[1,*], W. MNIF[2,3], S. SMAOUI[4], S. DHAOUADI[1], H. AICHI[1], M. BEN CHIKHA AND N. GRATI KAMMOUN[4]

[1]Higher School of Agriculture of Mograne, Research Laboratory of Agricultural Production Systems and Sustainable Development 1121 Zaghouan, University of Carthage, Tunisia.
[2]Department of Chemistry, Faculty of Sciences and Arts in Balqarn, University of Bisha, P.O. BOX 199, Bisha 61922, Saudi Arabia.
[3]University of Manouba, ISBST, BVBGR-LR11ES31, Biotechpole Sidi Thabet, 2020, Ariana, Tunisia.
[4]Institut de l'Olivier, P.O. Box 1087, 3018 Sfax, Tunisia.

***Corresponding author:** kaoutheragro@yahoo.fr

Abstract- The study of the influence of malaxation temperature (25°C, 30°C, 35°C) and ripening stage on the chemical characteristics of olive oils and oxidative stability were conducted on two Tunisian olive cultivars (Chemlali and Chétoui) during four months of storage. The present results confirmed the significance of genetic factor and ripening stage in determining the composition of the olive oil. Among studied olive oils, those obtained from olives harvested in early maturity and are characterized by low acidity, richness in antioxidants and good stability during storage. In addition, rising temperatures when mixing olive paste results an increase in free acidity and chlorophyll content and decrease in polyphenol content and oxidative stability values. Regard less of the ripeness stage and the malaxation temperature, Chétoui olive oil was the most stable to oxidation during storage**.**

Key words: olive oil, ripening, malaxation temperature, oxidative stability

1. Introduction

Olive oil is the oily juice of the olive, separated from the other components of the fruit. Properly extracted from fresh, mature fruit of good quality, the oil has a unique flavor. Its fatty acid composition is characterized by a good balance between saturated, monounsaturated, and polyunsaturated fatty acids (Lazzez et al., 2008). It is also unique among common vegetable oils in that it can be consumed in the crude form, thus conserving vitamin content and phenolic compounds of nutritional importance.

Aroma and other components of virgin olive oils are influenced by several production and processing parameters (Bianchi et al., 2020; Difonzo et al., 2021), such as the agronomic choices, olive cultivar, ripening degree and sanitary quality of olives, harvest system, post-harvest storage of fruits, processing technology, oil bottling and storage (Mele et al., 2018). Among these parameters, processing technology seems to greatly determine the final quality of the oil. It is well known that the organoleptic properties of virgin olive oils are significantly affected by the crushing method, the kneading process of the olive-paste, and the separation systems of crude oil or oil-water mixture from the olive-paste.

Others factors can be taken in consideration, such as high malaxation temperature that seem to have negative affect in the quality of the olive oil by reducing volatile compounds that display pleasant sensations and increase those giving less attractive perceptions (Angerosa et al ,2000; Franca., 2014), other research show also that the total phenol and o-diphenol contents in virgin olive oils, which affect both the sensory bitterness and the oxidative stability of the oils, greatly improved with the increase in the malaxation temperature (Antonio et al.,2009)

Recent studies showed the great variability in the content and type of phenols present and of volatile substances, which influence the aroma of the oil, during maturation (Skevin et al., 2003; Angerosa et al., 2004; Morello et al., 2004; Ouni et al., 2016; El Qarnifa et al., 2019). The most specific index to test the ripening is the oil accumulation in the olives. It is interesting to note that while the percentage of oil in fresh olive fruit continuously increases during ripening, the percentage of the oil in dry fruits reaches a maximum value and remains constant.

Therefore, fruit harvesting should be carried out from the middle of November in order to obtain the highest oil yield and avoid natural fruit drop. Traditionally, olives are harvested at the green-yellow or black-purple stage. Since all of the fruit does not mature simultaneously even on the same tree,

harvesting should take place when the majority of the fruit are at optimum maturity. This is not always possible because other factors may also affect harvest time such as weather conditions, availability of farm labor, availability of olive oil mills, etc.

Generally, as the fruit mature, the oil becomes less stable due to the increase of polyunsaturated fatty acids and the decrease of the total polyphenol content (Ayton et al., 2007). However early harvested conduct to oil with high polyphenol concentration causing a high level of bitterness and pungency. As a result, the oil is relatively more stable due to the anti-oxidative effect of the polyphenols (Diraman et al., 2009).

The quality indices for harvest timing show that quantity of oil increases significantly during early fruit ripening (Lavee and Wonder., 2004). Also, Oil quality improvement is initially associated with the increase in oil content but peaks and begins to decline, before maximum oil yield is reached (Salvador et al., 2001). Determining the optimal harvest timing is particularly difficult due to variability in cultivar response between growing seasons and varying crop loads.

Also, Oil quality index are paramount importance to ensure safety of the product for consumption. Although there is no official standard set for evaluating edible oil quality, free fatty acids FFA content, peroxide value (PV) and p-anisidine value (AV) are commonly used in industry to report edible oil quality (Dunford., 2016). This fact sheet summarizes edible oil quality parameters used in industry and for research purposes.

This study aims to evaluate the effect of olive ripening stages on the overall flavor as well as on the oxidative stability of two monovarietal extra virgin olive oil: Chemlali and Chétoui**.**

2. Materials and methods

The present study was carried out on two main varieties that contribute to approximately 90% of the national production of olive oil: Chemlali (Ouled Haffouz) and Chétoui (Bouarada). The olives are harvested on two different dates (early maturation and late maturation) with a time interval of 3 months (Figure 1). Ripeness index was determined according to Uceda and Hermoso (1998).

Figure 1: Olive fruits at various stages of ripeness index based on the color.

The olive processing was performed at an oil mill equipped with a continuous triple-phase chain. The mixing temperatures studied are 25°C, 30°C and 35°C (Figure 2).

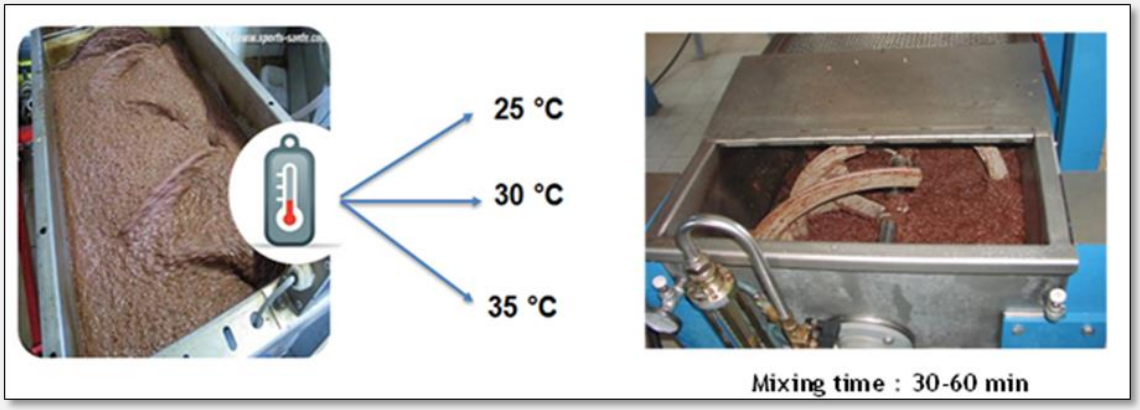

Figure 2: Mixing temperatures studied (25 °C, 30°C and 35 °C) and duration for all samples olive (45 min).

Quality indices

Free acidity, peroxide value (PV), and UV spectrophotometric indices (K232, K270) were evaluated according to the official methods described in Regulation EC 2568/91 (1) of the Commission of the European Union. All parameters were determined in triplicates sample for each variety.

Fatty acids

For the determination of fatty acid composition, the methyl esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2N methanolic potash and analyzed by gas chromatography (Shimadzu. GC-17A) and equipped with a FID detector. A fused silica capillary column (30m length x 0.32 mm diameter), coated with Carbowax (Polyethylene Glycol) phase was used. Nitrogen was employed as carrier gas with a flow through the column of 1mL/min. The temperatures of the injector and detector were set at 230 and 250°C respectively, whereas the oven temperature was 180°C. An injection volume of 1µL was used the fatty acid composition was expressed as relative percentages of each fatty acid, calculated by internal normalization of the chromatographic peak area used (IOOC 2010).

Total polyphenol content

Phenolic compounds were isolated using the method described by (Brighente et al., 2007) with triple extraction of oil-in-hexane solution with water–methanol (60:40 v/v) mixture. The concentration of total polyphenols was estimated with folin-ciocalteu reagent at 725 nm. Results were expressed as mg of caffeic acid per Kg of oil.

Volatile compounds

Supelco (Bellefonte. PA) SPME devices coated with polydimethylsiloxane (PDMS. 100 µm) were used to sample the headspace of 2 ml of olive oil inserted into a 5-ml vial and allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC–MS system. GC–EI/MS analyses were performed with a Varian (Palo Alto. CA) CP 3800 gas chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm, 0.25 µm; Agilent. Santa Clara. CA) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures were 250 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C/min; helium was used as carrier gas at a flow of 1 ml/min.
The identification of the volatile compounds was based on the comparison of the retention times with those of authentic standards, comparing their linear retention indices (LRI) relative to a series of n-hydrocarbons, and on computer matching against commercial (NIST 98 and Adams) and home-made library mass spectra, built from pure substances, components of known oils, and MS literature data. Moreover, the molecular weights of all the substances identified were confirmed by GC–CI/MS, using methanol as the ionizing gas (Ben Hassine 2015)

Determination of phenolic compounds by HPLC

The phenolic fractions were extracted by liquid-liquid extraction (Siddique et al., 2019) and separated by HPLC. Before injection, the phenolic extract was diluted with 1 mL methanol and filtered through a hydrophilic polyvinylidene fluoride (PVDF) 0.2- mm syringe filter. The HPLC analysis was conducted as reported by Selvaggini et al. (2006), using an Agilent Technologies system model 1100 (Agilent Technologies, Palo Alto, CA, USA) composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, a diode array detector (DAD) and a fluorescence detector (FLD).
The oil extract analyses were performed using C18 columns, Spherisorb ODS-1, 25064.6 mm with a particle size of 5 mm (Phase Separation Ltd., Deeside, UK). The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1 mL/ min, with the following gradient: 95% A/5% B for 2 min, to 75% A/25% B in 8 min, to 60% A/40% B in 10 min, to 50% A/ 50% B in 16 min, 0% A/100% B in 14 min, finishing with a plateau with this composition maintained for 10 min. Initial conditions were then reset and equilibration was reached in 13 min. The total run time was 73 min.

Pigment content

Chlorophyll and carotenoid were calculated from the absorption spectra of each virgin olive oil sample (7.5 g) dissolved in cyclohexane (25 mL) following the method of Benito et al. (2012). The maximum absorption at 670 nm is related to the chlorophyll fraction and at 470 nm is related to carotenoid fraction. The values of the coefficients of specific extinction applied were E0 = 613 for pheophytin as a major component in the chlorophyll fraction and E0 = 2000 for lutein as a major component in the carotenoid fraction. The chlorophyll and carotenoid contents are expressed as mg per Kg of oil.

Oxidative Stability

The oxidative stability was evaluated by the Rancimat method, because it is a fast and reliable analytical procedure (Tinello et al., 2018). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 679 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 5 g warmed to 100°C and an air flow of 20 l/h.. With this well-established methodology, the volatile oxidation products were stripped from the oil and dissolved in cold water, whose conductivity increased progressively. The time taken to reach a fixed level of conductivity was measured.

Statistical analysis

All the studied parameters were carried out in triplicate. The results are reported as mean values of three repetitions and standard deviation. Significant differences among varieties studied were determined by an analysis of variance that applied a Duncan test, using the SPSS programme, release 11.0 for Windows (SPSS, Chicago, IL, USA). Samples were also discriminated by multivariate parametric methods where the principal component analysis (PCA) was carried out using XLStat-Pro 7.5 (2007) for Windows (Addinsoft, New York, NY, USA).

3. Results and discussion

The malaxation phase is an important step in the production of olive oil, as shown by several authors (Kalua et al., 2006). The parameters (temperature and duration) of this operation must be well-controlled for their great influence on the quality of the virgin olive oil (VOO) (Kalua et al., 2006).

Table 1 shows a clear variation in quality indices in relation to three malaxation temperatures (35°C, 30°C and 25°C) during the storage period. The malaxation temperature had no significant effect on the quality parameters (acidity, IP, K232 and K270) of "Chemleli" and "Chétoui" VOOs.

It is known that PV is an assessment of the oil oxidative status which is correlated with rancidity (Ryan et al., 1999) which is influenced by the composition of the oil, with the richness in antioxidants (Ryan et al., 1999). In addition, the PV can be affected by the harvesting technique, the fruit status and the extraction process (Ryan et al., 1999). According to Table 1, a variation is observed for quality indices according to the storage condition without exceeding the limit established by the IOC for extra virgin olive oil category (Gutiérrez and Fernández, 2002).

Table 1: quality indices of VOO from Chemleli and Chétoui in function of malaxation (25°C, 30°C and 35°C).

Olive cultiar	T(°C)	Free Fatty acids (%)			PV (Meq O_2/kg)			K232			K270		
		25°C	30°C	35°C	25°C	30°C	35°C	25°C	30°C	35°C	25°C	30°C	35°C
Chétoui	T_i	0.140	0.200	0.210	4.481	10.458	8.745	0.169	1.745	1.435	0.154	0.135	0.095
	T_r	0.400	0.513	0.373	8.580	13.773	14.961	2.045	2.431	3.031	1.834	0.283	0.285
	T_{re}	0.167	0.247	0.237	4.489	11.135	10.273	1.886	2.033	2.477	0.157	0.171	0.291
Chemleli	T_i	0.193	0.193	0.203	6.087	8.125	9.506	1.600	1.590	1.425	0.115	0.105	0.095
	T_r	0.237	0.320	0.460	9.922	18.495	11.019	2.678	3.712	2.930	0.254	0.283	0.217
	T_{re}	0.217	0.240	0.297	8.130	16.483	10.310	2.761	2.165	1.927	0.216	0.228	0.275

Initial temperature (T_i); Room temperature (T_r); Refrigerator temperature (T_{re}); Peroxide value (PV), UV spectrophotometric indices (K232 and K270)

Table 2 shows that the mixing temperature has a negative impact on the contents of total polyphenols in olive oil. In fact, a content of 195.95mg/kg was recorded at 25°C face to 116.35mg/kg at 35°C for the variety Chétoui. The content of polyphenols was found to be lower, after refrigeration for 4 months, a content of 90.195mg/kg equal to 22.48% decrease than at ambient temperature (106.800 mg/kg) for a mixing temperature of 35°C and a regression of 34,185% for a mixing temperature of 25%. On the other side, the Chemleli variety underwent the same phenomenon, a regression of polyphenols content at 25°c from 106.943mg/kg to 85.780 mg/kg for a mixing temperature of 35°c, we also note a similar behavior when we faced with refrigeration, the polyphenol content dropped by 30.56% for a mixing temperature of 35°C and 32.170% with a temperature of 25°C. This phenomenon is due to the difficulty of the extraction of polyphenols following prolonged refrigeration,

So, it was found that polyphenol was influenced by the variety factor so that the conservation of polyphenols, at room temperature or in refrigeration during 4 months of storage varies considerably between the variety Chétoui and Chemleli. However, the Chemleli variety shows a better ability to preserve polyphenols in the face of a variation in the mixing temperature and storage temperature

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Table2: Variation of phenolic compounds according of the storage and malaxation temperature

		Chétoui		Chemleli	
T (°C)	TM (°C)	Polyphenols (mg /kg)	Percentage decrease of Polyphenols (%)	Polyphenols (mg /kg)	Percentage decrease of Polyphenols (%)
T_i		116.351		85.780	
T_r	35	106.800	8.209	63.761	25.670
T_{re}		90.195	22.480	59.990	30.065
T_i		115.160		96.780	
T_r	30	102.360	11.115	67.440	19.683
T_{re}		89.690	22.118	77.730	30.316
T_i		195.955		106.943	
T_r	25	158.355	19.189	90.937	14.967
T_{re}		128.967	34.185	93.929	32.170

Initial temperature (T_i); Room temperature (T_r); Refrigerator temperature (T_{re}); Malaxation temperature (TM)

Regarding flavonoids, the highest apigenin value 47.877 ppm (Figure 3) is observed in Chétoui VOO in the beginning of the ripening stage and stored at fridge temperature while the lowest value is observed in VOO extracted in the beginning of the harvesting stage and stored at ambient temperature. Concerning luteolin amounts, the highest value is observed in VOO obtained at the end of the maturity stage (S2) and stored at low temperature

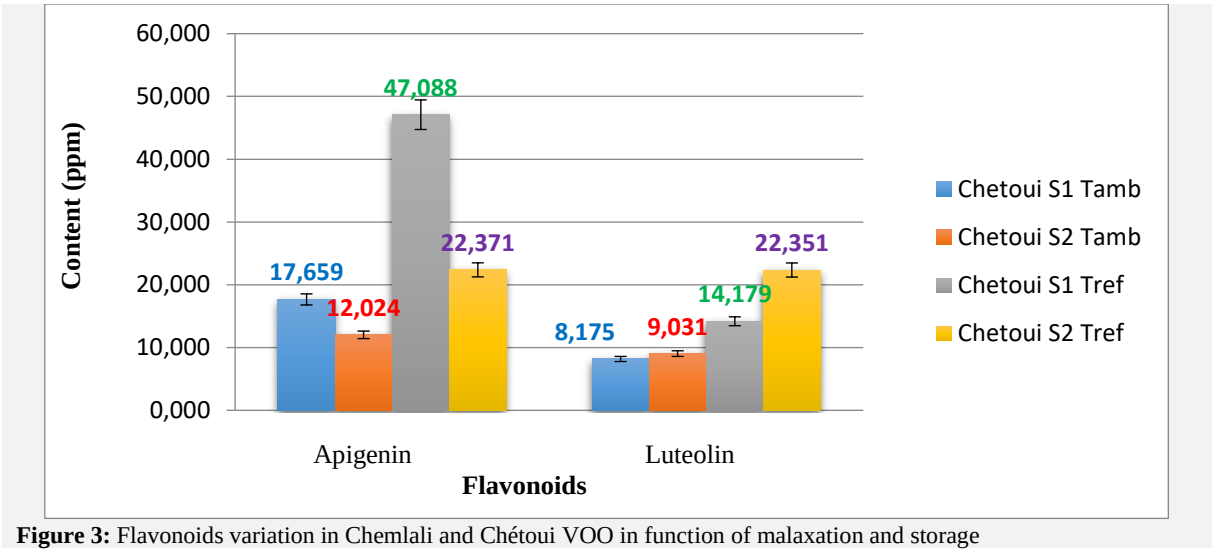

Figure 3: Flavonoids variation in Chemlali and Chétoui VOO in function of malaxation and storage

Principal component analysis

The PCA (Figure 4) was used as an exploratory analysis to explain, the influence of the malaxation temperature on the chemical composition of VOOs from Chemlali and Chétoui olive oil. The PCA shows a high positive correlation between the first component (that explained 37.88%) of the total variance) and the parameters percentage of fatty acid C16:1, C16:0, C20:0, C18:2, C18:3, and volatile compounds decanal, E-b-ocimene, E-2-hexenal, methyl salicylate and the pigment carotenoids. A negative correlation with C20:1, C18:1, C18:0, the oxidation parameters K232, K270, Free acidity, hexanal the volatile compounds E-3- hexenyl acetate, hexanal, a- copaene, and palmitic acid (C16:0). The second component (explained 19.02 % of the total variance) was mainly positively correlated to the volatile 1- hexanol and valencene, and negatively to PV value.

The PCA showed the presence of two main groups of olive oils according to malaxation temperature. Chetoui and Chemlali produced at 25^0C are more richer in terms of aromatic compounds 1-hexanol, related to the fruity, bitter and pungent sensory attributes (Angerosa et al., 2004; Luna et al., 2006) with lower PV values than when produced at 30°C and 35°C temperatures (marked by higher oxidation parameter PV, higher free acidity and higher amounts of alcohol Z-2-pentenol, E-2-hexenal, hexanal which accord with bitterness, pungency and fruitiness).

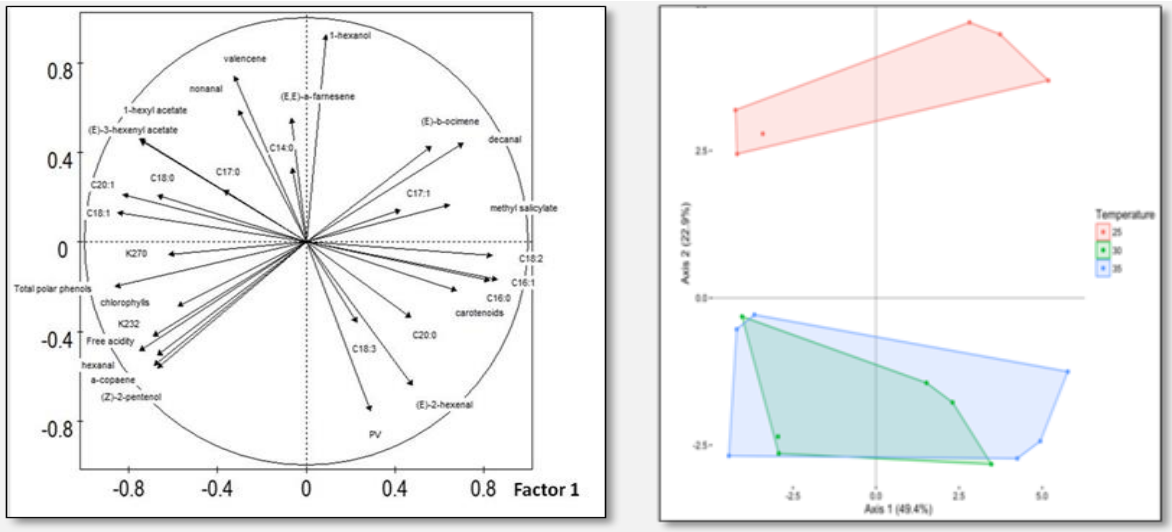

Figure 4 : PCA applied to the chemical variables of olive oils Chemlali and Chétoui as a function of the mixing temperature factor (25°C, 30°C, 35°C)- IM = 3.5.

The PCA (Figure 5) shows that the two first factorial axis explained 88.2% of the variability among olive oil samples. A clear separation is observed among oil samples according to ripeness stage. Oils produced from fruits harvested at early ripeness stage were characterized by their richness in carotenoids and hexanal with higher values of quality indices free acidity, PV, K232 and K270 while those obtained from olive fruits harvested at the end of the crop season presented volatile compounds such as nonanal, copaene and farnesene that denote negative attributes (Angerosa et al., 2004; Luna et al., 2006).
This is explained by the variable correlation graphic (fig. 5) Indeed, the first axis positively correlated to C16:0, C16:1, C20:0 and C18:2 and negatively correlated to chlorophyll, total polar phenols and the fatty acids C17:0, C18:1, C20:1 and free acidity. On the other hand, the second axis is positively correlated to carotenoids and hexanal, quality indices free acidity PV, K232 and K270 while negatively correlated to the volatiles nonanal, copaene and farnesene.

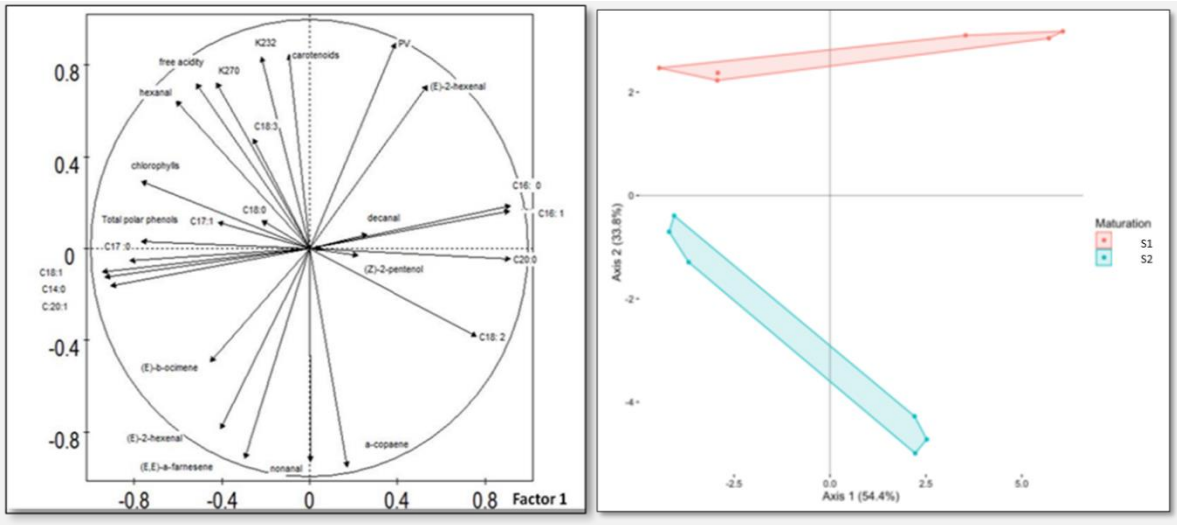

Figure 5: PCA applied to chemical variables of olive oils Chemlali and Chétoui according to ripening stage (RI: 3.5-4.5).

4. Conclusion

A significant impact of the cultivar is observed on antioxidant and fatty acid composition of studied VOOs. No significant effect of ripening was observed on acidity, PV, and extinction coefficients. The malaxation temperature has a significant influence on the VOO polyphenol contents. The storage temperatures presented a significant effect on chlorophyll and phenolic compound amounts.
Among studied samples, the olives harvested in the beginning of harvesting season produced oils characterized by low free acidity, high antioxidant content and stability during storage.
Malaxation at 35°C caused an increase in free acidity values and chlorophyll contents and a decrease in polyphenol contents and oxidative stability. The storage time has a significant effect on the quality and composition of olive oils by increasing free acidity, degrading the chlorophylls and carotenoids,

decreasing levels of antioxidants and reducing oxidative stability. Among samples, Chétoui VOO was the most stable to oxidation.

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