The Effects of Ripening Stage and Mode of Culture of Chemlali, Arbequina and Koroneiki on the Capacities of Oils to Scavenge ABTS Free Radicals

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Abstract

The aim of this study is to evaluate the antioxidant power of the olive oil against the cation radical $ABTS^{\bullet+}$, according to cultivars: Chemlali, Arbequina and Koroneiki; culture modes: extensive and intensive; ripening stages: November and December corresponding stages and study year: 2017 and 2018. Radical percentage inhibition (PI), Trolox equivalent antioxidant capacity (TEAC), inhibitory concentration required to scavenge 50% of radicals (IC50) and principal component analysis (ACP) were determined. The oil of the Tunisian main cultivar Chemlali, cultivated in extensive mode, has the best scavenging power with the lowest IC50 values of 9.3, 10.8, 9.65 and 10.4 µg/ml respectively in all the ripening stages. Also, at the lowest tested concentration of 20 µg/ml, this oil has the highest TEAC values, respectively 2.28 and 2.20 in November of 2017 and 2018. For the introduced cultivars, Koroneiki presents better performance than Arbequina,

in intensive mode, with IC50 ranged from 9.6 to 13.8 against 12.40 to 22.35 for Arbequina. Furthermore, the principal component analysis proves that the oils of Chemlali in extensive mode and Koroneiki in intensive have the best scavenging capacity of $ABTS^{\bullet+}$. This study proves that the extensive mode is the best culture mode and the ripening stage of November is the best stage in order to get oils with a height antioxidant capacity.

Keywords: Olive Oil, Chemlali, Koroneiki, Arbequina, Ripening stage, extensive mode, intensive mode, Antioxidant activity, $ABTS^{\bullet+}$

Introduction

The economy of Tunisia depends heavily on olive oil, where olive production offers a profession with a social, cultural, and economic dimension. Unquestionably one of the key vital sectors for the national economy in general and agriculture in particular, this sector represents an enormous amount of legacy for Tunisia (Aissaoui, 2009).

Moreover, olive oil is one of the centerpieces of the so-called "Mediterranean" diet, noted for its diverse health benefits (Jacotot, 1996). Thus, olive oil is renowned for its abundance in phenolic ingredients, which give it a flavor with an extraordinary nutritional value, and very medicinal benefits. As well, these advantages have been associated with its unique composition of fatty acids, of which oleic acid constitute the major compound. Oleic acid, and several minor biomolecules like vitamins, polyphenols, and other natural sources of antioxidants may help to defend against the cellular changes brought on by free radicals, and play a part in the etiology of many illnesses. Olive oil has a significant impact in cardiovascular disease, some cancers, cranial pathologies, and degenerative illnesses connected to atherosclerosis and accelerated ageing process degenerations (Jacotot, 1996; Covas, 2007). A continual increase in interest in olive oil as a healthy food has been seen outside of the Mediterranean countries due to its richness in useful nutritional and power antioxidants (Matos et al., 2007; Temine et al., 2008).

Numerous factors, including the type and ripeness of the fruit (Ajana et al., 1999), the extraction process (Stefano et al., 1999), oil preservation (Gutierrez et al., 2002), and climatic and agronomic factors, affect the content and the quality of the oil (El Antari et al., 2000).

In this context, the antioxidant power of the olive oil was tested according to many cultivars, local and introduced, culture modes and ripening stages. A comparative study was conducted against the cation radical $ABTS^{\bullet+}$.

Materials and methods

Plant material and growing areas

The study was carried out on the main Tunisian cultivar Chemlali, representing more than 80% of national oil production (Feki et al., 2013), and the introduced cultivars Arbequina and Koroneiki, known for their great productivity (Usanmaz et al., 2019). Chemlali has been cultivated in both extensive (12m × 12m) and intensive (6m × 6m) modes, while Arbequina and Koroneiki has been cultivated in intensive mode. The experimental orchard is located in the region of "Menzel Mhiri" in the province of Kairouan, Tunisia. The study was carried out over two successive years, 2017 and 2018, and in two ripening stages corresponding to two months of harvest, November and December. The agronomic processes carried out were the same for each cultivar: fertilizing, spraying for parasites, and pruning. The olives were harvested by hand directly from the plant in different dates according to different stages of maturity, and immediately taken to the laboratory, where the oil was extracted within 12 hours of harvesting, using a laboratory olive crusher machine. After crushing and mixing the olive paste, oil extraction was performed at room temperature by means of a pressure system. The olive paste was mixed for 30 min. The oil was separated from the water (by centrifuge), then filtered through a paper at room temperature and stored in dark bottles at 4°C until analysis.

Reagents

Commercial $ABTS^{\bullet+}$; Potassium persulfate ($K_2S_2O_8$); Deionized water; Ethanol and Dimethyl Sulfoxide (DMSO) were obtained from SMS Bio Sud Medical Services.

$ABTS^{\bullet+}$ free radical scavenging activity

The ABTS scavenging activity was determined as described by Arnao et al. (2001). The method is based on the oxidation of the ammonium salt of 2,2'-azinobis ethylbenzothiazoline-6sulphonic acid to the radical cation $ABTS^{\bullet+}$.

This method was performed in several steps. First, a stock solution was prepared. It contained 39.2 mg of commercial $ABTS^{\bullet+}$, 6.7 mg of Potassium persulfate ($K_2S_2O_8$) and 10 ml of deionized H_2O. A dark blue coloration appeared and an incubation for 24 hours was necessary. Next, 0.6 ml of the stock solution was added to 50 ml of ethanol. The spectrophotometer was calibrated by ethanol and the maximum absorbance of the prepared $ABTS^{\bullet+}$ solution was adjusted around 800 nm (± 0.005).

A large series of small quantities of oil samples ranging from 20 to 100µg was adjusted to a final volume of 1 ml with the $ABTS^{\bullet+}$ solution. For λ max, the volume of the oil was replaced equally by ethanol. Dimethyl

Sulfoxide (DMSO) was added at 5% of the oil volume to increase its solubility. After 30 min, the absorbance was measured at 734 nm, at several time intervals (5, 10, 15, 20 and 30 minutes) and each test was repeated three times.

Statistical analysis

Using the statistical software tool "SPSS 22," data were subjected to statistical analysis. Results were the average of three experiments. A one-way analysis of variance (ANOVA) was conducted before the Tukey test. At $P < 0.05$, differences between individual means were considered significant. The relationships between cultivars, culture modes, harvest years, maturity data, Trolox equivalent antioxidant capacity (TEAC), and the amount of oil required to reduce the initial concentration of $ABTS^{\bullet+}$ by 50% (IC50) were assessed using principal component analysis (PCA).

Results

Antioxidant activity of the different cultivars'oils, according to diverse culture modes and stages of maturity, during 2017 and 2018.

Antioxidant activity of Chemlali cultivated in extensive mode

A significant antioxidant activity is immediately noted with the contact of $ABTS^{\bullet+}$ (Figure 1). It reaches its highest inhibitory power of 96.06% at a low concentration of 20 µg/ml after 20 minutes, in November of both years, 2017 and 2018. Beyond this concentration, the inhibitory effect of Chemlali oil seem to be less important with an average of 80% of radical inhibition. The unregistered activity grows slightly with the time of incubation, especially in December 2018, rising from 80% after 5 min of contact to over 90% after 30 min, at a concentration of 100µg/ml. Chemlali oil, when cultivated in extensive mode, has a very high capacity to inhibit free radicals. Thus, to inhibit most free radicals, only lower doses of 20 µg/ml are necessary.

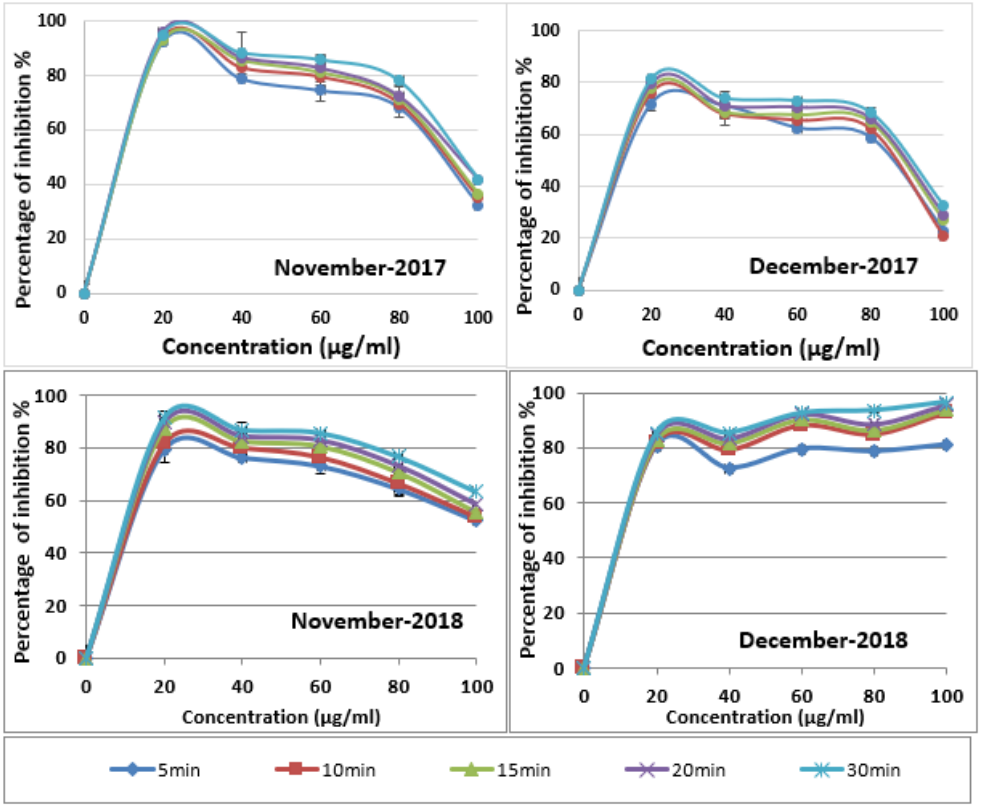


Figure 1. Antioxidant activity of Chemlali oils, when cultivated in extensive mode, against the $ABTS^{\bullet+}$ radical cation according to the stage of maturity, during 2017 and 2018

Antioxidant activity of Chemlali cultivated in intensive mode

A minor dependence on interaction duration is found, but the difference in maturity stage is clearer. In November 2017, a concentration of 100 µg/ml at a period of 15 minutes produced a maximum inhibition of 94% (Figure 2).

In contrast, the minimum value appears in December 2017 starting at a concentration of 20 µg/ml after 5 minutes. The curves from the first five minutes of interaction with the radical cation ABTS•+, at a concentration of 20 µg/ml and in November 2017, indicate moderate antioxidant activity. According to contact time and level of concentration, this activity rises. At a concentration of 40 µg/ml, the inhibition power rises within the first five minutes to about 76%. When the concentration reaches 100 µg/ml, it increases to a maximum of 91%. The antioxidant activity in December 2017 varies with concentration, with a maximum value of 100% of inhibition of ABTS•+ at a concentration of 100 µg/ml and at a period of 10 minutes.

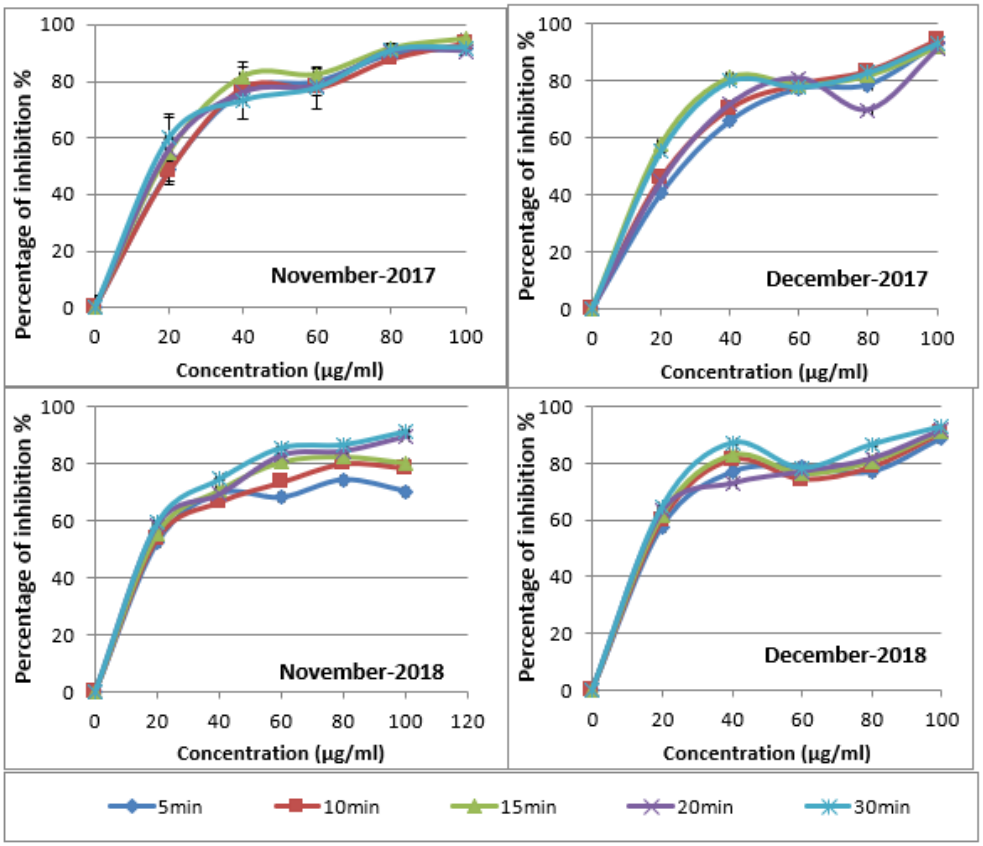


Figure 2. Antioxidant activity of Chemlali oils, when cultivated in intensive mode, against the $ABTS^{\bullet+}$ radical cation according to the stage of maturity, during 2017 and 2018

The inhibitory power reaches around 92% in November 2018 at a concentration of 100 µg/ml during 30 minutes of contact. However, it appears that this activity is time-dependent because it increases from 5 to 30 minutes. At a concentration of 20 µg/ml, it is about 50%. Then, at a concentration of 100 µg/ml, it inhibits more than 90% of free radicals.

Antioxidant activity of Koroneiki cultivated in intensive mode

Graphs illustrate antioxidant activity over four dates, beginning with the first five minutes of contact, when it is maximum at a concentration of 85.54 µg/ml, and peaking in November 2017 at 92% of $ABTS^{\bullet+}$inhibition (Figure 3). This activity increases according to both contact duration and concentration. In fact, it may reach 59% at a concentration of 20 µg/ml after30 minutes of contact, and over 99% at a concentration of 100 µg/ml after the same amount of time.

As a function of time, Koroneiki's antioxidant activity in December 2017 shows little fluctuation. This variation exhibits noticeable action during the

first five minutes and inhibits the ABTS$^{\bullet+}$ radical cations by 62% at a concentration of 40 µg/ml. After 5 minutes of contact and at a concentration of 80 µg/ml, a maximum activity of 94% is noted.

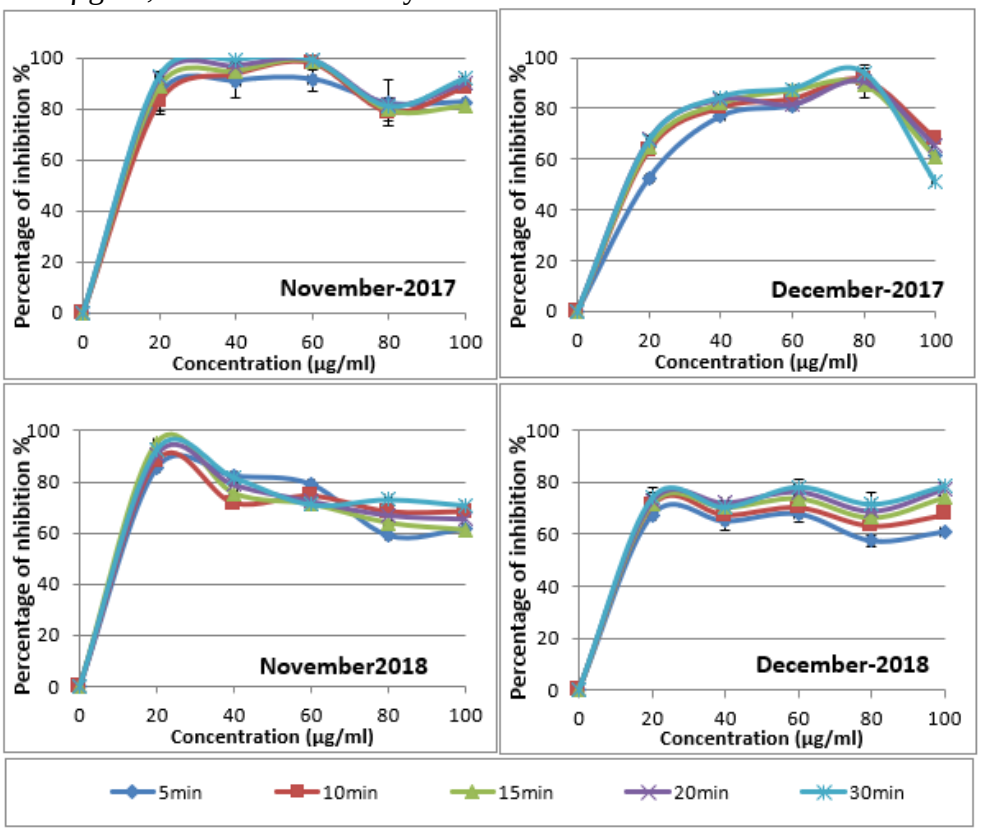


Figure 3. Antioxidant activity of Koroneiki oils, when cultivated in intensive mode, against the ABTS$^{\bullet+}$ radical cation according to the stage of maturity, during 2017 and 2018

In all doses, the ABTS$^{\bullet+}$ radical cations are inhibited with an average inhibitory power of 40 to 70% in November 2018. However, within 5 minutes of interaction, over 91.51% of the free radicals are inhibited at a concentration of 60 µg/ml. This power develops to 78% in 30 minutes while maintaining the same level of concentration. This percentage was found simultaneously at a concentration of 100 µg/ml. At a concentration of 20 µg/ml in December 2018, Koroneiki has an antioxidant activity that appears to develop, reaching a maximal activity of 95% inhibition of radical cations (ABTS$^{\bullet+}$) after 15 minutes. Therefore, over 30 minutes, this activity fluctuates between 80 and 95% at this dose. The inhibitory potency did not, however, surpass 81% of radical inhibition at the remaining concentrations, with 81.86% being measured at a concentration of 40 µg/ml. This leads to conclude that the antioxidant activity is powerful at a low concentration of 20 µg/ml.

Antioxidant activity of Arbequina cultivated in intensive mode

At a concentration of 100 µg/ml and in November 2017, the antioxidant activity rises to a maximum around 99% over the course of 30 minutes (Figure 4). At 20 µg/ml, it reaches 59% over that time. A modest temporal dependency is seen in December 2017, however the difference in function of the concentration is more striking. At a concentration of 100 µg/ml for the first five minutes, more than 79% of the radicals are inhibited as opposed to 53% at a concentration of 20 µg/ml.

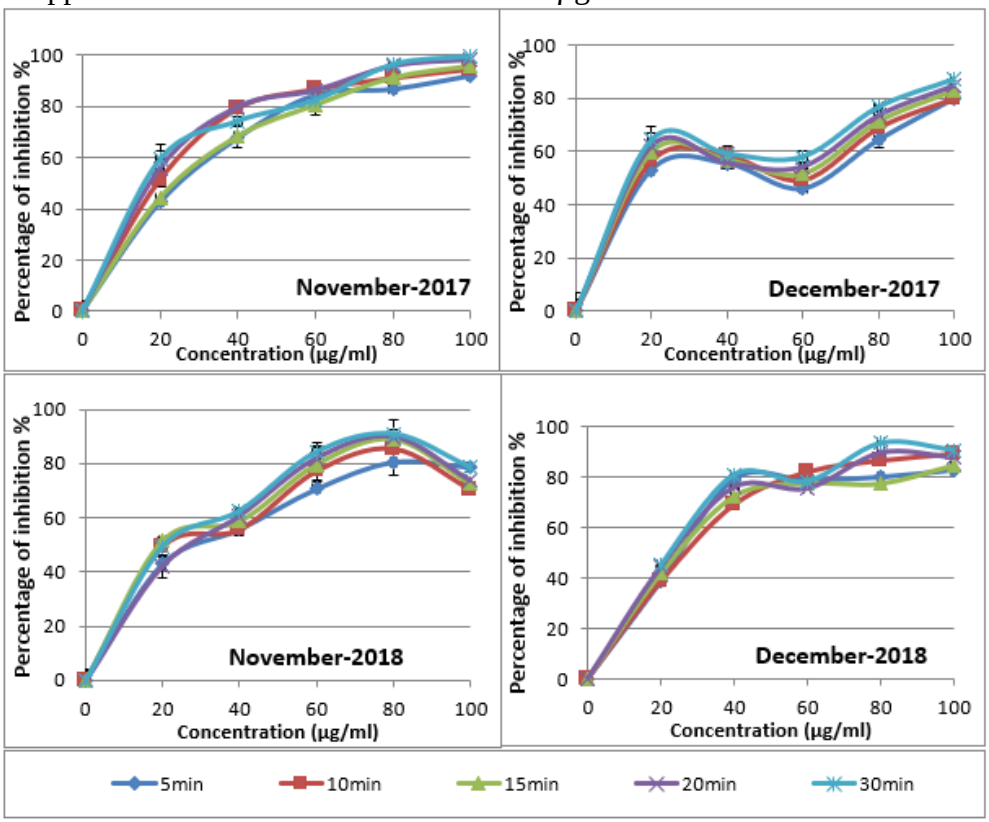


Figure 1. Antioxidant activity of Arbequina oils, when cultivated in intensive mode, against the $ABTS^{\bullet+}$ radical cation according to the stage of maturity, during 2017 and 2018

At a concentration of 80 µg/ml in November 2018, the antioxidant activity is extremely strong and inhibits free radicals by more than 90% after 30 minutes of contact. At the same time, it may block the $ABTS^{\bullet+}$ radical by about 84% at a concentration of 60 µg/ml. In contrast, it starts to decline at a concentration of 100 µg/ml and reaches 78% during the first five minutes.
Therefore, the development of antioxidant activity over time is almost nonexistent. The results for antioxidant activity at a concentration of 20 µg/ml and at other concentrations were found to differ significantly in December 2018. In fact, at a concentration of 20 µg/ml, 38% inhibition of free radicals was

observed after 5 minutes. This number then rises to 48% after 30 minutes. However, at the other concentrations, it changes from 68 to 80% at 40 µg/ml within 30 minutes, from 75 to 81% at 60 µg/ml, from 77 to 93% at 80 µg\ml and from 82 to 90% at 100µg\ml.

The inhibitory concentration required to scavenge 50% of the free radicals (IC 50)

The inhibitory concentration required to scavenge 50% of the free radicals in the reaction mixture (IC50) was determined (Table 1). The IC50 analysis of oils of the studied varieties reveals that the highest antioxidant activity is recorded for the oil of Chemlali, when cultivated in extensive mode. Indeed, Chemlali oil requires only the lowest concentration to inhibit the 50% of free radicals compared to oils of the other cultivars. The oil of this variety needs only 9.3, 10.8, 9.65 and 10.4 µg/ml respectively in all the dates of study to scavenge 50% of free radicals.

For the introduced cultivars, Koroneiki oils show the highest capacity to inhibit 50% of the free radicals compared to those of Arbequina. It necessities 9.6, 13.8, 9.46 and 11.50 µg/ml of oils to inhibit 50% of free radicals in all the dates of ripening respectively.

Table 1. The inhibitory concentration required to scavenge 50% of the free radicals (IC50) of the different cultivars' oils, according to diverse culture modes and stages of maturity, during 2017 and 2018

		November 2017	December 2017	November 2018	December 2018
Extensif	**Chemlali**	9.30 ± 0.43b	10.80±0.72**c**	9.65 ± 0.55b	10.40±2.02**b**
Intensif	**Chemlali**	15.60±0.69a	17.50± 1.22**a**	14.55±0.95ab	14.60± 3.83**ab**
	Koroneiki	9.60± 2.02b	13.80± 0.36**bc**	9.46 ± 0.46ab	11.50± 0.87**b**
	Arbequina	12.40± 1.30a	14.10± 1.73**ab**	20.50± 8.89a	22.35± 4.50**a**

Different letters correspond to significant differences of cultivars on the same date at the level of 5%

Consequently, according to IC50 analysis, the main Tunisian cultivar Chemlali has the highest capacity to inhibit 50% of the free radicals compared to the introduced cultivars in the majority of the studied dates. In addition, the oils produced in the month of November of all the studied cultivars have the most important capacity to inhibit free radicals than those produced in the month of December in both years of study.

Trolox equivalent antioxidant capacity (TEAC)

The activities of all oil samples were converted to Trolox-equivalent antioxidant capacity. All of them were able to scavenge the $ABTS^{\bullet+}$ radical cation. The average values obtained for the Trolox equivalent antioxidant capacity (TEAC) are shown in the Table 2. It's clearly that the TEAC values

depend on the cultivars, the concentration of olive oil and the stages of ripening. When cultivated in extensive mode, Chemlali possesses an oil which is able to scavenge the maximum of free radicals at the lowest concentration (20 µg/ml) in all the studied stages. Its TEAC values are 2.25, 1.96, 2.20 and 2.39, when cultivated in intensive mode. For the oil of Chemlali, it reaches the maximum of its capacity to scavenge free radicals at 100 µg/ml. However, for the introduced cultivars, TEAC values of Koroneiki oils seem to be better than those of Arbequina. In November 2017, the oils of Koroneiki reach their maximum of inhibiting free radicals at 60mµg/ml with TEAC value equal to 2.38. In December2017, this maximum is reached at 80 µg/ml with a TEAC value of 2.26. In the second year of study, the maximum of their antioxidant capacity is unregistered at only 20 µg/ml (the lowest tested concentration) with a TEAC value of 2.23 in November 2018. Nevertheless, in December 2018, these oils show their highest capacity to scavenge the maximum of free radicals at the maximal tested concentration,100 µg/ml, with a TEAC value of 1.90.

For the oils of Arbequina, these oils necessitate 100 µg/ml to inhibit the maximum of free radicals in November 2017 (TEAC = 2.39) and December 2017 (TEAC = 2.09). In the second year of study, the performance of these oils appears better. It's need only 80 µg/ml to scavenge the maximum of free radicals.

Table 1. Trolox equivalent antioxidant capacity (TEAC) values of the different cultivars' oils, according to diverse culture modes and stages of maturity, during 2017 and 2018

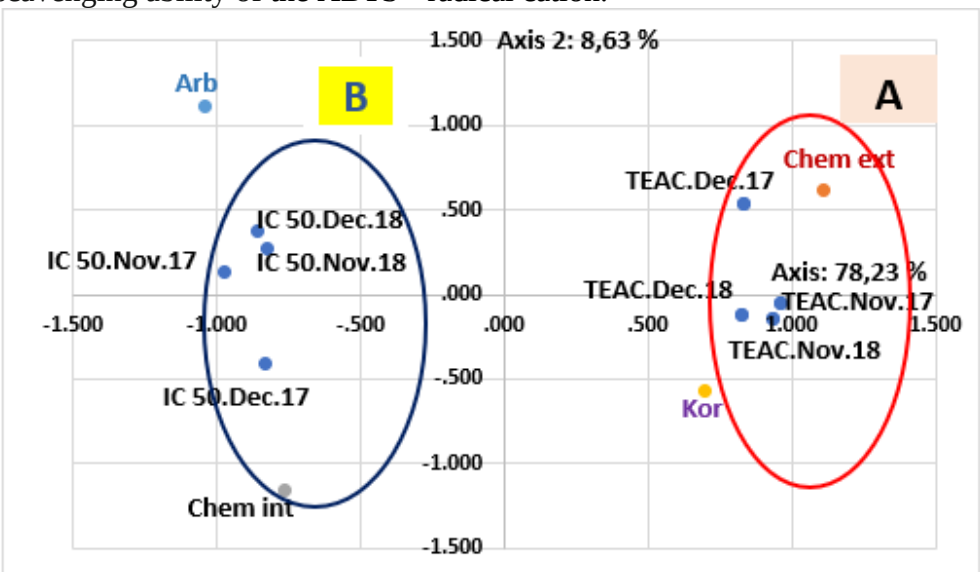
Time of harvest	Culture mode	Cultivar	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
November 2017	Extensif	Chemlali	2.28 ± 0.09a	2.12 ± 0.16a	2.06 ± 0.19ab	1.88 ± 0.03b	1.02 ± 0.03b
	Intensif	Chemlali	1.45 ± 0.06b	1.76 ± 0.17a	1.87 ± 0.16b	2.18 ± 0.18a	2.21 ± 0.17a
		Koroneik	2.24 ± 0.02a	2.39 ± 0.02a	2.38 ± 0.02a	1.95 ± 0.05a	2.21 ± 0.01a
		Arbequina	1.43 ± 0.09b	1.79 ± 0.15a	1.98 ± 0.17b	2.32 ± 0.07a	2.39 ± 0.01a
December 2017	Extensif	Chemlali	1.96 ± 0.05**a**	1.78 ± 0.01**a**	1.76 ± 0.06**a**	1.65 ± 0.50 **a**	0.79 ± 0.23**a**
	Intensif	Chemlali	1.34 ± 0.09**c**	1.92 ± 0.03**a**	1.88 ± 0.03**a**	2.00 ± 0.06 **a**	2.25 ± 0.00**a**
		Koroneik	1.62 ± 0.04**ab**	2.03 ± 0.21**a**	2.11 ± 0.25**a**	2.26 ± 0.01 **a**	1.23 ± 0.16**a**
		Arbequina	1.55 ± 0.12**bc**	1.42 ± 0.02**a**	1.40 ± 0.06**a**	1.84 ± 0.06 **a**	2.09 ± 0.02**a**
November 2018	Extensif	Chemlali	2.20 ± 0.08a	2.09 ± 0.02a	2.06 ± 0.16a	1.85 ± 0.01a	1.53 ± 0.08b
	Intensif	Chemlali	1.56 ± 0.10ab	2.10 ± 0.02ab	1.89 ± 0.09a	2.08 ± 0.09a	2.24 ± 0.03a
		Koroneik	2.23 ± 0.03a	1.97 ± 0.08ab	1.72 ± 0.20a	1.76 ± 0.07a	1.71 ± 0.07ab
		Arbequina	1.19 ± 0.42b	1.51 ± 0.09b	2.03 ± 0.03a	2.19 ± 0.08a	1.90 ± 0.12ab
December 2018	Extensif	Chemlali	2.39 ± 0.22**a**	2.05 ± 0.22**a**	2.22 ± 0.05**a**	1.86 ± 0.11**a**	2.32 ± 0.04**a**
	Intensif	Chemlali	1.43 ± 0.08**bc**	1.80 ± 0.01**a**	2.06 ± 0.16**a**	2.09 ± 0.02**a**	2.20 ± 0.08**a**
		Koroneik	1.78 ± 0.06**ab**	1.70 ± 0.10**a**	1.88 ± 0.04**a**	1.73 ± 0.07**a**	1.90 ± 0.12**a**
		Arbequina	1.10 ± 0.16**c**	1.95 ± 0.24**a**	1.89 ± 0.05**a**	2.25 ± 0.05**a**	2.18 ± 0.03**a**

Different letters correspond to significant differences of cultivars on the same date and same concentration at the level of 5%

Principal component analysis (PCA)

PCA was performed according to IC50 and TEAC of all oil samples (Figure 5). Whatever the year 2017 and 2018, TEAC values in November are present at the positive end of axis 1, showing a strong correlation (R=0.901). TEAC values in December of both years 2017 and 2018 are also located in the same direction of axis 1 but with a lower correlation with (R=0.754) and (R=0.779) respectively. All the TEAC values form a single group (A) which is very close to extensively grown Chemlali and shows the strongest antioxidant activity. This group is also close to Koroneiki, also showing significant activity. All this group (A) formed by TEAC values is inversely proportional to a group (B) formed by IC50 values further proving the importance of the activity with high TEAC values and low IC50 values. However, Chemlali grown intensively and Arbequina are found on the negative side of the axis1 and close to the group (B) containing IC50s, thus showing low antioxidant activities.

The extensive cultivation mode is the best mode where the anti-oxidant activity appears to be powerful. Chemlali cultivated in extensive mode and Koroneiki cultivated in intensive mode seem to have the best scavenging ability of the $ABTS^{\bullet+}$ radical cation.



Chem ext.: Chemlali cultivated in extensive mode; Chem int.: Chemlali cultivated in intensive mode; Kor.: Koroneiki; Arb., Arbequina; IC 50: The inhibitory concentration required to scavenge 50% of the free radicals; TEAC: Trolox equivalent antioxidant capacity; Nov.: November; Dec.: December; 17: 2017; 18: 2018.

Figure 2. Principal component analysis of the different cultivars' oils, according to diverse culture modes and stages of maturity, during 2017 and 2018, and their IC50 and TEAC values

Table 3 validate all the results shown by the principal component analysis (PCA). A higher positive correlation between percentage of inhibition of free radicals in months of November in 2017 and 2018 (R= 0.901) is noted, showing then similar behavior in the same month of both years. Also, the percentage of inhibition of November 2017 have a strong correlation with the TEAC values of November 2017 (R=1) and the TEAC value of November 2018 (R=0.901). Similarly, to the other year 2018, where the percentage of inhibition of November 2018 have a height positive correlation with TEAC values of November 2017 (R=0.901) and TEAC values of November 2018 (R=1). Additionally, we find that the IC50 of November 2017 has a height negative correlation with the TEAC values of November 2017 (R=-0.976) and the same with IC50 in November 2018 and the TEAC values of November 2018 (R=-0.838). All this data of correlation proves that the antioxidant activity in November is stronger than the other dates of ripening. November is the best stage of ripening in order to get a good quality of olive oil.

Discussion

In terms of the antioxidant activity of olive oils against the radical cation $ABTS^{\bullet+}$, the results show that the oil of the widely cultivated Chemlali cultivar has the strongest inhibitory activity, and it doesn't require a high concentration to reach its maximal potency. In addition, all the tested oils exhibit their maximum of free radical scavenging activity, reaching up to 99% in some cases, in the month of November of both studied years. November corresponds to the strongest antioxidant activity for all studied cultivars. Furthermore, the year 2017 corresponds to the best result (higher inhibition percentages) and then a more richness in antioxidant.

Chemlali is a prominent cultivar from Tunisia that has previously been demonstrated for its high antioxidant content, not just in its virgin oil but also in all of its organs, including the leaves, stems, flowers, roots, and fruits as well as in the pulps or cores. These intriguing antioxidants were either found in volatile fractions or organic extracts (Saidana et al., 2015; 2021a et b; Ben Mansour et al., 2020).

Table 2. Correlation table between all the studied parameter

	IP.Nov.17	IP.Dec.17	IP.Nov.18	IP.Dec.18	IC50.Nov.17	IC50.Dec.17	IC50.Nov.18	IC50.Dec.18	TEAC.Nov.17	TEAC.Dec.17	TEAC.Nov.18	TEAC.Dec.18
IP.Nov.17	1,000	,754	,901	,779	-,976	-,753	-,726	-,795	1,000	,754	,901	,779
IP.Dec.17	,754	1,000	,714	,630	-,719	-,880	-,586	-,514	,754	1,000	,714	,630
IP.Nov.18	,901	,714	1,000	,675	-,896	-,686	-,838	-,824	,901	,714	1,000	,675
IP.Dec.18	,779	,630	,675	1,000	-,809	-,572	-,557	-,700	,779	,630	,675	1,000
IC50.Nov.17	-,976	-,719	-,896	-,809	1,000	,778	,801	,890	-,976	-,719	-,896	-,809
IC50.Dec.17	-,753	-,880	-,686	-,572	,778	1,000	,605	,666	-,753	-,880	-,686	-,572
IC50.Nov.18	-,726	-,586	-,838	-,557	,801	,605	1,000	,884	-,726	-,586	-,838	-,557
IC50.Dec.18	-,795	-,514	-,824	-,700	,890	,666	,884	1,000	-,795	-,514	-,824	-,700
TEAC.Nov.17	1,000	,754	,901	,779	-,976	-,753	-,726	-,795	1,000	,754	,901	,779
TEAC.Dec.17	,754	1,000	,714	,630	-,719	-,880	-,586	-,514	,754	1,000	,714	,630
TEAC.Nov.18	,901	,714	1,000	,675	-,896	-,686	-,838	-,824	,901	,714	1,000	,675
TEAC.Dec.18	,779	,630	,675	1,000	-,809	-,572	-,557	-,700	,779	,630	,675	1,000

IP.: percentage inhibition; IC 50: The inhibitory concentration required to scavenge 50% of the free radicals; TEAC: Trolox equivalent antioxidant capacity; Nov.: November; Dec.: December; 17: 2017; 18: 2018.

The difference of the antioxidant capacity between the oils harvested in different maturation stages can be explained by the fact that as the maturity process advances, a number of changes, including adjustments to the polyphenol, o-diphenol, flavonoid, saponin, phytosterol, and tannin profiles, take place in the various parts of the olive. Flavonoids (apigenin, luteolin), secoiridoids (oleuropein), phenolic alcohols (tyrosol, hydroxytyrosol), phenolic acids (vanillic, p-coumaric, ferulic, caffeic acid), and lignans (pinoresinol, acetoxypinoresinol) are some of the numerous groups of phenolic compounds found in olive oil (Bouaziz et al., 2004).

Throughout the growth and ripening phase, the phenolic compounds in *Olea europaea* L. fruits change in both quality and quantity (Amiot et al., 1989). One of the primary phenolic chemicals in unripe olives that produces Fruits' bitterness is caused by the secoiridoid oleuropein a hydroxytyrosol (3,4-dihydroxyphenylethanol) ester glucosylated elenoic acid, the aglycone, which oleuropein can produce. It is well known that hydrolysis makes a molecule pharmacologically active (Esti et al., 1998). A straight forward phenolic substance called hydroxytyrosol is another intriguing component found in olive fruits. The extra virgin olive oil contains this lipid- and water-soluble cathecol-shaped molecule, either as a simple phenol or esterified with elenolic acid (Boskou, 1996). For its capacity to scavenge free radicals, hydroxytyrosol was examined and tested with success (Visioli et al., 1999).

The biggest abundance of polyphenols in the oil can be the reason of a higher antioxidant activity. Phenols undoubtedly play an important role in olive stability and could be considered the most effective antioxidants. Tocopherols, carotene, lutein, squalene, lipophilic and hydrophilic phenols, are the antioxidants found in olive oil in highest concentrations. The phenolic components of olive oil include phenolic acids and their derivatives (vanillic acid, gallic acid), phenolic alcohols (tyrosol, hydroxytyrosol), secoiridoids (oleuropein, oleocanthal), lignans (pinoresinol), and flavones (luteolin). Polyphenols in olive oil have antioxidant properties. The phenolic compounds hydroxytyrosol, tyrosol, oleuropein, and oleocanthal are primarily responsible for antioxidant activity, protection against blood lipid oxidation, anti-inflammatory activity, the potential to be anticarcinogenic, the resistance to oxidative stress, and other positive effects on human health.
The phenolic compound most widely used in olive cultivars is oleuropeine, which is significant in the early stages like our first stage of analysis: November and can reach 14% of dry matter in young fruits. It is made up of three subunits: a polyphenol (hydroxytyrosol), a secoiridoid (elenic acid), and a glucose molecule. During the maturation of fruits or the production of oil, chemical and enzymatic reactions allow the concentration of oleuropein to be reduced and the concentration of hydroxytyrosol to be increased

(Omar, 2010). Hydroxytyrosol is an effective antioxidant (Bubonja-Sonje et al., 2011).

α-Tocopherol is another compound in olive oil that contributes to its antioxidant activity. Among the natural antioxidants present in virgin olive oil, tocopherols stand out because of their antioxidant and important nutritional activities (Aguilera et al., 2005). Another natural antioxidant that can be found in the oils of the studied oils and correlate with stability and flavor are the phenolic substances which are measured by colorimetry (Salvador et al., 2001; Aparicio et al., 1999; Ben Mansour et Saidana 2021).

Dabbou et al., in 2010 characterized the oils of Chemlali cultivated in different area in Tunisia (Sfax, Boughrara, and Zarzis) and compared it with the oils of the introduced varieties Arbequina and Koroneiki. They find that α- Tocopherol is the most abundant one of the virgin olive oil; its values ranged from 147.8 mg.kg^{-1} for Chemlali Sfax, to 577.8 mg kg^{-1} for Chemlali Boughrara. Then, in spite of the lower Chemlali Sfax content (147.83 mg.kg^{-1}), Tunisian cultivars showed the highest contents of α-tocopherol. This result can explain why the oil of our Chemlali cultivated in extensive mode in Kairouan have the highest antioxidant activity with the highest TEAC values at 20 µg/ml and the lowest IC50 at the same concentration. Besides, the abundance of this compound in Arbequina and Koroneiki oils is 269.51 and 381.41 mg. Kg^{-1} respectively.

Therefore, Bayram et al. in 2012, have analyzed the **α**-tocopherol content of 55 olive oil samples and its correlation to total reducing capacity, individual phenolic compounds and antioxidant activity assays. α-Tocopherol content of oils may be affected by many factors, such as olive variety, ripening, geographical region, agricultural and technological factors , and varied greatly between the olive oil samples (38–330 mg/kg oil). No correlation was observed between the α-tocopherol concentrations and TEAC. However, a moderate correlation was found between **α**-tocopherol and total reducing capacity ($R=0.34$, $p<0.01$). Also, the same scientists showed that TEAC values highly correlated with the hydroxytyrosol and moderately with the oleuropein and tyrosol contents. Hydroxytyrosol was found to have the highest correlation coefficient with TEAC values. In the literature, hydroxytyrosol is reported with higher antioxidant activity than tyrosol and oleuropein. The hydroxytyrosol content correlated highly with the tyrosol ($R= 0.72$, $p<0.01$) and moderately with the oleuropein ($R=0.33$, $p<0.05$) content in the 55 olive oils studied.

Furthermore, the most phenolic studied compounds are carotenes and chlorophylls. For the carotenes the highest values are found in the oils of Koroneiki (20.03 mg/Kg) and in the oils of Chemlali Zarzis (17.49 mg. Kg^{-1}). The highest abundance of chlorophylls was found with the amount of 38.46, 38.42 and 22.24 mg. Kg^{-1} in the oils of Chemlali Zarzis, Koroneiki

and Arbequina respectively. Also, this study proves that the abundance of the antioxidants is better in the oils of Koroneiki than Arbequina. This can be an explanation about the reason that the antioxidant activity of Koroneiki is higher than that of Arbequina. Our results can also be validated by Dabbou et al. in 2009. They compared the antioxidant activity of oils of Arbequina, Koroneiki, Leccino, Oueslati and Chemchali. They found that the oils of Koroneiki had a higher total antioxidant status test with ABTS (TAA-ABTS = 0.24 mM Trolox Kg^{-1}) than the oils of Chemchali (TAA-ABTS = 0.22 mM Trolox.Kg^{-1}), oils of Leccino (TAA-ABTS = 0.13 mM Trolox.Kg^{-1}), Oueslati TAA-ABTS =0.09 mM Trolox. Kg^{-1}) and Arbequina (TAA-ABTS =0.07 mM Trolox.Kg^{-1}).

Conclusion

To assess the quality of the oils of the Tunisian main cultivar, Chemlali, which is cultivated extensively and intensively, as well as of the introduced cultivars Arbequina and Koroneiki, which are grown intensively during two stages of maturity, November and December, over two successive years, it is crucial to characterize the oils' antioxidant capacity toward free radicals. The mode of extensive cultivation is advantageous since it appears to have strong anti-oxidant action. The $ABTS^{\bullet+}$ radical cation appears to be better scavenged by Chemlali cultivated in an extensive mode and Koroneiki cultivated in an intense mode. As well, November corresponds to the powerful antioxidant activity in the both study years. The analysis of these oil samples by a Gas chromatography coupled with mass spectrometry is necessary in order to have a better explanation about the antioxidants, their proportions, interactions and activity relationship.

Conflicts of interest

The authors declare no conflicts of interest.

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