Effect of pigments and total phenols on oxidative stability of monovarietal virgin olive oil produced in Morocco

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This work was carried out on monovarietal virgin olive oils from *Moroccan Picholine* cultivar produced in northern Morocco to assess their oxidation stability as affected by chlorophyll and carotenoid contents and total phenols under different extraction systems, production sites and crop seasons. Results showed that the assessed traits were greatly influenced by the extraction system as compared to the production site and crop season. Two-phase decanter centrifugation allowed the production of virgin olive oil with higher values of carotenoids, total phenols and oxidative stability. Chlorophylls were more abundant in those from a super-pressure system. The effect of the production site on the studied oils was also observed, although in a lesser degree. The highest levels of pigments were scored in Bouchfaa, while total phenols content was greater in Bni Frassen. About the crop season, 2014 exhibited the highest values of chlorophylls and better oxidative stability, while higher contents of total phenols and carotenoids were recorded for 2015. Lastly, a stepwise regression was applied to explain the relationships between oxidative stability and the rest of the traits. The contribution of carotenoids with total phenols content to the variability of oxidative stability was around 51%.

Keywords: Carotenoids, chlorophylls, virgin olive oil, oxidative stability, total phenols.

1. INTRODUCTION

Olive is the major fruit crop in Morocco with a growing acreage of 920,000 ha. Taza province, located in northern Morocco, is one of the most important olive-growing regions with an area of about 78,800 ha, accounting for 36% of the provincial agricultural lands and 9% of the national olive orchards. Olives produced by the province account for 7% of national production, standing at 90,000 tons in a normal year [1]. Olive oil industry in Taza province is made up of 1000 units of traditional mills (Maasra), 19 in super pressure and 30 in centrifugation system (23 with three-phases and only 7 units with two-phases decanter). All units have contributed to the crushing of about 50,000 tons of olives giving a production of 7,000 tons of olive oil during the crop season 2015 [1].

The implementation of the 'Green Morocco Plan' has led to a remarkable development of the olive sector in northern Morocco. Unfortunately, the quality of olive products (mainly olive oils) is poorly discussed, comparing to the production progress. Moreover, despite the economic importance of the olive oils produced in northern Morocco, there is scarce information on its chemical composition and properties in scientific literature, regarding the effects of the climatic conditions and agronomic practices with particular emphasis on the Taza province.

Olive oil composition can change dramatically depending on numerous factors such as geographical production area (altitude and soil composition), climatic

conditions prevailing in the production year, cultivar, and extraction process [2, 3, 4, 5].

The oxidative degeneration is one of the major causes of significant deteriorative changes in the chemical, sensory and nutritional properties of olive oil; its evaluation is mainly made by the study of the oxidative stability that is an axial trait in assessing the quality of olive oil [6]. In the presence of oxygen, the oxidation generates unstable compounds leading to changes in sensory and nutritional characteristics of the oil, hence the deterioration of the product. Autoxidation is a slow radical chain process that occurs in three stages, namely induction, propagation and termination. During the induction period, alkyl radicals are formed and undergo a reaction with oxygen molecules to form hydropevroxides (ROOH) and peroxy radicals during the propagation phase. The termination of chain reactions occurs via combination of free radicals to form stable adducts [7]. Phenolic compounds and pigments known for their high antioxidant activity, promote the resistance of olive oils against oxidative deterioration [6]. The role of polyphenols in inhibiting oxidation in virgin olive oils is well defined as primary antioxidants. Their contribution on the oxidative stability has been confirmed by several studies [8, 9]. The presence of pigments in olive oils appears also of great importance, and largely affects its oxidative stability. Chlorophylls and carotenoids effects have been a great concern of food scientists. Several authors have mentioned the role of carotenoids as a protective antioxidant against the oxidation of olive oils [6, 10, 11]. Chlorophylls and their derivatives, in the presence of light, contribute greatly to the susceptibility of olive oil oxidation because of their photosensitised oxidation promoting activities [10, 12].

The objective of this work was (i) to examine the influence of the extraction system, production site and crop season on some quality traits (oxidative stability, carotenoids, chlorophylls and total phenols content) of *Moroccan Picholine* olive oils produced in north of Morocco, and (ii) to study the relationships between oxidative stability and the rest of the tested traits.

2. EXPERIMENTAL PART

2.1. SAMPLING

Samples of monovarietal virgin olive oil of the *Moroccan Picholine* cultivar ($n = 54$) were collected at the end of December during two consecutive crop seasons 2014 and 2015 (twenty-seven samples in each season). Oils sampling was conducted from industrial oil mills located in three sites in the Taza province (north Morocco): Bni Frassen (34°21'35" N, 4°22'57" W, 354 m), Bouchfaa (34°5'17" N, 4°17'6" W, 602 m), and Taza (34°12'36" N, 3°52'0" W, 552 m) (eighteen samples from each site). These sites are representative of one of the most important olive

growing regions in Morocco. At sampling, olive fruits from all sites had approximately the same maturation index (5-6). The latter was determined following the method described by Uceda and Frías [13]. Oils from each site were extracted and collected in three replicates using three-phases (C3) and two-phases (C2) decanter centrifugation and the super-pressure system (SP) (eighteen oils samples from each system). C3 extraction system consisted in milling the washed olives in the hammer crusher, and then the olive paste was kneaded for 30 min with the addition of hot water, to allow the small olive droplets to coalesce and to facilitate the separation of oil from the other phases. The liquid phases (oil and water) is separated from the solid phase (olive cake) using a horizontal centrifuge (decanter). These liquid phases are submitted to a vertical centrifugation to separate olive oil from olive mill wastewaters. In the C2 extraction process, the olives were washed and crushed, using a hammer mill, to a fine paste. The olive paste was kneaded for 30 min and pumped into a two-phases industrial decanter. The latter separates the olive oil from the mixture of olive cake and vegetable water into a single phase called 'wet pomace'. Lastly, the olives are washed and ground in the super-pressure system using large millstones for approximately 45 min. The obtained olive paste is spread onto stacked fibre disks and placed into the press. The disks are then put under super-pressure of about 400 kg/cm^2 in a hydraulic piston. The solid phase of the paste is compacted, while the liquid phases (oil and water) are drained through the disks. During this operation, water is added to facilitate oil separation. The olive oil is then separated by simple decantation.

The collected olive oil samples were put in dark glass bottles of 250 ml with no space at the top and brought to the laboratory where they were refrigerated at a temperature of 4°C, until their analysis.

2.2. PEDO-CLIMATIC CONDITIONS

The climate in the overall area of study is Mediterranean-type with mild and humid winters and dry and hot summers. For the three studied sites, the 2014 season was marked by an average annual rainfall of 515 mm and mean temperatures during summer (coinciding with the olive development stage) ranged from 24.7°C to 29.2°C, while the 2015 season was less rainy with average annual precipitations of only 348 mm and a relatively hotter summer with mean temperatures ranged from 25.3°C to 31.8°C.

Regarding the pedologic characteristics of the three studied sites, Bni Frassen have a Typic Chromoxeret vertisol with a high content of expanding 2:1 lattice clay developed on very fine parental material from the marl alteration. Bouchfaa is characterized by a Typic Xerochrept fersiallitic soil with calcareous reserve and a clay-rich B-horizon, moderately developed on very fine parental material from the alteration of the Lias limestone. The soil in Taza is a Typic Xerofluvent; a

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weakly developed soil of alluvial contribution with a silty clay loam texture and parental material from middle and old quaternary.

2.3. ANALYTICAL METHODS

2.3.1. Pigment content

Content of chlorophyll and carotenoid compounds, expressed as mg/kg of oil, were determined from the absorption of the olive oil dissolved in cyclohexane, at 670 and 470 nm respectively, using Jenway Model 6100 spectrophotometer (visible range), following the method of Minguez-Mosquera et al. [14].

2.3.2. Total phenols content

Total phenols were extracted according to the method described by Zunin *et al.* [15]. Olive oil samples in *n*-hexane were extracted with aqueous methanol (60/40, v/v) three times. The concentration of total phenols was determined following the Folin-Cicalteu method [16] using caffeic acid as standard (Sigma-Aldrich, St. Louis, MO, USA). Values for total phenols content are given as mg caffeic acid/kg oil.

2.3.3. Olive oil stability

Oxidative stability was assessed using the Rancimat method [17] and was given as the oxidation induction time (hours) determined with a Metrohm Rancimat 743 apparatus using 3±0.01 g of the test olive oil that were submitted to thermal degradation at 100±1.6°C by bubbling a stream of air at a rate of 15 l/h.

2.4. STATISTICAL ANALYSIS

All analytical determinations were performed in triplicate. Combined analyses of variance (ANOVA) were carried out over extraction systems, production sites and crop seasons. Least significant difference values were calculated at the 5% level. In a second stage and to point out the relationships between the studied factors and analysed traits, principal component analyses (PCA) were carried out on the correlation matrix, calculated on the mean data of all replicates. In addition, stepwise linear regression analysis (SLRA) was applied to select the chemical variables (phenols, carotenoids, chlorophylls) that better explain the oxidative stability. The STATGRAPHICS Centurion XVII package (Statpoint Technologies, Inc., Virginia, USA) was used for all the calculations and artworks.

3. RESULTS AND DISCUSSION

Results from this work displayed the high influence of the studied factors on most tested traits in olive oils. Furthermore, a large variability was revealed in virgin olive oil compositions between extraction systems, production sites and crop seasons.

The ANOVA analyses (Tab. I) showed that the extraction system had major effect on all analysed traits and explained about 26% of the total variance for the total phenols and more than 34% for the other traits (chlorophylls, carotenoids and oxidative stability). Moreover, mean comparisons among extraction systems (Tab. II) revealed that C2 allowed the extraction of virgin olive oil with high levels for carotenoids, total phenols content (antioxidants), hence the good scores of oxidative stabilities that can give it a great shelf-life. This is consistent with that reported in similar works [2, 18, 19, 20]. The amount of water added, and water solubility explains the differences in total phenols content with respect to each system. In fact, C3 requires the addition of large amounts of warm water that increase the loss of phenols by solubilisation [21]. These differences could be attributed to other variables involved in the extraction process like olive crushing, malaxation machinery, temperature applied and time of contact with water [22]. Virgin olive oil from C2 was also richer in carotenoids

Table I - Mean squares of the combined analyses of variance of virgin olive oil samples produced at different sites in northern Morocco (Bni Frassen, Bouchfaa, and Taza) using three extraction systems (two and three-phases decanter centrifugation and super-pressure system) during two crop seasons (2014 and 2015).

Source of variation	Df	Chlorophylls	Carotenoids	Total phenols	Oxidative stability
Season	1	0.676***	0.004	20509.40***	0.938
Site	2	0.155**	0.108***	5846.59***	5.620*
System	2	0.706***	0.148***	11740.30***	13.675**
Season × Site	2	0.362***	0.025	4014.78***	1.968
Season × System	2	0.086	0.050**	191.36	0.829
Site × System	4	0.0667	0.024*	786.36	3.572
Season × Site × System	4	0.117**	0.042**	2060.12**	0.890
Replicate (Season × Site × System)	18	0.032	0.030**	387.05	0.529
Residual	18	0.025	0.008	345.31	1.462
Total	53				

* Significant at 0.05 probability level, ** Significant at 0.01 probability level, *** Significant at 0.001 probability level

Table II - Means and range values of analyzed chemical traits of virgin olive oil samples (n = 54). produced at three different sites in northern Morocco (Bni Frassen, Bouchfaa, and Taza) using three extraction systems (two [C2] and three [C3] phases decanter centrifugation and super-pressure [SP] system) during two crop seasons (2014 and 2015).

	Chlorophylls (mg/kg)		Carotenoids (mg/kg)		Total Phenols (mg/kg caffeic)		Oxidative Stability (h)	
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*
Crop season								
13-14 (n=27)	2.06[a]	1.89-2.23	1.07[a]	1.02-1.21	523.72[b]	503.85-543.60	27.37[a]	26.08-28.67
14-15 (n=27)	1.47[b]	1.30-1.64	1.12[a]	0.98-1.17	626.85[a]	606.97-646.73	26.37[a]	25.38-27.97
Extraction System								
C2 (n=18)	1.16[b]	0.96-1.37	1.36[a]	1.24-1.48	652.77[a]	628.43-677.12	29.59[a]	28.00-31.17
C3 (n=18)	2.05[a]	1.85-2.26	1.04[b]	0.92-1.15	528.63[b]	504.29-552.98	26.37[b]	24.78-27.95
SP (n=18)	2.09[a]	1.88-2.29	0.89[b]	0.77-1.01	544.45[b]	520.11-568.80	25.12[b]	23.53-26.70
Site								
Bni Frassen (n=18)	1.82[a]	1.61-2.03	1.04[b]	0.91-1.15	604.93[a]	580.58-629.28	26.64[b,c]	25.06-28.23
Bouchfaa (n=18)	1.98[a]	1.77-2.19	1.32[a]	1.21-1.44	600.65[a]	576.30-624.99	28.66[a]	27.07-30.24
Taza (n=18)	1.50[b]	1.29-1.70	0.93[b]	0.81-1.04	520.28[b]	495.94-544.63	25.77[c]	24.19-27.36

[abc] Means for each trait followed by the same letter are not significantly different at P <0.05.

than those from C3 and SP systems characterised by the addition of warm water during malaxation mainly because of their excessive degradation under higher temperatures [23]. Chlorophyll content was more abundant in olive oil obtained by the SP system, probably due to mixing leaves with olives when they are crushed, given the absence of a well-developed leaf removal system of olives in this type of extraction process. Efficiency problems of leaf removal system in three-phase decanter units could also explain the high level of chlorophylls in oils extracted by these units, since these are the most widely used in the study area. Influence of the extraction system and olive processing on pigments level were also observed by other studies [22, 24]. Contrariwise, other studies stated that pigment contents in olive oil did not vary from one extraction system to another [2, 5, 25].

The production site effect was not of great magnitude and did not explain more than 25% of variability in all cases (Tab. I). Effect of the production site on the composition of the studied olive oil was also observed, although in a lesser degree. Similar results were reported for different varieties and geographical areas [26, 27, 28, 29]. Among sites, Bouchfaa exhibited higher contents of chlorophylls and carotenoids along with good oxidative stability (Tab. II). The highest levels of pigments were recorded in 'Bouchfaa', while total phenols content was greater in 'Bni Frassen'. Criado *et al.* [27] attributed the difference in pigment level to the prevailing temperatures during November and December. In fact, severe frosts could have caused deterioration of the olive fruit and degradation of the pigment, mainly in the chlorophyll fraction. Variations of carotenoid content were related in the literature to temperatures, altitudes and probably water regimes applied in each site [5, 30, 31]. Similar finding was reported by Rouas *et al.* [4], who found that oils obtained from olives cultivated at higher altitudes showed higher chlorophyll and carotenoid contents compared with those from lower altitudes.

Total phenols content ranged from 495.94 to 629.28 mg caffeic acid/kg oil. Bni-Frassen displayed olive oil with the highest mean value, and Taza showed the lowest one (Tab. II). Impact of production site on phenols content in our olive oil samples was confirmed by various studies [4, 26, 27, 32, 33]. The highest level recorded in Bni-Frassen could be attributed to its lower altitude as indicated by Mousa *et al.* [34] who observed that higher phenol contents are produced in lower altitudes. Ranalli *et al.* [26] reported that total phenols were strongly influenced by the origin zone, i.e., by the pedoclimatic factors of the production environment. The effect of altitude, tree ages and the irrigation management in a given growing area were also indicated in other works [4, 32].

Concerning the crop season, the influence was important only for chlorophyll and the total phenols content that which accounted respectively for about 32% and 45% of the observed variance (Tab. I). When comparing between the two crop seasons (Tab. II), total phenols content was higher during 2015 as compared to 2014. Contrariwise, 2014 scored higher values for chlorophyll content. No significant differences were observed for carotenoids and oxidative stability. In fact, the prevailing climatic conditions in each season could explain these differences. Therefore, a reduced amount of rainfall was recorded in the 2015 season compared to the previous season. This water shortage might be the cause of variability in pigment and phenols content. Various studies have evaluated phenol and pigment contents in relation to the water availability. Greven *et al.* [35] have reported that polyphenols in virgin olive oils decreased with increased irrigation level. In the same trend, Tura *et al.* [36] have indicated a positive correlation between the temperature sum and total phenols content of olive oils. Water shortage tends to generate a stress situation in the olive tree leading the production of phenols in the olive fruit [37]. Furthermore, total phenols were higher in the years

with the highest heat summation at a given olive ripening stage [36]. In contrast, a higher chlorophyll level was positively associated to abundant rainfall that could inhibit the peroxidase activity [38]. Morelló *et al.* [32] attributed the difference in pigment contents to the stress that the fruit could endure during the summer period.

To detect the combination of variables that best explained the existing variability, principal component analysis (PCA) was performed on the correlation matrix based on data mean values as shown in Figures 1, 2 and 3. The first two PC axes accounted for 83% of total variance; 67 and 16% for axes 1 and 2, respectively. The first PC axis (PC1) clearly separated oxidative stability, carotenoids and total phenols in its negative direction from chlorophyll content in the positive direction. On the second axis (PC2), all variables tested appeared jointly on the positive side.

Figure 1 shows the projection of points related to the extraction system on the surface delimited by PC1 and PC2. PC1 discriminated clearly between C2 on its negative side, and C3 and SP, both on the positive one. In addition, virgin olive oil samples extracted by C2 had higher values of carotenoids,
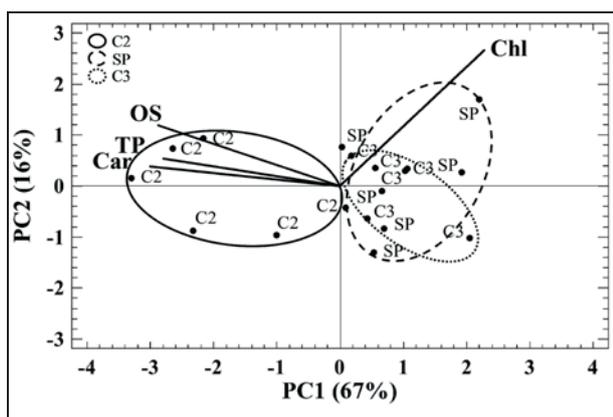

Figure 2 - PCA projections on axes 1 and 2 accounting for 83% of total variance. Eigenvalues of the correlation matrix are symbolized as vectors representing traits (Car: carotenoids, Chl: chlorophylls, OS: oxidative stability, and TP: total phenols) that most influence each axis. The 18 points representing virgin olive oil samples means for each site (B-F: Bni Frassen, BC: Bouchfaa, and T-C: Taza) are plotted on the plane determined by axes 1 and 2.

Figure 1 - PCA projections on axes 1 and 2 accounting for 83% of total variance. Eigenvalues of the correlation matrix are symbolized as vectors representing traits (Car: carotenoids, Chl: chlorophylls, OS: oxidative stability, and TP: total phenols) that most influence each axis. The 18 points representing virgin olive oil samples means for each extraction system (C2: two-phases decanter, C3: three-phases decanter, and SP: super-pressure) are plotted on the plane determined by axes 1 and 2.

total phenols, and oxidative stability, and low amounts of chlorophylls in contrast to C3 and SP. This confirmed what was previously illustrated by ANOVA analyses (Tab. I). The same axis (PC1) allowed a clear separation between sites; Bouchfaa interacted with carotenoids, total phenols, and oxidative stability on the negative direction, while both Taza and Bni Frassen were located on the positive direction with important amount of chlorophylls (Fig. 2). With respect to the crop season, the observation

Figure 3 - PCA projections on axes 1 and 2 accounting for 83% of total variance. Eigenvalues of the correlation matrix are symbolized as vectors representing traits (Car: carotenoids, Chl: chlorophylls, OS: oxidative stability, and TP: total phenols) that most influence each axis. The 18 points representing virgin olive oil samples means for each crop season (2014 and 2015) are plotted on the plane determined by axes 1 and 2.

of Figure 3 showed an important separation among olive oil samples on the second axis 2 (PC2). In fact, 2014 season was associated with great amounts of
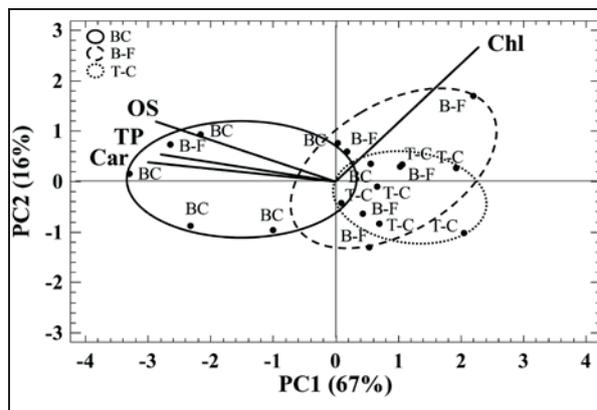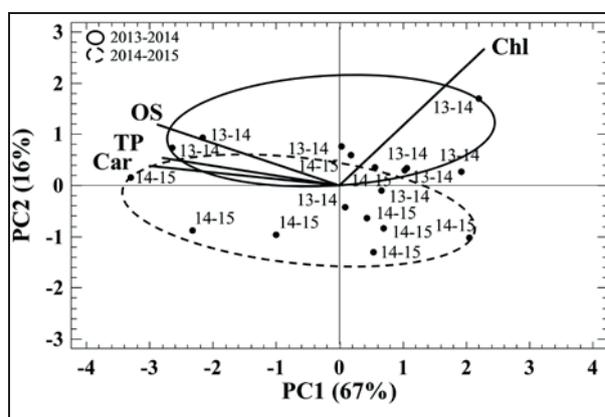
chlorophylls and good scores for oxidative stability on the positive side, whereas, the following season (2015) was on the negative side with considerable amounts of carotenoids and total phenols.

Associations between studied traits elucidated by PCA were confirmed by correlation studies shown in Table III. Oxidative stability was significantly correlat-

ed to carotenoids (r = 0.744, p < 0.001) and total phenols (r = 0.625, p < 0.001). Chlorophyll content was negatively but not significantly associated with all traits. Such associations were also found by several studies that have focused on the assessment of the virgin olive oil quality from different varieties and origins [2, 3, 6, 8, 10, 39]. Carotenoids, and in particular β-carotene, are potent antioxidants because of their ability to quench oxygen radical species, and they also may act as light filters [6, 10]. Rahmani and Saari Csallany [11] indicated that β-carotene was a strong natural inhibitor of photooxidation for all light intensities under low temperatures; however, antioxidant activity of β-carotene was reduced at 40°C owing to its rapid destruction. Phenolic compounds,

Table III - Correlations between chlorophylls, carotenoids, total phenols and oxidative stability for data from virgin olive oil samples (*n* = 54) produced at different sites in northern Morocco (Bni Frassen, Bouchfaa, and Taza) using three extraction systems (two and three-phases decanter centrifugation and super-pressure system) during two crop seasons (2014 and 2015).

	Carotenoids	Chlorophylls	Total Phenols	Oxidative Stability
Carotenoids		- 0.507	0.637**	0.744***
Chlorophylls			0.437	- 0.380
Total Phenols				0.625**

* Significant at 0.05 probability level. ** Significant at 0.01 probability level. *** Significant at 0.001 probability level.

the main antioxidant compounds in virgin olive oils, can give a hydrogen atom to the lipid radical (alkylperoxyl radical) formed during the propagation stage of lipid oxidation and then producing a stable radical [6]. A negative relationship was found in our study between chlorophyll content and oxidative stability, thus confirming the results of a previous study [12] that indicated that, in the presence of light, chlorophylls and their derivatives are the most active promoters of photosensitised oxidation in virgin olive oils contributing greatly to its oxidation susceptibility.

In addition, a stepwise linear regression analysis (SLRA) based on the results found previously by ANOVA and PCA, was applied to highlight the relationship between the oxidative stability as a dependent variable, and all analysed traits (independent variables). SLRA selected the chemical variables: carotenoids, and total phenols as having together the maximum correlation with stability and explained 51% of oxidative stability variation. Chlorophylls were removed from the model because they were not statistically significant at a 95.0% or higher confidence level. The equation of the fitted model is shown in Table IV. The favourable role of carotenoids in olive oil stability was assigned by several previous studies. It has been reported that the olive oil stability was well associated to carotenoid contents [40], and more precisely with carotenes [41, 42]. The protective effect of β-carotene

Table IV - Stepwise regression analysis considering oxidative stability as dependent variable, and chlorophylls, total phenols and carotenoids, as independent variables, for data from virgin olive oil samples (*n* = 54) produced at different sites in northern Morocco (Bni Frassen, Bouchfaa, and Taza), using three extraction systems (two and three-phases decanter centrifugation and super-pressure system) during two crop seasons (2014 and 2015).

Traits included	Oxidative stability (OS)	
	Partial R^2	Model R^2
Carotenoids (Car)	0.45*	0.45
Chlorophylls (Chl)	-	-
Total phenols (TP)	0.06	0.51
Final equation	OS = 16.7797 + 4.416 × Car + 0.00919416 × TP	

*Significant at 0.05 probability level. **Significant at 0.01 probability level. ***Significant at 0.001 probability level.

against oxidative damage induced by light was also demonstrated in soybean oil [43]. Carotenoids have been considered as major singlet oxygen stabilisers by many authors [44, 45]. Regarding total phenols, numerous reports have confirmed their participation in providing protection against oxidation in olive oil. In fact, Gutiérrez *et al.* [41] reported a great positive correlation of stability with total phenols level (r = 0.77). Similarly, a strong relationship was shown between the values of oxidative stability and total phenols contents in Tunisian cultivars cultivated under rainfed conditions [39]. The effect of the total phenols on the oxidative stability was demonstrated by other works that confirmed their ability to inhibit the evolution of the secondary stage of oxidation processes [7, 46]. However, previous researchers have reported that total phenols and carotenoid contents did not completely explain the oxidative stability of olive oils, and the degree of correlation was different in these works depending on olive oil characteristics [2, 6, 9, 10]. Moreover, Aparicio *et al.* [8]. had investigated the influence of several compounds on the stability of virgin olive oil measured by Rancimat. They reported that the contribution of phenolic and orthodiphenolic compounds was the most important followed by the fatty acid composition, and in lesser percentage α-tocopherol, carotenoids and chlorophylls. In a similar study, Ceci and Carelli [47] indicated that the oxidative stability was mainly influenced by the fatty acid profile, polyphenols and carotenes. In addition, fatty acid composition was the main contributing factor to the oxidative stability of 'Hojiblanca' oils, while the contribution of total phenols and total tocopherols was of minor importance [44].

4. CONCLUSIONS

This research might be of great interest; given the importance of the olive oil sector in northern Morocco as well as the lack of data characterising local products that are still underestimated. It could be concluded that the assessed traits of monovarietal

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virgin olive oil from *Moroccan Picholine* cultivar produced in the Taza province (northern Morocco) were firstly influenced by the extraction system and then by the crop season and the production site. In addition, the oxidative stability of local olive oils has shown a great dependence on carotenoid content in synergy with total phenols. Additional works should be addressed to assess the whole composition of local olive oil regarding different maturity indices and different farming practices including irrigation and fertilisation.

Acknowledgements

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