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Anti-lipid peroxidation effect of essential oil from *Mentha piperita* leaves on cosmetic argan oil under accelerated oxidation

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Abstract

The oxidation of lipids in edible and cosmetic vegetable oils leads to the production of toxic products which are harmful to human health. The present research aims to assess the chemical composition of essential oil from *Mentha piperita* leaves and to dertermine the anti-lipid peroxidation effect on cosmetic argan oil (1% v/v). The oils were subjected to two parallel accelerated oxidation tests, heat in the oven at 40°C and UV-A irradiation during 4 months. The evolution of oxidation throughout this period was followed periodically every 4 weeks by simultaneously measuring the free acidity, peroxide value and absorption at 232 and 270 nm. Results showed that linalool (46.84%) and linalyl acetate (26.72%) were the major components. Free acidity parameter was lower for samples containing argan oil mixed with essential oil for both conditions of storage. However, peroxide value increased with the addition of essential oil before the storage and thus results could be not significant but the strong effect of the essential oil has been registered under UV-A irradiation in the 12th week where values where lower for the samples compared to the control which contain only argan oil (7.113±0.098 and 10.145±0.268 meq O_2/kg respectively). The absorption at 232 nm and especially at 270 nm where all the secondary oxidation products suddenly increase at the 16th week showed the strong effect of *Mentha piperita* essential oils which reduced their production.

Keywords: Anti-lipid peroxidation; Vegetable oil, Essential oil; Argan; *Mentha piperita*; Heat; UV-A

1. Introduction

For a very long time, vegetable oils have been used as skin moisturizers, their first use dates from the second century B.C. in China [1]. Vegetable oils are produced by plants, the highest concentration is located in seeds and fruits. Vegetable oils are composed primarily of triglycerides with small amounts of mono and bi-glycerides [2]. The differences among various vegetable oils are based on the types of acids constituting the radical group of triglycerides [3].

In recent years, argan oil has emerged as a very important ingredient of many cosmeceutical products for skin and hair due to its excellent antioxidant properties. It's a vegetable oil extracted from the fruit of the endemic argan tree *Argania spinosa* L. which belongs to *Sapotaeceae* family found in southern Morocco [4,5]. The argan forest covers an area of 830,000 ha [6] and it was designated in 1998 a UNESCO biosphere reserve [7]. Two main types of argan oil exists, the cosmetic argan oil and the edible argan oil. Unroasted kernels deliver a cosmetic argan

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oil and roasted kernels give an edible argan oil. During the production process a large amount of argan co-products is formed: shells (52%), pulp (43%) and around 2% of the argan cake [8]. The largest proportion in the composition of argan oil is represented by glycerides (99%) of which triglycerides represent about 95% [9]. Unsaturated fatty acids include mainly: oleic acid (ω-9) in a proportion of 43-49%, linoleic acid (ω-6) in a proportion of 29-36% and linolenic acid (ω-3) in the smallest trace amount [10,11]. The other compounds present in argan oil (about 1%) form an unsaponifiable fraction. The fraction includes: polyphenols, tocopherols, triterpene alcohols, sterols and carotenes. These compounds are the main responsible for the stability of the oil, its high cosmetic and medical values, including its anti-neoplastic activity [12]. Argan oil has many effects on skin and hair, it is known to have moisturizing, anti-acne, sebostatic, wound healing and anti-aging properties [13]. Applied externally, argan oil in combination with antioxidants forms a special protective layer. This makes it appropriate for medical and cosmetic purposes [14].

Essential oils have many pharmacological properties and may represent a promising source for new natural drugs [15,16]. *Mentha piperita* or peppermint belongs to *Lamiaceae* family that is widespread throughout the Mediterranean area [17]. *Mentha piperita* essential oil is used in perfume industry, cosmetics, aromatherapy, nutrition, spices, etc. Several studies have shown the antioxidant, antiviral, antibacterial, antifungal properties of essential oils and extracts of the herbal parts [18]. *Mentha piperita* essential oil is composed of monoterpenes, menthone, menthol and their derivatives [19].

The oxidation of lipids represents a major cause of oil and fat degradation from processing and storage, leading to an alteration of major quality parameters such as flavor, aroma, color, and nutritive value, due to essential fatty acids degradation and the production of toxic compounds responsible or contributing to adverse *in vivo* human health effects such as cancer, atherosclerosis, heart disease and allergic reactions [20]. High levels of polyunsaturated fatty acids like linolenic and linoleic acids in oils and fats make them more subject to oxidation. The oil oxidizes further if exposed to factors that include high temperatures, oxygen, light or trace metals, especially Cu and Fe [21]. The quality of oils can improve by retarding or reducing their oxidations using antioxidants. [22]. An important parameter for the quality assessment of oils and fats is oxidative stability, which is the capacity of oils and fats to resist oxidative deterioration during processing and storage periods [23]. Antioxidant act as a radical scavenger and can be categorized as donor and acceptor. For example, the donor provides hydrogen, usually unstably bonded to the free radical carbon in the essential oil compositions, thereby recovering the free radical in the original vegetable oil compositions. The acceptor only acquires the free radicals (the unoxidized free radical and the oxidized free radical) to form stable chemical compounds [3].

Synthetic antioxidants are widely used to prevent the oxidation of oils and fats and extend the shelf-life of lipid containing in cosmetics and foods. In recent years, their use in cosmetics and foods had severe criticism such as their toxicity and carcinogenicity [24]. Such factors have created a growing interest in the research of natural antioxidants. For external application, argan oil, mixed with antioxidants from essential oils, forms a special protective layer. It can therefore be used for cosmetic and medical purposes [25].

The aim of this study was to identify the chemical compounds of essential oil from *Mentha piperita* leaves and to assess the anti-lipid peroxidation effect in combination with argan oil under accelerated oxidation by heating to temperatures of 40°C in the oven without light access and by irradiating with UV-A.

2. Material and methods

2.1. Samples

Almonds of argan tree were collected in Tafraout located in 170 km south-east of Agadir (south of Morocco).

Mentha piperita plant was collected in the region of Rabat (northwest of Morocco).

2.2. Cold press extraction of argan oil

Argan oil was obtained by a mechanical press. The argan kernels were pressed in a cold press extractor at 45°C. The obtained oil was then clarified by filtering, collected in dark bottles and stored at 4°C.

2.3. Isolation of essential oil

The leaves of *Mentha piperita* were stored in a dry place, without light access and at room temperature for 14 days. The essential oil was isolated via hydro-

distillation using a Clevenger unit. The dried leaves were introduced into a flask and after distilled water was added until it covered the leaves completely. The distillation was conducted during three hours. Then, the essential oil was stored at 4°C until further analyses. The essential oil yield (%) was calculated using the following formula [26]:

$$\text{Essential oil yield (\%)} = \frac{\text{volume of essential oil obtained (mL)}}{\text{masse of dry plant (g)}} \times 100$$

2.4. Gas chromatography-mass spectrometry (GC–MS) analysis

Chromatographic analyses of the essential oil were carried out in CNRST-UATRS center in Rabat, Morocco. The profile of volatile compounds was characterized by gas chromatography coupled to mass spectrometry GC-MS/MS (1300 TSQ 8000 Evo Thermo Scientific). The fragmentation was operating in the electron ionization (EI) of 70 eV. The capillary column was a TR-35MS (35% Phényl polysilphénylène-siloxane) (30m x 0.32mm, film thickness: 0.5µm) and the flame ionization detector (FID) was set at 300°C supplied by a mixture of helium/air gas. The essential oil was injected (1 µL) into the column by 1:50 split mode using helium as carrier gas with a flow rate of 1.4 mL/min. The temperature of the column increases from 50 to 200°C at a rate of 4°C/min and a plateau of 5 minutes at the final temperature. Identification of the compounds were assigned by their retention times relative to known compounds and by matching mass spectral with those present in the computer data bank (NIST) and published spectra [27].

2.5. Free acidity

The free acidity was evaluated as described in the Annex II of the CONSLEG 2015 for olive oil analyses [28]. An aliquot of 2.5 g of sample was dissolved in neutralized ethanol and titrated with a 0.1 N of KOH in aqueous solution using 1% phenolphthalein in ethanol as an indicator solution. The free acidity was expressed as milligrams of KOH per g of oil (mg KOH/g) and calculated with the formula:

$$\text{Free acidity} = (56.11 \times V \times c) / m$$

V: Volume (mL) of potassium hydroxide solution
c: Concentration (mol/L) of potassium hydroxide solution
m: Mass (g) of the oil

2.6. Peroxide value

Peroxide value was determined according to the Annex III of the CONSLEG 2015 for olive oil analyses [28]. An aliquot of 5 g oil of sample was dissolved in a 25 mL solution of acetic acid/chloroform (3:2, v/v) and 1 mL of a saturated aqueous solution of potassium iodide was added. The mixture was shaken for 1 minute before being placed in the dark for five minutes. After, it was titrated with 0.01 N sodium thiosulphate solution using 1% starch soluble solution as an indicator. The peroxide value was expressed as milliequivalents of active oxygen per kilogram of oil (meqO_2/kg) and calculated with the formula:

$$\text{Peroxide value} = (V \times c \times 1000) / m$$

V: Volume (mL) of sodium thiosulphate solution
c: Concentration (mol/L) of sodium thiosulphate solution
m: Mass (g) of the oil

2.7. Spectrophotometric investigation in the ultraviolet

The measure of the absorption between 200 and 300 nm gives qualitative information about the vegetable oil. The lower the absorption, the better the chemical quality of the oil. Spectrophotometric indexes in the ultraviolet were determined as suggested by the Annex IX of the CONSLEG 2015 for olive oil analyses [28]. Each sample was diluted 1:100 (m/v) with cyclohexane and the specific extinctions were measured at 232 and 270 nm against a blank (only cyclohexane). A UV/Vis LLG-uniSPEC 2 Spectrophotometer was used.

2.8. Evaluation of the oxidative stability

In order to evaluate the oxidative stability, two parallel accelerated oxidation tests were used: heat in the oven at 40°C (T) without light access and UV-A irradiation (UV) at 365 nm during 16 weeks. The evolution of oxidation throughout this period was followed periodically every 4 weeks by simultaneously measuring the free acidity, peroxide value and absorption at 232 and 270 nm. Argan oil and *Mentha piperita* essential oil were used in this study. The control (C) is composed only of argan oil but the samples (S) were made from a mixture of *Mentha piperita* essential oil and argan oil 1% (v/v). The control and the samples were put in 20 closed transparent glass bottles and each bottle has been opened on the day of the analysis. The results

covered by our analysis represent arithmetic means from two repeated tests.

3. Results and discussion

The initial values of free acidity, peroxide value and K270 evaluated before storage remained below the limits set by the Moroccan Standard NM 08.5.090 characterizing the "extra virgin" quality of argan oil (acidity < 0.8 / PV < 15 meqO_2/kg / K270 < 0.35) [29].

3.1. Essential oil yield and chemical composition

The average yield of the essential oil from *Mentha piperita* was about 3.13% (v/w). This yield was higher than that obtained from Slovakia 0.4% [17], from Algeria 1.0% [30], from Iran 1.38% [31] and from Morocco 1.02% [32]. Indeed, it has been reported that the characteristics of the growing region, growing stage and harvest time can affect *Mentha piperita* essential oil yield [33].

A chromatogram of isolated essential oil from *Mentha piperita* leaves was shown in Figure 1. Analysis of the essential oil allowed the identification of 95.41 % of the constituents (Table 1). Linalool was the major component (46.84%), followed by linalyl acetate (26.72 %) and other constituents at relatively low concentration. It has been reported that the major components of the essential oil isolated from peppermint were menthol, menthone, d-carvone, limonene and pulegone [33]. The composition of *Mentha piperita* essential oil differs depending on the area. Menthol (49.89%) and menthone (20.84%) have been the main constituents in Algeria [30], menthol (70.08%) and menthone (14.49%) were the most principal component identified in Slovakia [17], the main compounds were menthol (30.35%), menthone (21.12%) and carvone (5.60%) in Taiwan [34] and the principal contents were reported to be carvone (34.94%), pulegone (14.8%), methyl petroselinate (15.51%) and D-limonene (11.20%) from Oman [35]. In contrast, the essential oil characterized in this study showed a high amount of linalool and linalyl acetate. This has been confirmed with a study performed on *Mentha piperita* from Morocco where the amount of linalool (52.0%) and linalyl acetate (25.9%) were almost the same [36] and also from Brazil where the major components were linalool (25.43%) and linalyl acetate (51.35%) [37].

Table 1. Chemical composition of *Mentha piperita* essential oil

Compound	R.T.* (min)	Mass percentage (%)
α-Pinene	9.09	1.88
trans- α -Ocimene	11.07	1.92
Eucalyptol	11.24	3.39
3-Carene	11.41	0.78
Cyclohexene	12.97	0.25
Linalool	14.29	46.84
L- α -Terpineol	18.37	6.21
Linalyl acetate	19.27	26.72
Geraniol	20.18	0.87
cis-p-Mentha-2,8-dien-1-ol	23.31	0.45
2,6-Octadien-1-ol,3,7-dimethyl-, acetate, (Z)-	23.47	1.47
Geranyl acetate	24.25	4.09
Naphthalene	27.45	0.25
Guaiol	32.81	0.29
Total mass percentage (%)	95.41	

*Retention time

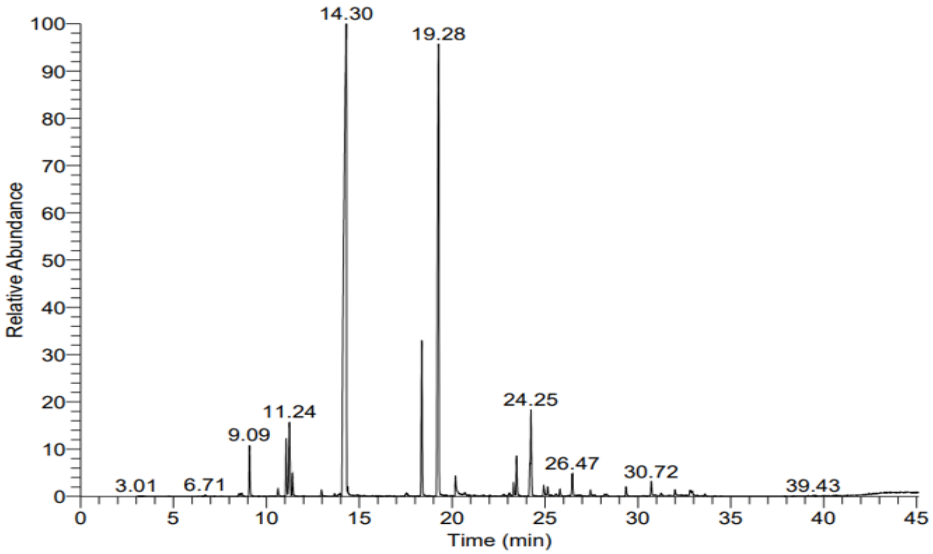

Fig. 1. Chromatogram of the constituents of *Mentha piperita* essential oil

3.2. Free acidity

Free acidity is an important parameter to determine the quality of the vegetable oil and the quality change during storage. It is used to measure the content of free fatty acids present in the oil. They are formed due to triglyceride hydrolysis by the lipase activity [38].

Results of free acidity measurements of pure argan oil and argan oil mixed with *Mentha piperita* essential oil (1% v/v) stored at 40°C without light access and under UV-A irradiation are shown in Figure 2. It's clear that free acidity increased with aging duration under both conditions of storage. The increase in free acidity could be explained by the hydrolysis of the triglycerides and the release of fatty acids which constitute the major components of the vegetable oil. It could trigger reactions of oxidative degradation of the oil due to the prooxidative action of free fatty acids [39]. Argan oil mixed with *Mentha piperita* essential oil exhibit a lower free acidity than pure argan oil under both conditions of storage during 16 weeks. It reached a pick in the 16th week at 40°C which was high for the control and low for the sample (4.384±0.195 and 2.510±0.182 mg KOH/g respectively). Under UV-A irradiation values were approximately constant. The release of acids was sensitive with temperature. The free acidity increased rapidly at temperature of 40°C comparing to UV-A irradiation. It has been reported that hydrolysis of the triglycerides is dominating under temperature condition between 35°C and 45°C [40]. Secondary plant metabolites present in plant have been found to be lipase inhibitors [41]. Generally, *Labiatae* species are rich in terpenoids and phenolic compounds. Those constituents are present in the *Mentha* species and responsible for the antioxidant activity [42]. Essential oils are secondary metabolites composed essentially of several volatile compounds including monoterpenes, sesquiterpenes, alcohols, aldehydes, ketones and esters [43–45]. The linalool and linalyl acetate are oxygenated monoterpenes which constitute the major compounds of the essential oil studied and could be the main responsible for the inhibition of lipase activity. Thus, the addition of *Mentha piperita* essential oil to argan oil played an active role in improving its quality during storage.

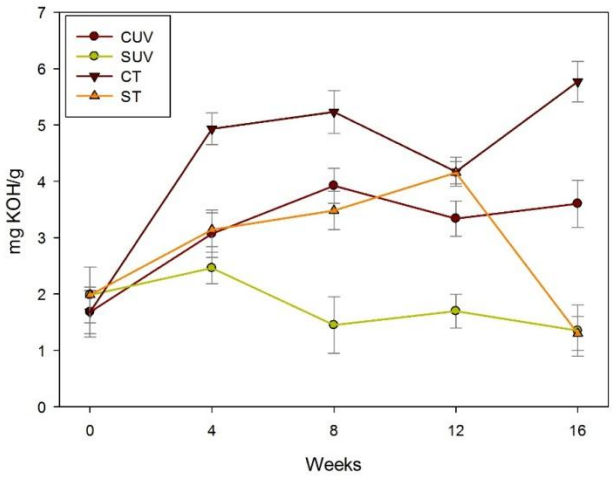

Fig. 2. Evolution of free acidity at 40°C and under UV-A irradiation

3.3. Peroxide value

Peroxide value is a parameter commonly used to evaluate the primary oxidation state of vegetable oils during technological treatments and storage. The peroxide value analysis is based on the quantification of the primary oxidation products, mainly hydroperoxides [46]. The oxidation of unsaturated fatty acids by O_2 during peroxidation induces the formation of free radicals from fatty acids due to an auto-catalytic reaction and starts the oxidation of the remaining non-oxidized fatty acids.

The results of peroxide value of the studied oils are shown in Figure 3. The peroxide value varied significantly between the samples. Initial values before storage showed that the presence of *Mentha piperita* essential oil in argan oil gives higher values than the pure vegetable oil. This will affect our results and the inhibition of oxidation by the essential oil will be less significant. Under UV-A irradiation the peroxide value is continuously increasing and reversing but at 40°C the values go up and down. Storage at UV-A irradiation showed that at the 4th week the values were higher for samples mixed with essential oil and reached 7.380±0.368 meq O_2/kg. After 4 weeks, the trend is reversed and the values start to stabilize for argan oil mixed with *Mentha piperita* essential oil but pure argan oil begins to increase and reached 10.145±0.268 meq O_2/kg on the 12th week. After 4 weeks, the trend is reversed again. The increasing of peroxide value reflects the production of hydroperoxides in response to fatty acid oxidation. For the storage at 40°C the values increased and decreased every 4 weeks with the same trend. It was always low for the control and high for samples. The low picks were practically the same in the 4th week and the 12th week (under 2 meq O_2/kg). In the 16th week, the second high pick reached 4.926±0.060 meq O_2/kg for the control and 11.966±0.158 for the sample. Indeed, hydroperoxides are unstable at high temperatures and decompose resulting in a rapid decreasing of peroxide value [47]. In general, the time between the start of storage and the sudden increase of the peroxide value correspond to the induction period [48]. Meanwhile, antioxidants present in the essential oil protect the fatty acids against oxidation [29]. The increasing of peroxide value can be explained by the production of hydroperoxides in response to fatty acid oxidation.

The generation and decomposition of peroxides occur simultaneously in vegetable oils. Some studies have indicated that oxidation evolves differently depending on the type of oil at 65°C for 10 weeks.

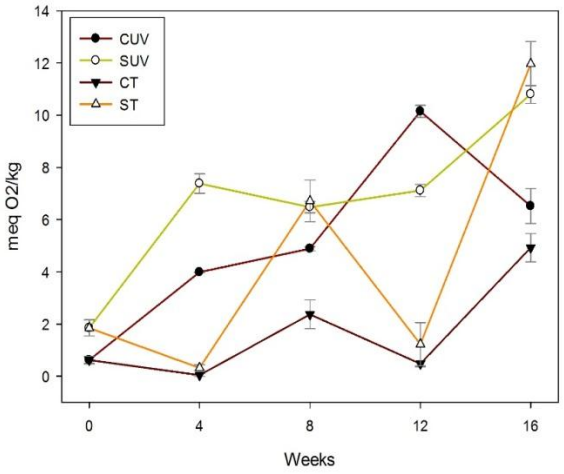

Fig. 3. Evolution of peroxide value at 40°C and under UV-A irradiation.

The formation of peroxides was fast in sunflower oil (abruptly decreased in 6 weeks), followed by those of rapeseed, olive and argan oils [49]. In other work, peroxide value significantly increased in oil extracted from almonds roasted at 125°C for 30 min, 150°C for 10 min, and at 175°C for 10, 20 and 30 min durations [47]. Another work showed that peroxide value of Envirotemp FR3 vegetable oil fluctuates, stabilizes and reached a peak at 110°C during 2500 hours. For the same vegetable oil subjected to oxygen, peroxide value shows the similar fluctuation at the begging, but increases quickly from 500 hours until reaches the peak value at 1000 hours [50]. The lipid oxidation at low and high temperatures may go through different steps or reaction pathways, depending on the reactivity of antioxidants and metal ions at different temperatures [51]. In addition, the degree of oxygen solubility in vegetable oils is affected by oil temperature. Oxygen solubility decreases approximately with 25% for every 10°C increase in temperature [52]. Consequently, these prediction results may involve errors and uncertainties and may only be taken as approximate values.

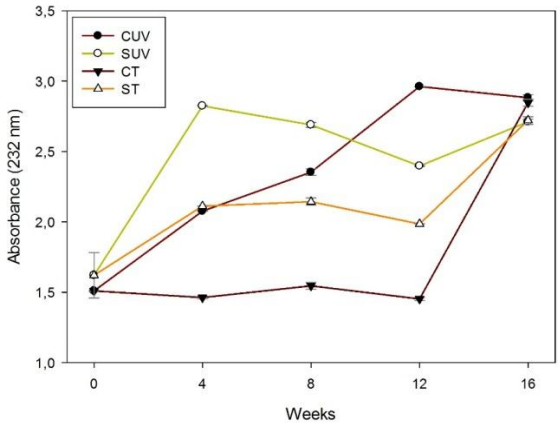

Fig. 4. Evolution of conjugated dienes at 40°C and under UV-A irradiation

3.4. Spectrophotometric investigation in the ultraviolet

The absorption at 232 nm permits to evaluate the concentration of conjugated dienes produced by the oxidation of the linoleic acid (ω-6). The hydroperoxides and the conjugated dienes are the primary products of oxidation. Conjugated trienes are formed by oxidation of linolenic acid (ω-3). The conjugated trienes and the secondary oxidation products (aldehydes) have a maximum absorption near 270 nm. They are resulting from the decomposition of primary products (hydroperoxides and conjugated dienes) [53].

The extinction coefficient values K232 recorded in this study were shown in Figure 4. For the storage at 40°C the same results were observed with the peroxide value regarding the addition of *Mentha piperita* essential oil to argan oil. At 40°C, the absorbance of the control was lower than the sample and values were constant but in the 16th week it increased for the control more than the sample (2.721±0.005 and 2.846±0.009 respectively). This proves that conjugated dienes are not affected by temperature, their concentration is stable at 40°C unlike hydroperoxides. However, under UV-A irradiation the values take another trend, the absorbance was increasing for both the control and the sample like in the peroxide values it was higher for samples containing essential oil and then reversed between the 8th and the 12th week. The decrease in the K232 value is explained by the instability of the conjugated dienes that turn into secondary oxidation products [54]. Antioxidants limit the action of free radicals on the polyunsaturated fatty acids and increase the stability of vegetable oils. [55,56]. This result demonstrated the power of the antioxidants present in the essential oil of *Mentha piperita* especially linalool and linalyl acetate in the prevention of the oxidation of fatty acids.

K270 extinction coefficient values recorded in this study are presented in Figure 5. Values for both conditions of storage were practically constant and then suddenly increase in the 16th week. The absorbance was lower for samples containing essential oil under UV-A irradiation and at 40°C (2.003±0.005 and 2.297±0.009 respectively) compared to the control for UV-A irradiation and at 40°C (2.736±0.003 and 2.728±0.002 respectively). The elevation of the absorbance at 270 nm is due to the gradual generation of the secondary oxidation products formed mainly by decomposition of the hydroperoxides and indicates the formation of undesirable oxidation products such as carbonyl compounds. Previous studies reported that increasing temperature and time of seed roasting led to an increase in K270 [57,58]. This result showed the effect of *Mentha piperita* essential oil in retarding or inhibiting the formation of secondary oxidation products which are toxic to human health and it could be mainly due to the presence of the major compounds linalool and linalyl acetate.

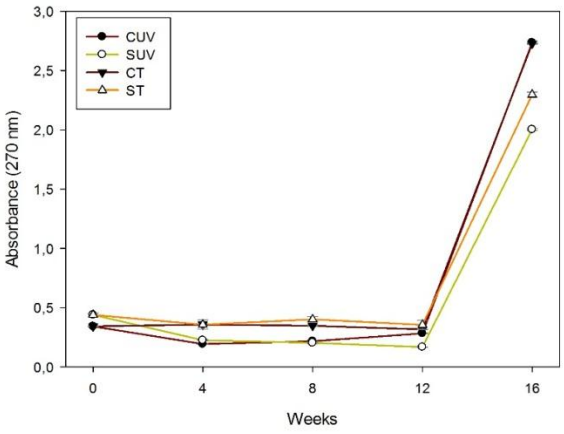

Fig. 5. Evolution of conjugated trienes at 40°C and under UV-A irradiation

4. Conclusion

According to the findings of this study, the high amount of antioxidants mainly linalool and linalyl acetate present in *Mentha piperita* essential oil

showed an interesting anti-lipid peroxidation activity on cosmetic argan oil. Those antioxidants can inhibit the lipase activity by reducing free acidity and thus decreasing the hydrolyses of lipids which release fatty acids subjected to oxidation. The storage at 40°C influence the stability of the primary oxidation products mainly hydroperoxides. Therefore, the storage under UV-A irradiation gives significant values. The absorbance at 270 nm showed the power of *Mentha piperita* essential oil to limit the generation of secondary oxidation products. These results, make essential oils a possible alternative to the usual chemical additives. This would allow a longer shelf life and inhibit or retard the production of toxic compounds to ensure a healthy cosmetical use. Further studies should be conducted in order to better understand the underlying mechanisms responsible for this activity and also the synergistic effect with other essential oils.

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Conflicts of Interest

The authors declare no conflict of interest.

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