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Antioxidant activities of ethanolic extracts of four Tunisian olive varieties

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Abstract: Olive leaves extracts of four olive varieties from Tunisian origin: Chitoui, Chimlali, Sahli and Zarrazi were studied for their phenolic contents, antioxidant activities and their effect on corn oil stability. The amounts of total polyphenolics, flavonoids and proanthocyanidins were determined by spectrophotometry. The antioxidant activities of extracts were evaluated and compared with butylated hydroxytoluene (BHT) using a *β*-carotene bleaching assay and a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Chimlali ethanol extract showed the highest total phenolics, flavonoids and proanthocyanidins contents expressed respectively in gallic acid, in quercetin and catechin equivalent per gram of dried matter 245.5; 9.12 and 40.92. Chimlali ethanol extract showed the highest antioxidant activity coefficient (AAC) of 863.636 at 200 mg/ml by the *β*-carotene bleaching method and the highest scavenging activity of 7.5 µg.mL^{-1} at 0.1 mg/mL by the DPPH method. Analysis of the oxidation results of corn oil, heated to 110°C and during frying, showed a significant antioxidant effect of ethanolic olive leaves extracts relative to the BHT. Olive leaves extracts from varieties Chimlali and Sahli showed the highest antioxidant activity. The synthetic antioxidant BHT exhibits a lower antioxidant power than the extracts from varieties Sahli and Chimlali but is comparable to other varieties. The results suggest the introduction of olive leaves extracts in food preparation, both during storage or heating food, for better preservation.

Keywords: olive leaves, extracts, phenolics, flavonoids, antioxidant activity, refined corn oil, thermal oxidation, frying.

Introduction

Antioxidants from natural sources such as fruits, vegetables, and herbs have gained attention due to their protective properties against several chronic diseases like cancer and cardiovascular diseases[1]. Scientific researches have been developed for the extraction, identification and quantification of these compounds[2] .Among plants, olive considered as a source of biactive compunds, is a Mediterranean tree thatis characterized by a fruit the olive, whose oil is an essential component of the Mediterranean diet[3]. About 840 million olive trees are located in the Mediterranean and 90 millions in the rest of the world[4]. The olive tree *Olea europaea* belongs to the family Oleaceae[5]. It has great economic and social importance[6].

Olive leaves are sub-products that can reach 10% of the total weight of processed olives[7]. They accumulate during pruning olive trees and large timber.

Historically. Olive leaves have been widely applied in folk medicine for thousands of years in the European Mediterranean Island. They have been used for the treatment of fever, malaria and other diseases[8].

Olive leaves extract possesses a variety of biological activities including antioxidant, antimicrobial, antiviral, anti-inflammatory and anti hypertensive activities[9]. It has also anti HIV activity by blocking the HIV virus entry to host cells[10]. Recent publication showed that the extract of olives leaves is a potential therapeutic drug for the treatment of the parasite infections *Acanthamoeba*[11].

Many studies demonstrated that numerous bioactive components such as oleuropein hydroxytyrosol and olive biophenols were present in olive leaves[12,13]. Oleuropein posseses many beneficial effects such as antioxidant[14], anti-inflammatory[15], antimicrobial[16], anti-carcinogenic[17], alleviator of cardiovascular disease[18] and anti-diabetic[19]. Oleuropein and hydroxytyrosol are also effective against intestinal infections in humans[20]. They reduce lipid peroxidation and improve antioxidant defense system[21].

In food industry, olive leaves can extend the shelf life of food. Indeed they were used to increase the oxidative stability of the turkey breast fillets and to improve color stability in both bovine and porcine muscle systems[22]. The protective effects of olive leaf extracts on protein and lipid oxidation of pork

patties during refrigerated storage[23a] and frozen storage have been studied too[23b].

The olive leaves extract has a great potential as effective natural antioxidants and may have applications in the development of new functional healthy meat products[24].

Several studies highlighted the beneficial effects of the addition of natural oxidants to vegetable oils[25]. Extracts of olive leaves are also revealed to be effective stabilisers in vegetable oils under oxidative induction[26, 27].

To the best of our knowledge, there are no previous reports related to both the antioxidant activity of olive leaves extracts and the oxidative stability of corn oil enriched in olive leaves extracts.

In the present study, our objective was to evaluate the antioxidant activity of olive leaves extracts from four Tunisian varieties and the effect of adding olives leaves extracts to refined corn oil. The thermal oxidation of the corn oil enriched with Tunisian olive leaves was monitored by measuring peroxide value, free acidity and color. By this treatment, it is believed that the nutritional quality of corn oil could be maintained during heating.

Materials and methods

Reagents

Folin-Ciocalteu reagent, β-carotene, cis-9,12-Octadecadienoic acid (linoleic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT), aluminium chloride ($AlCl_3$), hydrochloric acid (HCl), 3,4,5 –trihydroxydenzoic acid (gallic acid), quercetin, catechin anhydrous sodium carbonate (Na_2CO_3), , ammonium ferric sulphate $NH_4Fe(SO_4)_2$, acetic acid, potassium iodide, sodium thiosulphate were procured from Sigma-Aldrich Chemie. Analytical grade ethanol, chloroform butanol and Tween 40 were obtained from Merck.

Plant material

Four olive leaves varieties were selected: Chitoui variety from Tunis (Mornag) ,Chimlali variety from the south of Tunisia (Tataouine), varieties Sahli and Zarrazi from the center of Tunisia (Sidi Bouzid).

These leaves were collected in January 2012. The harvested plants were identified according to Pottier-Alapetite 1979[28]. Voucher specimens are in the herbarium of the High School of Food Industries for future reference. The selected leaves were dried in the laboratory at room temperature in the shade in a ventilated area. After reaching a stable weight, the dried olive leaves were kept in darkness and cut into small pieces just before handling.

Preparation of olive leaves extracts (OLE)

Fifty grams of dried olive leaves were extracted with 80% aqueous ethanol (3 x 300 mL) by agitated maceration at room temperature for 72 h. The extracts obtained from three extractions were combined, filtered through a Whatman No. 4 filter paper and concentrated under reduced pressure.

Total phenolics, flavonoids and proanthocyanidins contents

Total phenolic content was assessed using the Folin-Ciocalteu reagent according to the method modified by Turkoglu, Duru, Mercan, Kivrak, and Gezer (2007)[29]. An aliquot (0.1 mL) of a suitable diluted extract was added to 0.5 mL of the Folin-Ciocalteu reagent and 1 ml of deionized water. The mixture was shaken and allowed to stand for 1 min, before adding 1.5 mL of 20% Na_2CO_3 solution. The absorbance at 760 nm was recorded after 60 min. Total phenolic content was expressed in mg gallic acid equivalents per gram of extract (mg GAE/g).

Total flavonoid content was measured according to Al-Dabbas, Kanefumi, Hou, and Fujii (2006)[30]. 1.5 mL of the extract was mixed with 2% $AlCl_3$. After 10 min, the absorbance was read at 367.5 nm. Total flavonoid content was expressed in mg quercetin equivalents per gram of extract (mg QE/g).

The HCl/butan-1-ol assay was used to quantify total proanthocyanidins. 0.25 mL of extract was added to 3 mL of a 95% solution of n-Butanol/HCl (95:5 v/v) and 0.1 mL of a solution of $NH_4Fe(SO_4)_2,12H_2O$ in 2 M HCl in stoppered test tubes. The tubes were incubated for 40 min at 95 °C)[31]. The absorbance of the red color was read at 550 nm with data expressed as mg catechin equivalents per gram (mg CE_xg^{-1}).

Antioxidant activity

DPPH radical scavenging assay

Olive leaves extract (OLE) was tested for the scavenging effect on DPPH radical according to the method of Al-Dabbas et al. (2006)[30]. 2 mL of extract solution in methanol (5, 50,100, and 400 mg/mL) were added to 2 mL of a 0.4 mmol/L DPPH methanolic solution.

A solution containing 2 mL of methanol and 2 mL of the DPPH solution was the negative control. As a positive control, synthetic antioxidant BHT was used. Different solutions were kept in the dark at room temperature for 30 min. The scavenging activity on the DPPH was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UVeVis spectrophotometer. All determinations were performed in triplicates. The DPPH radical scavenging activity was calculated using the following equation:

$$\% \textbf{ inhibition} = (1 - A_1/A_0) \times 100$$

A_1 and A_0 are respectively the absorbance of the tested sample and the negative control after incubation.

β-Carotene bleaching method

Antioxidant activity of Olive leaves extract was determined according to slightly modified version of β-carotene bleaching method described Al-Dabbas, Kanefumi, Hou, and Fujii (2006)[30]. Two milligrams of β-carotene were dissolved in 5 mL of chloroform and 0.5 mL β-carotene solution was mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier in a round bottom flask. Then, chloroform was removed in a rotary vacuum evaporator. The resulting mixture was diluted with 50 mL oxygenated distilled water. To 4 mL of this emulsion, 0.2 mL of test sample in ethanol (2 mg/mL) was added. BHT was used for comparative purposes. A solution with 0.2 mL of ethanol and 4 mL of the above emulsion was used as control. A mixture was prepared as described above but without β-carotene to serve as blank. The tubes were covered with aluminium foil and were maintained at 50 °C in a water bath. Absorbance of the emulsion at 470 nm was taken at zero time (t ¼ 0 min) and after every 15 min. Measurement of absorbance continued until the colour of β-carotene disappeared in the control reaction (t ¼ 120 min). All determinations were performed in triplicate. Antioxidant activity coefficient (AAC) was calculated according to the following equation:

AAC = (AA (120) _ AC(120)/AC(0)_ AC(120)) x 1000

Where AA (120) is the absorbance of antioxidant at 120 min, AC(120) is the absorbance of the control at 120 min, and AC(0) is the absorbance of the control at 0 min.

Oil oxidative stability

Refined corn oil

A vegetable refined corn oil purchased from a local market with synthetic antioxidant (commercial oil) and without synthetic antioxidant (the control) was used**.**

Stability parameters

In order to assess the effect of the different extracts in the corn oils during heating , quality parameters (free acidity, peroxide value, color) were determined according to European union standard methods .The samples of corn oil added with 100 ppm and 200 ppm with OLE, at 110 °C for four days were performed to test the antioxidant effect of ethanol extracts. A comparison with both control and treated oils (commercial oil and oil enriched with OLE and with synthesis antioxidant BHT) was performed. Same samples were also subjected to a fry testing. Each sample was heated for 5 min to reach the desired temperature. Thermal stability was evaluated by measuring, peroxide value PV, free acidity, color.

Frying test

The two oil samples (2.5L) were each placed in a deep-fryer. Peeled potatoes (250g) were cut into chips (10 mm × 10 mm × 90 mm) and fried once at 180 °C[32]. After frying, the oil samples were cooled to room temperature and analyzed in triplicate for changes in PV, free acidity, color.

All analytical determinations were performed at least in triplicate.

Peroxide value

Peroxide value (PV) was determined according to the AOAC method[33]. About 5g of oil was weighed into a 250 mL flask. Previously prepared acetic acid/chloroform ($CHCL_3$) solution (30mL), saturated potassium iodide (0.5mL) and distilled water (30mL) were added with occasional shaking. The mixture was titrated with 0.05 $mol.L^{-1}$ $Na_2S_2O_3$ with vigorous shaking, 0.5mL of $10mL.L^{-1}$ starch solution was added and titration was continued with vigorous shaking, to release all iodine from the $CHCl_3$ layer, until the blue colour just disappeared. PV was calculated using the equation:

$$\mathbf{PV\ (meq\ O_2.Kg^{-1}) = (V \times 2M \times 1000)/m}$$

Where V (mL) is the volume of $Na_2S_2O_3$ consumed (blank corrected), M ($mol.L^{-1}$) is the molarity of $Na_2S_2O_3$ and m(g) is the mass of the test sample.

Free acidity

5 g of fat was weighed into a 250 mL Erlenmeyer flask Meyer, 30 ml of neutralized alcohol and the sample is disolved by stirring. 5 to 6 drops of phenolphthalein are then added. Free fatty acids are tittered with a sodium hydroxide solution of normality 0.177 N. The end of the neutralization of the free fatty acid in oil is indicated by the pink color of the solution.

Free acidity content was calculated as % oleic acid using the equation:

FFA content (% oleic acid) = (V x 2M x 28.2)/m

Where V (mL) is the volume of NaOH consumed, M ($mol.L^{-1}$) is the molarity of NaOH and m (g) is the mass of the test sample.

Color measurement

The values of L*[34], a* and b* are measured with a colorimeter (Lovibond PFX 195). Each measurement is repeated 3 times by performing a measurement on 2 g of oil samples.

• The CIE L* a* b* is a model of color representation developed by the CIE (International Commission on Illumination) in 1976.

• The L * is the luminance, which ranges from 0 (black) to 100% (white).

• The component * represents the range of the red axis (127) to green (-128) through white (0) if the luminance is 100%.

• The component a* represents the yellow range of the axis (127) to blue (-128) through the blank (0) if the luminance is 100%.

ΔE* represents the Euclidean distance between two points in the Lab space. This value reflects the total color difference of the sample relative to a reference sample. In our study, the control at time t_0 is taken as a reference to calculate of ΔE*[34,35].
The value of ΔE*is calculated according to the following equation:

ΔE* = |(L*1-L*2)^2 + (a*1-a*2)^2 + (b*1-b*2)^2]^ (1/2)

Where L* 1, a * 1, b* 1 are the components of the reference sample in the space CIE L * a * b * and L* 2, a* 2, b* 2 are the components of the sample measured.

Statistical analysis

Data were presented as the mean of triplicate ± standard deviation (mean ± SD). The data were analyzed for statistical significance using Statgraphics Centurion XVI.

Differences between treatments were assessed using one way ANOVA, followed by Tukey HSD post hoc test. P values below 0.05 were considered significant.

Results and discussion

Extraction yield

Significant differences on extraction yield depending on the studied variety were observed (Figure 1). Zarrazi leaves from Sidi Bouzid showed the highest yield (49.4% w/w) while the lowest (40.2%) was obtained from Sahli leaves collected in Sidi Bouzid. Intermediate values were found in Chimlali and Chitioui leaves with yields of 43.7% and 40.5% respectively.

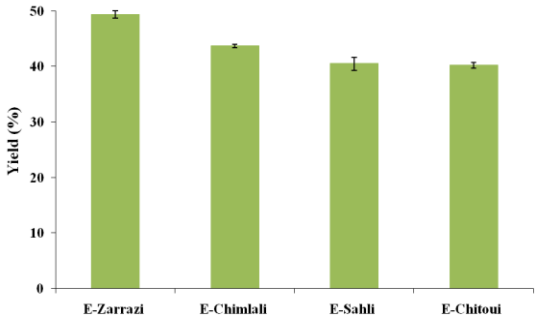

Figure 1. Extraction yield

Similar results were obtained using solvents of the same density and operating with the method of maceration or other extraction methods [36].

Total phenolics , total flavonoïds and proanthocyanidins contents

The amounts of total phenols in extracts depend on the studied variety and they ranged from 245,5mg GAE.g^{-1} to 157,6 mg GAE.g^{-1}. The highest amount was observed with the Chimlali extract followed by the Zarrazi extract variety which has a rate of 193.6 mg GAE g^{-1}, the Chitoui extract variety that contains 188 mg GAE.g^{-1} and the Sahli one contains 157.6 mg GAE.g^{-1} of total phenols (Figure 2).

These results are similar to those reported in the literature**.** The amount of total phenols depends on phenological state. Indeed, methanolic extract variety of Neb Jmel (Tunisia) has an amount of 350 mg GAE.g^{-1} in October and 250 mg GAE.g^{-1} in February[37].

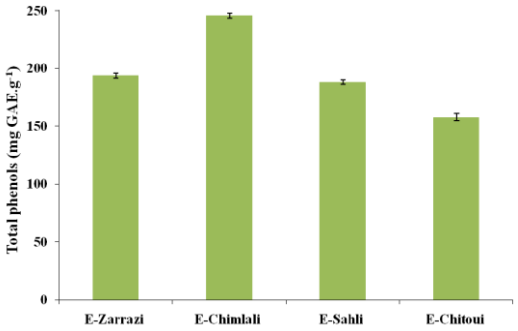

Figure 2. Total phenols rate of ethanolic olive leaves extracts from different varieties (mg GAE g^{-1} : milligram gallic acid equivalent per gram of dry matter)

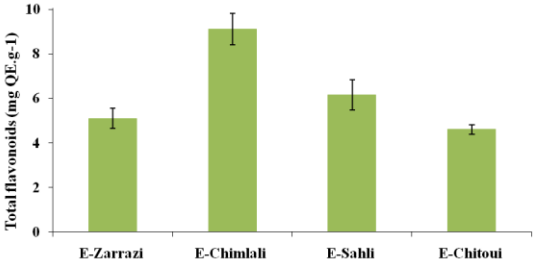

Figure 3. Total flavonoids rate of ethanol olive leaves extracts from different varieties (mg QE g^{-1} : milligramm quercitin equivalent per gramme of dry extract

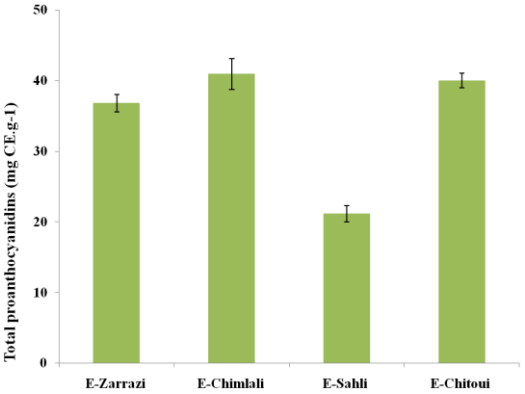

Figure 4. Total Proanthocyanidins rate of ethanolic olive leaves extracts from different varieties (µg EC g^{-1} : microgramm catechin equivalent per gram of dry extract)

Similary, for a variety of Irish methanol extracts, the quantity of phenolic compounds is 290 mg GAE.g-[1] [24] .Italian ethanolic extracts from Salella , Frantoio , Pisciottana varieties contain respectively 330.2 ± 2.3mg GAE.g^{-1} , 219.6 ± 7.0 mg GAE.g $^{-1}$, 198.2 ± 3.6 mg GAE.g $^{-1}$ of the total phenols[38].

Results of flavonoids extracts shows that the Chimlali extract variety has the highest rate of flavonoids (9.12 mg QE.g $^{-1}$), the Chitoui extract contains 6.16 mg QE. g $^{-1}$, the Zarrazi extract variety with a rate of 5.1 mg QE.g^{-1} and ethanol extract of the variety Sahli contains 4.60 mg QE.g $^{-1}$ of the flavonoid.

These results are not in concordance with those reported in the literature. Indeed, our results are significantly higher. The total flavonoids of methanolic extract of Neb jmel variety is in the order of 2 mg QE.g^{-1} [37] . This difference is due in part to the olive leaves variety and the nature of the used solvent, ethanol (80 %), which has the capacity to dissolve more flavonoids than absolute methanol.

According to the results of proanthocyanidins (Figure 4), we note that the Chimlali ethanolic extract variety has the highest proanthocyanidins rate: 40.92 µg EC.g^{-1} while the Sahli extract variety contains 40 µg EC.g^{-1} of proanthocyanidins. The Zarrazi extract variety has a rate of 36.788 µg EC.g^{-1} and Chitoui extract variety contains 26.146 µg EC.g^{-1} of proanthocyanidins.

For flavonoids and proanthocyanidins The results are superior to than those of (Boo - Yong et al, 2010)[8] whose amount of proanthocyanidins is in the range of 14 µg EC.g^{-1} for ethanolic extract (80 %) of German variety . This difference is due to the variety and the origin of olive leaves since the same type of solvent is used for extraction.

Antioxidant activity

Test DPPH

The radical scavenging effect of olive leaves extracts of the four studied varieties on the DPPH was examined. In comparison with BHT, all samples show a lower antioxidant activity.

The Chimlali extract showed higher inhibition percentage of DPPH than all sample varieties tested, followed by Sahli and Zarrazi. The Chitoui extract has the lowest radical scavenging activity.

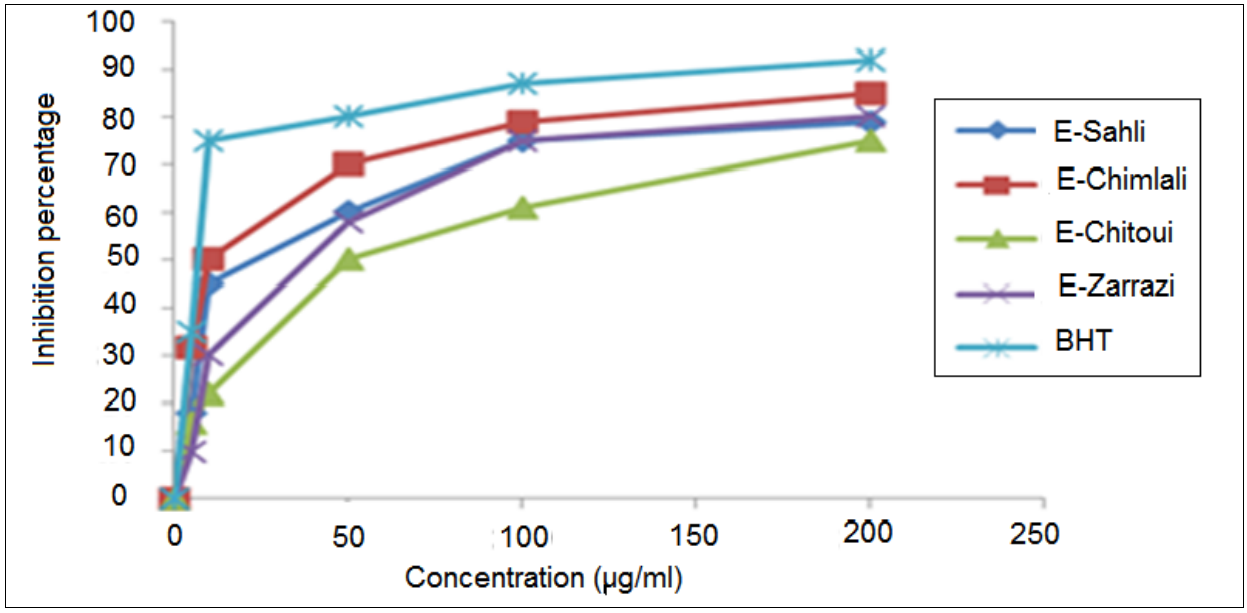

Figure 5. Inhibition percentage of DPPH radical according to different extract concentrations

These results are confirmed by the EC50 which is the effective concentration corresponding to 50% of the anti-radical activity.

The antioxidant activity of different varieties can be classified as follows:

Chimlali > Sahli > Zarrazi > Chitoui

The obtained results are similar to those already reported in the literature : EC50 ethanolic extracts Zarrazi, Sahli and Chitoui are similar to the ethanolic extract cultivated in Italy namely Rotondella and Salella: EC50 are respectively 36.3 ± 3.0 and 37.3 ± 1.6 µg mL^{-1} [38] varieties. As for the methanol extracts of olive variety grown in Irland, the EC50 is 34.58 ± 1.64 µg mL^{-1} [24] .

Antioxidant activity by co-oxidation of linoleic acid system - β-carotene

This method is used to confirm the antioxidant activity of olive leaves extracts by studying their effect on the co-oxidation of linoleic acid-βcarotene system.

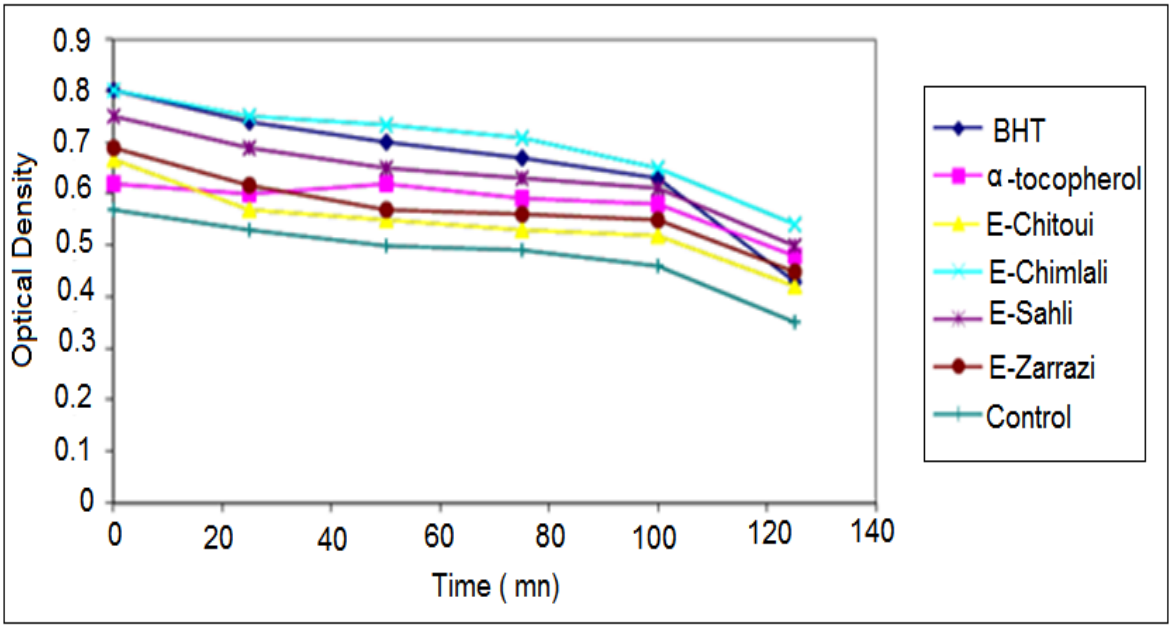

Figure 6. Evolution of optical density of olive leaves extracts, BHT relative to control, during monitoring the antioxidant activity by co-oxidation of linoleic acid system - β-carotene.

This method is based on the loss of the yellow color of the β-carotene after its reaction with the free radicals from the oxidation of linoleic acid.

The degree of bleaching of β-carotene was detected by the presence of antioxidants[39] .Color loss of β-carotene was detected by a decrease in absorbance over time.

As shown in Figure 6, all OD optical density previews keep stable up to 100 min then display a slight decrease towards the end. This demonstrates the presence of a very intense antioxidant activity. We find that the antioxidant power of the Chimlali variety is higher than the BHT and higher than other samples:

Chimlali > BHT > Sahli > Zarrazi > Chitoui

Olive leaves extracts effect on oil oxidative stability

Antioxidant effect of extracts at 110 ° C

The antioxidant effect of four OLE from Chimlali, Sahli, Zarrazi and Chitoui varieties soaked in the corn oil was tested at high temperature 110 ° C at two concentrations 100 ppm and 200 ppm for four days. The corn oil oxidative stability was followed by three parameters: Peroxide value, free acidity and color parameters.

Peroxide value (PV)

Because of the importance of oil oxidation on the nutritional properties, peroxide value was determined. PV is widely used as a measure of lipid oxidation indicating the amount of peroxides formed during oil oxidation[40].

Food lipid oxidation products, such as peroxides, free radicals involve in their formation and propagation, malonaldehyde and several cholesterol oxidation products. These food lipid oxidation products promote atherosclerosis and coronary heart disease[41]. The changes in PV of corn oil during heat treatment are shown in Figure 7.

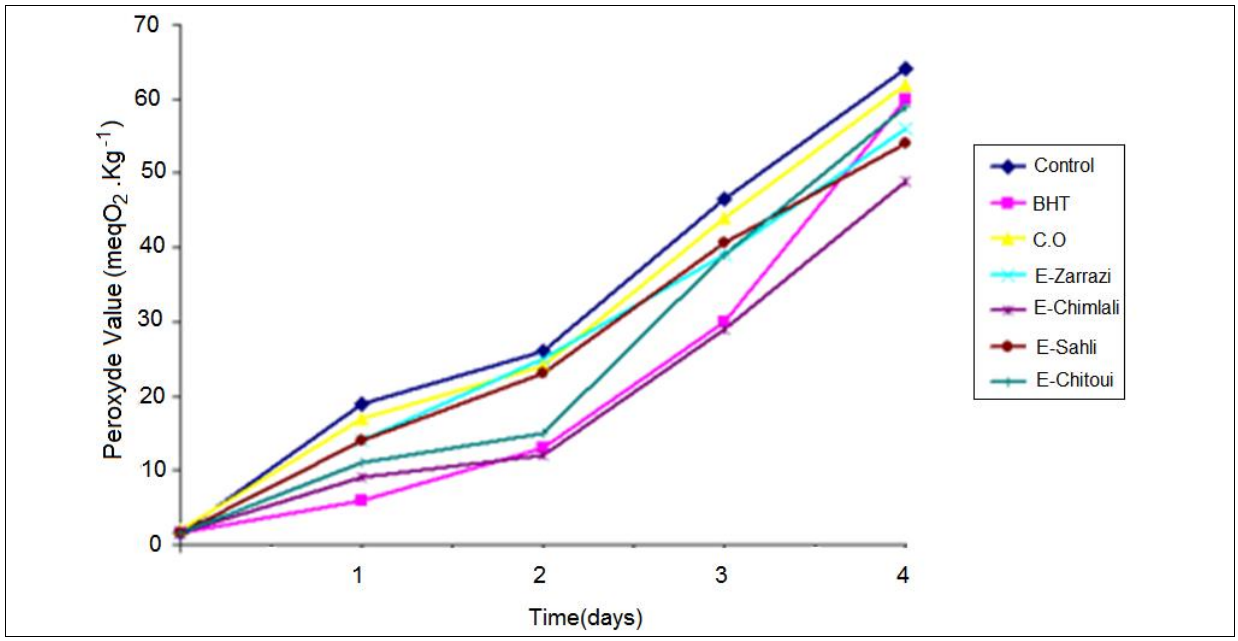

Figure 7. Peroxide value evolution of corn oil for different olive leaves extracts at 100ppm (in meq $O_2.kg^{-1}$)

At concentration 100 ppm during the first two days, the least oxidized samples are respectively: BHT, Chimlali, Chitoui, Sahli, Zarrazi, the commercial antioxidant oil relative to the control that has undergone the greatest oxidation. During the third day, the order of increasing oxidation altered as follows: Chimlali, BHT, Sahli, Zarrazi, Chitoui Commercial oil compared to the control that has experienced an even greater oxidation (Figure 7).

While the duration storage increases at 110° C, synthetic antioxidant (BHT) loses gradually his antioxidant effect.

We notice in Fig 7 that the values of the peroxide values (PV) series containing antioxidants are significantly lower than the control. In the light of those results we can classify the different additives incorporated in oils according to their antioxidant activity as follows:

Chimlali > Sahli > Zarrazi > Chitoui > BHT > Antioxidant in commercial oil

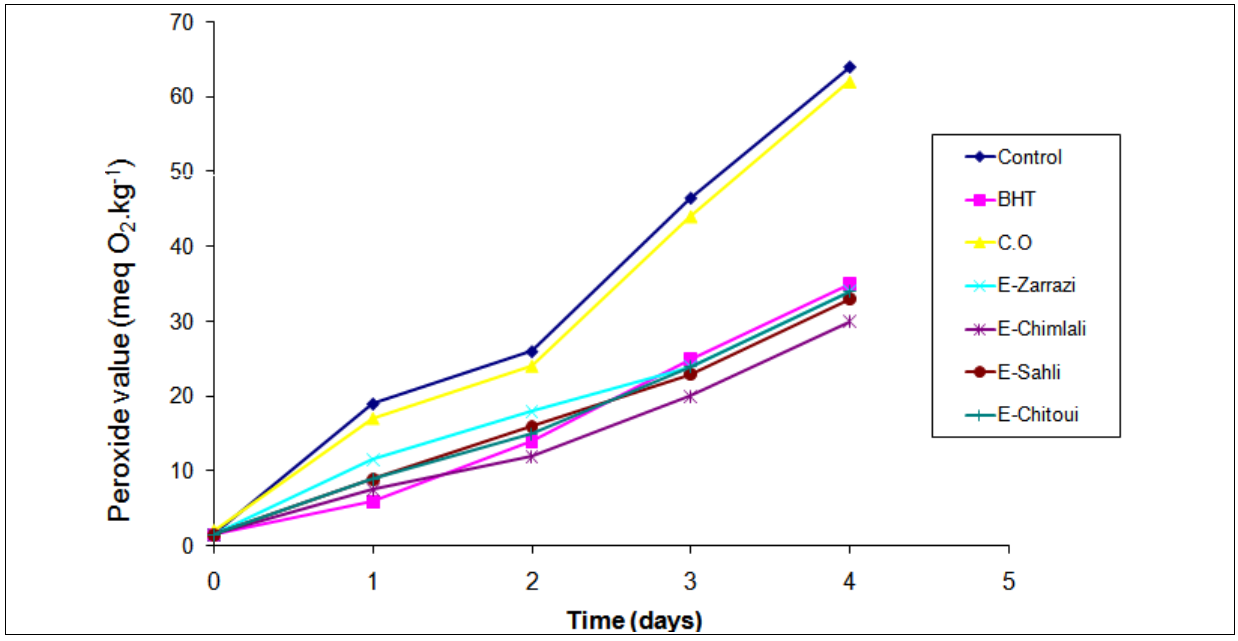

Figure 8. Peroxide value evolution of corn oil for different olive leaves extracts at 200ppm (in meq O2.kg-1)

The peroxide value evolution of corn oil observed at 110 °C for concentration 200 ppm is shown in Figure 8. We notice that the classification of the different samples is the same although the peroxide values of these samples are significantly lower than those at 100ppm.

The effect on PV is dose–dependent. Samples containing antioxidant (BHT and OLE) have lower

peroxide values at 200 ppm concentration than samples at 100 ppm concentration. Indeed, the peroxide values at 200ppm are twice as higher as peroxide values at 100 ppm.

From 200 ppm concentration, different oxidants (BHT and OLE) have a better antioxidant activity than 100 ppm concentration. Increasing the concentration of the antioxidant has contributed to a better oxidative stability.

Free acidity

The free acidity evolution of corn oil observed at 110 ° C for 100 and 200 ppm are shown in Figure 9 and 10 respectively.

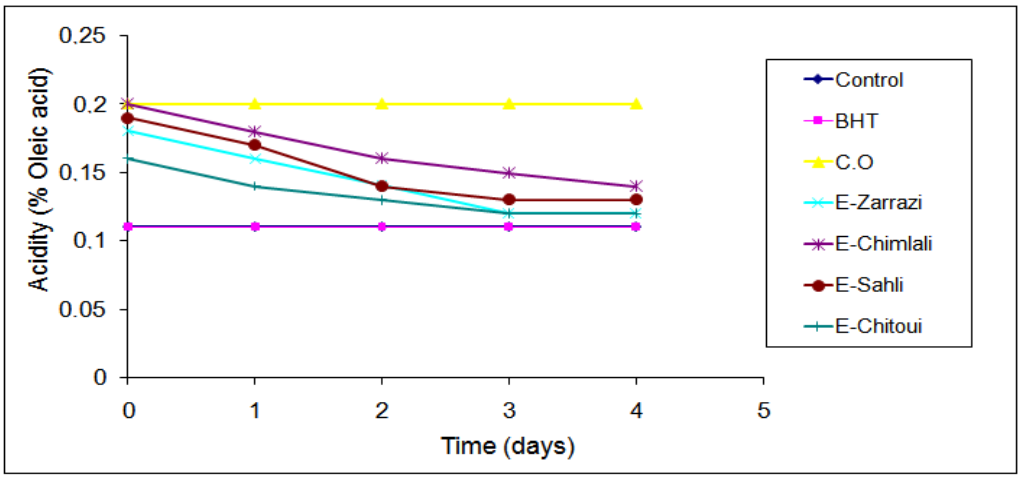

Figure 9. Evolution of corn oil acidity during storage at 110ºC for concentration 100 ppm

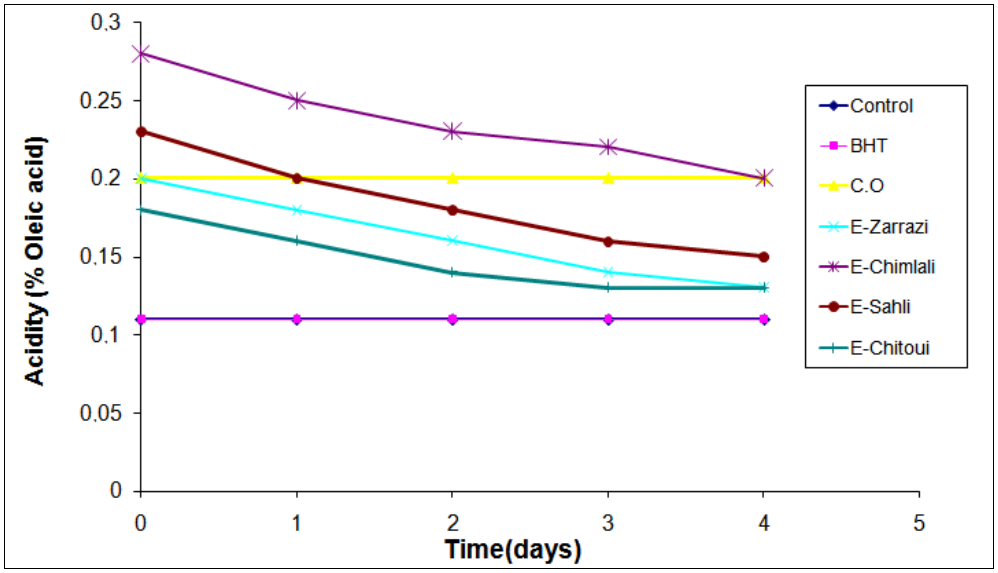

Figure 10. Evolution of corn oil acidity during storage at110ºC for concentration 200ppm

At 100 ppm concentration of olive leaves extracts, we shows two trends in the evolution of the acidity during storage, one is constant and the other is decreasing. Indeed, the acidity on the following samples: the control, the commercial oil and the oil enriched BHT remains constant.

Moreover the corn oil enriched in olives leaves extracts presents a high initial acidity due to the presence of phenolic acids in extracts. During storage, this acidity decreases.

The order of decreasing acidity oils is as follows:

Chimlali > Sahli > Zarrazi > Chitoui

The result confirms that Chimlali is the most polyphenol-rich extract and especially in phenolic acids. After heating at 110°C, the acidity of extracts decreased following a degradation of phenolic acids during thermal oxidation process. Concerning the control, (the commercial oil, the oil enriched with BHT), the acidity remains constant hence there is no release of oleic acid which has a remarkable stability at high temperature.

Similarly to 100 ppm concentration, the 200 ppm one shows the same trend. Regarding 200 ppm, the samples of corn oil containing the ethanolic extracts have a higher acidity than the ones containing synthetic antioxidant (BHT), the commercial oil and the control. The increase of acidity confirms their high phenolic compounds (phenolic acid**).** The OLE samples have a better antioxidant activity than the

others. Indeed the ethanolic extracts at 200ppm are more efficient than those at 100ppm.

Color evaluation

Before heating, the OLE color oils at 100 ppm concentration are similar for all samples tested. Thus, OLE has no effect in color oil. After heating, the color oil is different. We note an increase in color parameters L *, and a decrease in a * and b *. Also the yellow-golden color of all samples containing corn oil becomes less intense as the pigment responsible for the yellow color is due to the β-carotene which is heat and oxidation sensitive[42] .

We notice that when ΔE*increases the oxidation oil is higher. (ΔE* is proportional for the oxidation).

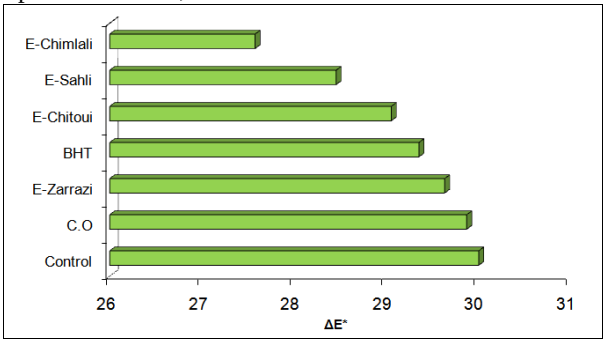

Figure 11. ΔE*Evolution of corn oil during heating at 110°C for extract 100 ppm

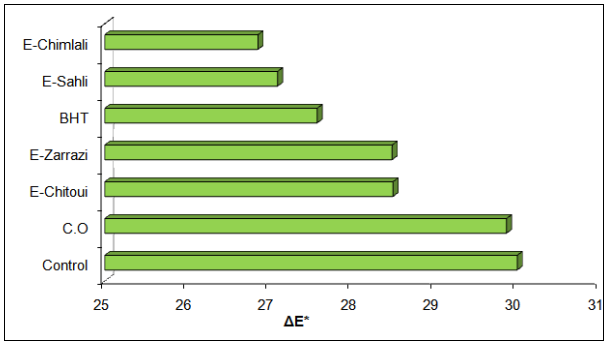

Figure 12. ΔE* evolution for corn oil during heating at 110°C for concentration 200 ppm

We can classify ΔE* as follows:

ΔE* control > ΔE*commercial > ΔE*Zarrazi> ΔE* BHT> ΔE*Chitoui>ΔE*Sahli > ΔE*Chimlali

According to these results, we can classify the different additives according to their antioxidant activity as follows:

Chimlali > Sahli > Chitoui > BHT> Zarrazi>antioxidant in commercial oil

Prior to heating, the 200 ppm concentration and the 100 ppm concentration of OLE tested samples are similar in color. Whereas after heating, there is degradation in color indicating that an oxidation has occurred.

According to Luaces et al[43], heat treatment can produce changes in the pigment content of oils. Moreover, the values of ΔE* for oils containing olive leaves extracts and BHT are lower in 200ppm concentration than in 100 ppm concentration as shown in the Figures 11 and 12.The different OLE are classified According to their antioxidant activity as follows:

Chimlali > Sahli > BHT > Zarrazi > Chitoui > antioxidant in commercial oil

Therefore the corn oils containing OLE and BHT at 200 ppm concentration are less oxidized than those at 100 ppm concentration and show more effective antioxidant activity than the concentration 100 ppm.

Based on the obtained results for the PV, the acidity and the color of different samples of corn oil with the control, the BHT, the commercial oil and the different olive leaves extracts, we can classify the different olive leaves extracts according to their antioxidant activity as follows: **Chimlali > Sahli > Zarrazi and Chitoui** are showing comparative antioxidative effect and are comparable with the BHT.

While the extract Chimlali has the best antioxidant activity we compare this effect in frying potatoes with BHT.

Antioxidant effect of olive leaves extracts in frying potatoes

Peroxide value

During potatoes frying, the peroxide value of all samples increased with time. The Chimlali extract variety undergoes to a smaller increase of peroxide value than the other series: the control, the commercial oil and the BHT. We notice that the OLE Chimlali is a natural oxidant more efficient as natural antioxidant than BHT a synthetic one (Figure 13).

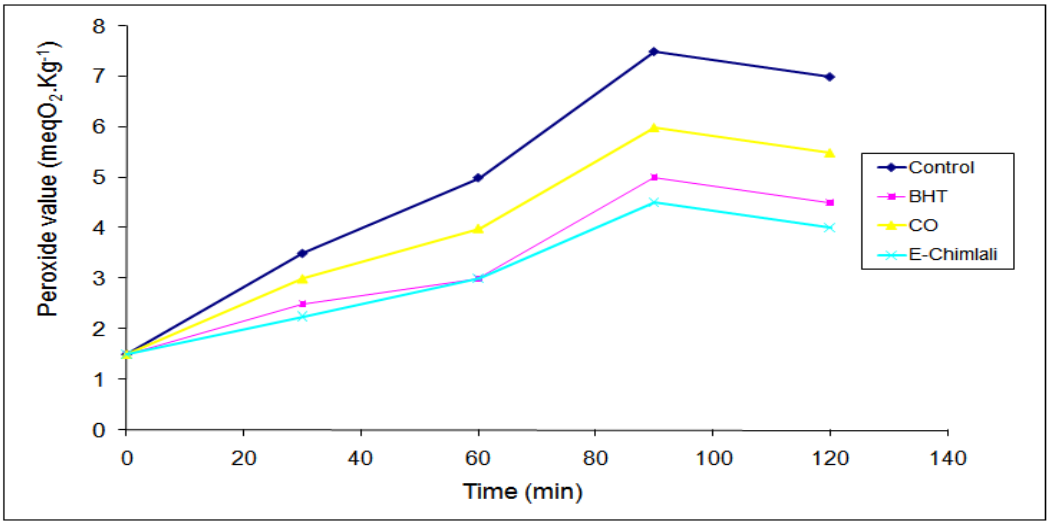

Figure 13. Evolution of corn oil peroxide value during frying potatoes

We can classify the additives incorporated in corn oil according to their antioxidant activity as shown in Figure 13:

Chimlali >BHT > antioxidant in the commercial oil

Several authors[44,45] have reported an increase in PV of oils during heating and/ or frying.The lower increase in PV of the corn oil with OLE could be due to the presence of antioxidants in the oil that can quench the initiation and propagation steps of autooxidation chain reactions[46].

The abundance of natural active substances in OLE and consequently in the corn oil may have acted as free radical scavengers. These results are in concordance with those previously reported by Ayadi et al[45] and Naz et al [32] for olive and corn oils respectively.

Acidity

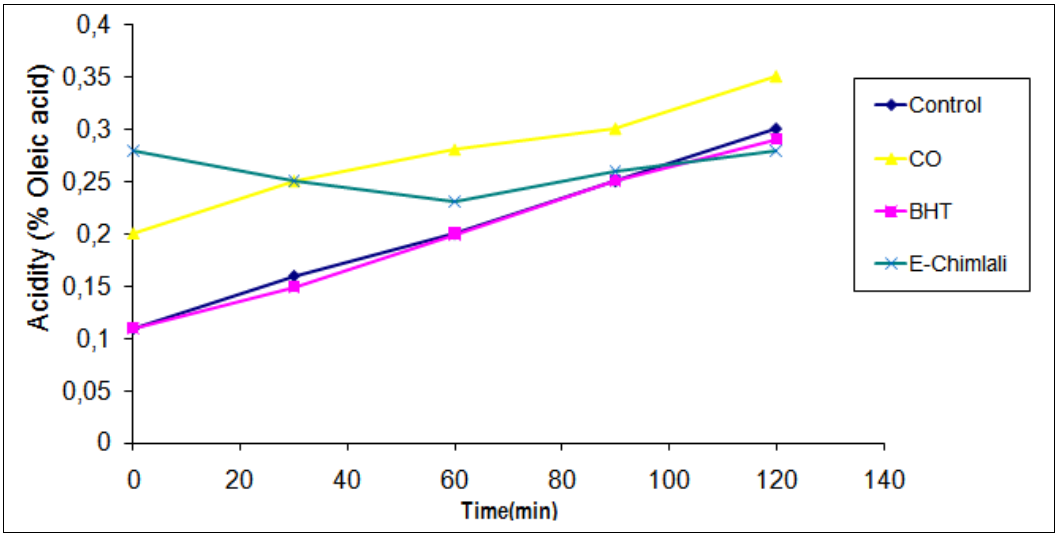

Figure 14. Evolution of corn oil acidity during frying potatoes

The Figure 14 showed an increase of acidity for the control, the commercial oil and the BHT. Moreover the oil containing the Chimlali ethanolic olive leaves extract showed a reduction of acidity at the beginning of the frying then an increase starting from 60 min frying. The increase in acidity of the samples is partly due to the degradation of unsaturated fatty acids which are responsible for the production of free fatty acids. On the other hand, it is due to the hydrolysis of triglycerides into free fatty acids giving di and monoglycerides.The reduction of acidity value for oil containing ethanolic extract of olive leaves Chimlali during frying is explained by the degradation of phenolic acids at high temperature[47].

According to the following Figure 15, we notice that the oil containing the Chimlali ethanolic olive leaves extract has a lower acidity after frying potatoes followed by the BHT, the control and the commercial oil.

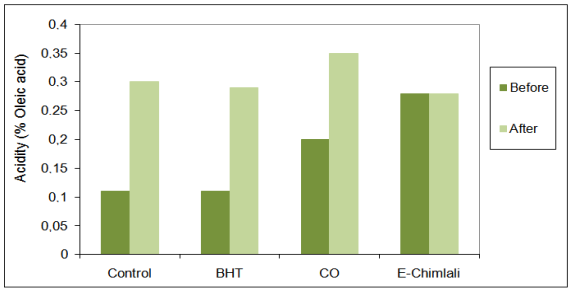

Figure 15. Evolution of corn oil acidity before and after frying potatoes

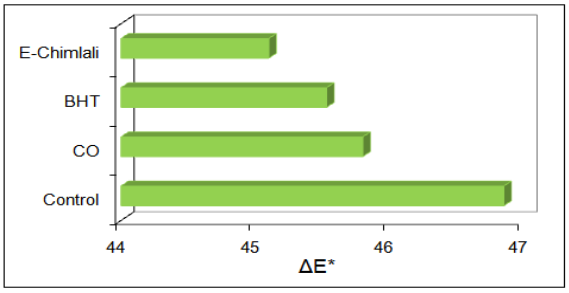

Figure 16. Evolution of ΔE* during frying potatoes

Color evaluation

During frying, the amber yellow color of oil corn turns to brown orange. We note a considerable reduction of color parameters L* and an increase of a * and b * This color change is due to the oxidation of color pigments in the frying oil and to the color formed by interaction of food components with oil (darkening of oil).

After frying, the color of natural antioxidant of Chimlali extract variety is clearer than the other samples. Thus ΔE* is classified in Figure 16:

ΔE* Chimlali< ΔE* BHT<ΔE* commercial oil <ΔE* control

The extract of variety Chimlali show a better antioxidant activity during frying followed by BHT then the antioxidant present in the commercial oil :

Chimlali > BHT> Commercial oil

Conclusion

In order to improve the quality of vegetable oil especially corn oil used for cooking and frying, four olive leaves extracts from Tunisia were used. The total phenolic content showed that the variety Chimlali is the richest in phenolic compounds:

Chimlali >Zarrazi> Chitoui > Sahli

For flavonoids, as well, we find that the total flavonoid content is as follows:

Chimlali >Chitoui>Zarrazi > Sahli

Concerning the proanthocyanidins content:

Chimlali > Sahli> Zarrazi> Chitoui.

According to their antioxidant activity, determined by the DPPH method and confirmed by the method BCB (β-carotene bleaching method) , ethanol extracts of the different olive leaves are classified as follows:

Chimlali > Sahli > Zarrazi > Chitoui

Different heating experiments of samples of corn oil enriched with 100 and 200 ppm extract at 110 ° C and during frying were performed to test the antioxidant effect of ethanol extracts.

Analysis of the results of the oxidation of corn oil during 4 days storage in a stove at 110°C showed a significant antioxidant effect of ethanolic extracts of olive leaves relative to the BHT. The highest efficiency was associated mainly with the extract of the variety Chimlali and Sahli. The antioxidant BHT is always lower than that of extracts of varieties Sahli and Chimlali but comparable to other varieties.

The antioxidant activity is presented as the following classification:

Extract Chimlali > Extract Sahli > BHT, Extract Zarrazi, Extract Chitoui > Commercial oil.

Further, the extracts of olive leaves at 200 ppm have a better antioxidant activity than those for 100 ppm at 110°C.

While the extract Chimlali has the best antioxidant activity, we compare this effect in frying potatoes , at 200 ppm for 2 hours, with the BHT.We showed that extract Chimlali is a natural oxidant more efficient than the synthetic oxidant: the BHT.

The results of this study showed that the abundance of the phenolic compounds in olive leaves extract and consequently in the corn oil have acted as free radical scavengers and may contribute to an increase in the radical scavenging activity and the oxidative stability of corn oil enriched with olives leaves extracts[27, 48]. We can conclude that the phenols assured some protection against oil oxidation. The results suggest the introduction of olive leaves extracts as food additives to increase the shelf life of foods.As well,we suggest that olive leaves can be used as replacers to the synthetic oxidants used by the industry. This is another line of investigation in order to valorize olive leaves as a sub-product from olive oil extraction industry .

References

1- N. J. Temple,Nutrition research **2000**, 20, 449-459.
2- C.Popovici, I.Saykova, B. Tylkowski , Review of industrial Engineering, **2010** , 4,1313-1371.
3- K.Ghedira. Olive tree : Association For promotion of medical phytotherapy, **2008** , 6, 2, 83 -89.
4- FAO STAT: Agricultural Data base FAO SAT–FAO, **2001**.
5- R.Flauhault, Olive tree. Ann. Ecol. Nat. Agric, t.II. Montpellier, **1986** , p61.
6- T.Albi, Guinda A, Lanzo A, European Journal of Lipid Science and Technology, **2004**, 106, 22-26.

7- M . Bouaziz, I.Fki , H. Jemai, M.Ayadi, S.Sayadi, Food Chemistry **2008**,108, 253-262.
8- L.Boo-Yong, L.Ok-Hwan, Bioresource Technology,**2010**, 101, 3751-3754.
9- K. Muhamed Ahmed, The Saudi Dental Journal, **2013**, 25, 141-147.
10- S. Lee-Huang . P.Zhang Huang. Y.Chang, P .Huang, Bio Chem. Biophys. Res. Communication , **2003**, 307(4), 1029-1037.
11- I . Sifaoui. A. Arencibia. J.C . Ticona. M. Carmen, Experimental parasitology, **2014**, 145, S111– S114.
12- Pu-Jun Xie, Ti-Xin Huang, Cai-Hong Zhanz , Feng You, Yao Pei Zhang , Food and Bioproducts processing, **2015**, 93, 29 – 38.
13- F. Aouidi. N. Dupay. J. Artaud. S .Roussos . I. Mgallem. Perraud Gaime. M. Hamdi,Industrial Crops and Products **2012**, 37,292 – 297.
14- R.Mazzei, L.Giorno, E.Piacentini, S.Mazzuca, E.Driolli, Journal of Membrane Science, **2009**,339,215-223.
15- L.Stupans, M.Murray, A.Kirlich, K.L.Tuck, P.J.Hayball, Food and Chemical Toxicology, 2001, 39, 1119-1124.
16- A.N.Sudjana, C.D'Orazio, V.Ryan, N.Rasool, J.Ng, N.Islam, T.V.Riley, K.A Hammer, International Journal of Antimicrobial Agents, **2009**, 33, 461-463.
17- H.K.Hamidi, R.Castellon, Biological and Biophysical of research communications, **2005**,334,769-778.
18- I.Andreadou, M.Papaaethimiou, A.Zira, M.Constantinou, Journal of Molecular and Cellular Cardiology, **2007**, 42,162-170.
19- N.Agus, E.Susalit, I.Effendi, R.Tjan Drawinator, D, Nofiarny, Perrin Jacquet -Moccetti T. Verbruggen M, Food , Chem, **2011**, 18,251-258.
20- M.Bardoulat: Olive tree: treasure of health. Editions Alpe ,**2005**.
21- M.Bouaziz., S.Sayadi , Eur. J. Lip Sci. Technol, **2008**,107,497-504.
22- E Botsoglou, A.Govaris, E.Christaki, N.Botsoglou, Food, Chem., **2009**, 120,500-506.
23- a. E.Bostoglou**,** A.Govaris, I.Ambrosiadis, D.Fletouris, and G.Papageorgiou, Journal of the Science of Food and Agriculture, **2014**, 94, 227-234.
b.. E.Bostoglou, A.Govaris, I.Ambrosiadis, D.Fletouris, N.Botsoglou, Meat Science, **2014**, 98, 150-157.
24- P.Allen, E.Hayes, V.Stepanyan, N.D'Grady, M.O'Brien, P.Kerreys, Meat Sc., **2009**, 83, 201-208.
25- N.Rodrigues, R.Malheiro, S.Casal., M.C.Manzanera, A.Asensio Bento, Pereira J.A, Food, Chem. Toxicol, **2012**,50, 2894-2901.
26- R.Malheiro, N.Rodrigues, G.Manzke, A.Bento. Pereira J.A, Industrial Crops and products, **2013**, 44, 37-43.
27- F.N.Salta, A.Mylona, A.Chiou, G.Boskou, Andrikoupoulos N.K, Food. Sci. Technol. Int, **2007**, 13,413-421.
28- G.Pottier-Alapetite, Printing office of the republic Tunisia, Vol I.Tunis, **1979**.
29- A.Turkoglu, M E. Duru, N.Mercan, I.Kivrak, and K.Gezer, Food,Chem , **2007**,101,1,267-273.
30- M.M. Al-Dabbas, T,Suganama, K.Kanefumi, DX.Hou and M.Fujii, Journal of ethnopharmacology, **2006**, 108 ,2, 287-293.
31- L. J. Porter, L. N. Hrstich, B. C. Chan, *Phytochem.*, **1986**, *25*, 225.
32- S.Naz, H.Sheikh, R.Siddiq and S.Asad Sayeed, Food, Chem , **2004**,88,253-259.
33- AOAC , Official Methods of analysis (16^{th} edn). Association of Official Analytical Chemists, Washington, DC,**1999**
34- S.Besbes, C.Blecker, C.Deroanne, G.Lognay, N.Drira, H.Attia , Food, Chem, **2005**, 91,3, 469-476.
35- C.Berlinet. Study of the influence of the package and the on the quality of orange, juice, Thesis, ENSIA, France, **2006**, pp. 66.
36- N.Bahloul, N.Boudhioua, I.Ben Slimen, et N. Kechaou , Review of renewabl Energy SMSTS'08 Alger, **2008**, 111-116.
37- F.Brahmi, B.Mechri, S.Daddou, M.Dhibi, M.Hammami , Eur J Lip Sci Technol, **2012**, 38, 146-152.
38- B.D'Abrosca, M.Scognamiglio, S.Pacifico, V.Fiumano, P.Luca, P.Monaco, A.Fiorentino, Food Res Int, **2012**, 64, 1-10.
39- T.kulisic, A. Radonic, V.Katalinic, M.Milos, Food Chemistry, **2004**,85,4, 633–640
40- SA.Raza, A.Adnan, FA.Qureshi, MF.Asim, S.Najaf and J.William, Electron J Environ Agric Food, Chem, **2009**,8:1043-1051.
41- R.Subramanian, KE.Nandini, PM.Sheila, AG.Gopalakrishna, Raghavarao KSMS, M.Nakajima, et al, J Am Oil, Chem Soc ,**2002**, 77:323 -328.
42- M.Murkovic, A. Zeb . Food Chemistry. **2011,**127, 4, , 1584-1593.
43- AG.Luaces Perez, JM.Garcia and C.Sanz, Food Chem, **2005**,90:169-174.
44- R .O'Brien, Fats and Oils: Formulating and Processing for Applications. Taylor and Francis / CRC Press, New York, NY ,**2009**.
45- MA.Ayadi, N.Grati-Kamoun and H.Attia, Food Chem Toxicol, **2009**, 47: 2613 - 2619.
46- AG.Marangoni, Food Res int, **2000**,33,407.
47- JL.Perrin. Détermination of the alteration in "fatty substances Manual'' karleskind,A.TEC and DOC,Lavoisier,Paris, **1992**, 2 ,1198-1218.
48- I.Jabri Karoui, W. Dhifi, M.Ben Jemia and B.Marzouk, J Sci Food Agric, **2011**, 91,927-933.