

## THERMAL STABILITY OF THE ESSENTIAL OILS ISOLATED FROM TUNISIAN *THYMUS CAPITATUS* HOFF. ET LINK.: EFFECT ON THE CHEMICAL COMPOSITION AND THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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The chemical composition, the antioxidant and the antibacterial activities of essential oils, isolated from the aerial parts of Tunisian *Thymus capitatus* during the flowering phase, and stored in the dark during 37 days in the oven, at 60 °C were evaluated. Samples taken periodically were used to evaluate the chemical composition, the antioxidant and the antibacterial activities. With some fluctuations, carvacrol (68–74%) was the major component of the oil independent of the storage period.  $\alpha$ -Terpinene and  $\gamma$ -terpinene decreased over time, whereas *p*-cymene increased in the same period. Despite the thirty-seven days of storage at 60 °C, *T. capitatus* essential oil still showed high antioxidant and stable antimicrobial activity.

**Keywords:** *Thymus capitatus*, essential oils, thermal stability, biological activities

Plant volatile oils have been recognized since antiquity to possess biological activities. The most important are their antibacterial, antifungal and antioxidant properties (ADAMS et al., 1998; EDRIS, 2007).

Nowadays, there is an increasing interest in biological activities of the essential oils, in particular to prevent the deterioration of the constituents of foodstuffs. In view of this, the concern for determination of the efficacy of natural products, especially during food processing, has increased significantly. In the case of foods, it is necessary to determine the ability of natural antioxidants for food protection against oxidative damage causing rancidity in food products, leading to their deterioration. In spite of some essential oils promoting the inhibition of lipid oxidation, and lipid-containing products, in accelerated oxidation conditions, for example, with increased temperature (e.g. during food processing), the effect of this condition on essential oils composition and activity is rarely discussed. To the best of our knowledge, only TOMAINO and co-workers (2005) studied the effect of heating (80–180 °C) on free radical-scavenger activity and on the chemical composition of some spice essential oils employed in the food industry.

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In the mean time, apprehension has grown about foodborne microorganisms (ADAMS et al., 1998; RUBERTO et al., 2000; FALEIRO et al., 2005). In fact, the consumption of food contaminated with foodborne bacteria, such as meat, fish, vegetables, and dairy products, especially cheese, can constitute a serious health risk to humans. In addition, the importance of this foodborne pathogen is reinforced by the ability to adapt when exposed to sublethal conditions during food processing and gain tolerance to more severe conditions (FRIEDMAN et al., 2002; SINGH et al., 2003; FALEIRO et al., 2005). There is therefore interest for searching new methods of making food safe as well as of using natural products as antibacterial additives.

In Tunisia, the genus *Thymus* (Lamiaceae) is mainly represented by *Thymus capitatus* Hoff. et Link. [= *Coridothymus capitatus* (L.) Rehb. f., *Satureja capitata* L., *Thymbra capitata* (L.) Cav.], a perennial, herbaceous shrub commonly used as a spicy herb and locally known under the common name “zaâtar”. In view of the antibacterial and antioxidant capacity of this species oils (BOUNATIROU et al., 2007), the purpose of this work was to evaluate its thermal stability when stored for over a month at 60 °C, and to assess the corresponding effect on the chemical composition, antibacterial and antioxidant activities.

## 1. Materials and methods

### 1.1. Plant material

Samples (approximately 30 g dry weight) of the aerial parts from *Thymus capitatus* Hoff. et Link., growing wild in Tunisia, were collected during the vegetative (January, 2005), the flowering and the post-flowering phases (July–August, 2005) from three different localities: Jendouba (interior north), Haouaria (littoral north) and Aïn Tounine (littoral south). The plant material was dried in the dark at room temperature before extraction.

### 1.2. Essential oil extraction and thermal stability assay

Essential oil was obtained from each collected material, in a total of 16 samples, by hydrodistillation during 3h using a Clevenger-type apparatus (EUROPEAN PHARMACOPOEIA, 1996). The essential oils were kept at –4 °C until analysis. The study of the composition of these oils (BOUNATIROU et al., 2007) indicated strong oil chemical homogeneity and that higher antioxidant and antibacterial activity was observed with the flowering and the post-flowering phase essential oils. In view of this, these oils were combined in order to have enough oil to perform the different thermal stability studies. The oil mixture was kept in closed, transparent 10 ml glass tubes, in an oven at 60 °C in the dark for 37 days. Aliquots of oil were taken periodically to perform the time course study of oil composition and biological activities.

### 1.3. Essential oils analysis

**Gas chromatography:** Gas chromatographic analyses were performed using a Perkin Elmer 8700 gas chromatograph equipped with two flame ionization detectors (FIDs), a data handling system and a vapourising injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness 0.15 µm; J & W Scientific Inc.). Oven temperature was programmed, 45–175 °C, at 3 °C min<sup>-1</sup>, subsequently at 15 °C min<sup>-1</sup> up to 300 °C, and then held isothermal for 10 min; injector and detector temperatures were 280 °C and 290 °C,

respectively; carrier gas, hydrogen, was adjusted to a linear velocity of 30 cm s<sup>-1</sup>. The samples were injected using split sampling technique at a ratio 1:50. The volume of injection was 0.2 µl of a pentane-oil solution. The percentage composition of the oils was computed by the normalisation method from the GC peak areas, calculated as mean values of two injections of each oil without using correction factors.

Gas chromatography-mass spectrometry: The GC-MS unit consisted of a Perkin Elmer Autosystem XL gas chromatograph, equipped with DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific, Inc.), and was interfaced with Perkin-Elmer Turbomass mass spectrometer (software version 4.1). Injector and oven temperatures were as above; transfer line temperature, 280 °C; ion trap temperature, 220 °C; carrier gas, helium, was adjusted to a linear velocity of 30 cm s<sup>-1</sup>; split ratio, 1:40; ionisation energy, 70 eV; ionization current, 60 µA; scan range, 40–300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices relative to C<sub>9</sub>–C<sub>16</sub> *n*-alkane indices and GC-MS spectra from a home-made library, based on data of components of reference oils (RO), laboratory-synthesised components (LSC) and commercially available standards (CAS) from a home-made library. RO 1. *Thymus caespitosus* oils, RO 2. *Cinnamomum zeylanicum* oil, RO 3. *Achillea millefolium* oils. CAS 1. Extrasynthese (Cymit Química, S.L., Barcelona, Spain), CAS 2. Sigma-Aldrich (Steinheim, Germany). CAS 3. Fluka (Steinheim, Germany), CAS 4. Riedel-de Haën (Seelze, Germany).

#### 1.4. Antioxidant activity

From each sample, different concentrations of essential oils were prepared in methanol: 100, 500 and 1000 mg l<sup>-1</sup>. The antioxidant activity of *T. capitatus* essential oils was carried out using two different methods: free radical scavenging activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) and by the TBARS (thiobarbituric acid reactive substances) assays.

Free radical scavenging activity: Modified DPPH (2,2-diphenyl-2-picrylhydrazil) method was used (BLOIS, 1958). Briefly, a solution of DPPH in methanol (24 µg ml<sup>-1</sup>) was prepared and 2 ml of this solution was added to 50 µl of extracts solution in methanol at different concentrations (100, 500 and 1000 mg l<sup>-1</sup>). Then the absorbance was measured at 517 nm in a spectrophotometer Shimadzu 160-UV after 5 min (LEBEAU et al., 2000). Radical scavenging activity was calculated using the following equation: Scavenging effect % = [(A<sub>0</sub> - A<sub>1</sub>)/A<sub>0</sub>] \* 100, where A<sub>0</sub> was the absorbance of the control sample (without essential oil) and A<sub>1</sub> was the absorbance in the presence of the sample (t=5 min).

TBARS assay: A modified thiobarbituric acid-reactive substances (TBARS) assay (WONG et al., 1995) was also used to measure the potential antioxidant capacity of *T. capitatus* essential oils. Egg yolk homogenate was used as lipid-rich media. An aliquot of yolk material was made up to a concentration of 10% (w/v) in KCl (1.15%, w/v). The yolk was then homogenised for 30 s, followed by ultrasonication for further 5 min. Five hundred µl of 10% (w/v) homogenate and 100 µl of sample, solubilised in methanol, were added to a test tube and made up to 1 ml with distilled water, followed by addition of 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) 2-thiobarbituric acid (TBA) in 1.1% (w/v) sodium dodecyl sulphate (SDS). Each essential oil sample and tested substance was assayed at concentrations of 100, 500 and 1000 mg l<sup>-1</sup>. This mixture was stirred in a vortex, and heated at 95 °C for 1 h. After cooling at room temperature, 5 ml butane-1-ol was added to each tube, stirred and centrifuged at 3.000 r.p.m. for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer Shimadzu 160-UV. All values were expressed as antioxidant index (AI%), calculated by the formula: Antioxidant index % = [(A<sub>0</sub>-A<sub>1</sub>)/A<sub>0</sub>] \* 100, where A<sub>0</sub> being the absorbance value of the fully oxidized control and A<sub>1</sub>, the absorbance of the test sample.

### 1.5. Antimicrobial activity determination

The microorganisms used in this study were three different strains of *Staphylococcus aureus*, C15, ATCC6538, ATCC25923, and these strains were a gift from INETI-DTIA (Instituto Nacional de Engenharia e Tecnologia Industrial, Departamento de Tecnologia das Industrias Alimentares). The antimicrobial activity of essential oils was tested by the disc agar diffusion method as described by BURT and REINDERS (2003) and FALEIRO and co-workers (2003; 2005). Original cultures are kept at  $-80\text{ }^{\circ}\text{C}$ . Prior to the assay, the bacterial cultures were plated in fresh agar BHI (Brain Heart Infusion) plates and grown for 24 h at  $37\text{ }^{\circ}\text{C}$ . From each plate a loop was used to inoculate 10 ml of BHI and left for about 2 h at  $37\text{ }^{\circ}\text{C}$  until the cultures reached the exponential phase. From the above exponential culture 100  $\mu\text{l}$  were used to inoculate BHI agar plates. Sterile filter paper discs (6 mm, Oxoid), containing 4  $\mu\text{l}$  (0.8  $\mu\text{l}$ /disc) of essential oil diluted (1:5) in propanol were distributed on the agar surface. Sterile water was used as the negative control, whereas the antibiotic cloramphenicol disc was used as positive control (30  $\mu\text{g}$ /disc). Inhibition zones were determined after an incubation period of 24 h at  $37\text{ }^{\circ}\text{C}$ . The antibiotic was not submitted to the temperature of the assay ( $60\text{ }^{\circ}\text{C}$ ).

### 1.6. Statistical analysis

The obtained antioxidant and antimicrobial results were stated in mean  $\pm$  standard deviation. Analysis of variance was performed by ANOVA procedures (SPSS 14.0 for Windows). Significant differences between means were determined by Tukey Post Hoc tests. P values inferior to 0.05 were regarded as significant.

## 2. Results and discussion

### 2.1. Essential oil chemical composition

Thirty-two components were identified in the eight *T. capitatus* essential oil samples analysed after storage at  $60\text{ }^{\circ}\text{C}$ , for 37 days in the dark, amounting to 92–100% of the total oils, which are listed in Table 1 in order of their elution from a DB-1 column. All of the oils analysed were dominated by the monoterpene fraction (95–97%), the oxygen-containing monoterpene being the most representative group (71–77%) of this fraction and in all other oils. Sesquiterpenes ranged from 2–3% in the oil samples.

Carvacrol was the main component of all the essential oils, independently of the storage period (68–74%). This component was already reported by other authors as being the major one present in the same plant species collected in Tunisia (HEDHILI et al., 2005; BOUNATIROU et al., 2007). *p*-Cymene and  $\gamma$ -terpinene were also present in considerable amounts in the beginning of the assay (9% for both). In general, *p*-cymene increased over time, whereas  $\gamma$ -terpinene decreased. Such profile suggests that heating samples promoted the aromatisation of  $\gamma$ -terpinene generating *p*-cymene.

The increase in the relative amount of *p*-cymene over time at  $60\text{ }^{\circ}\text{C}$  can be explained by its thermodynamic stability in relation to  $\alpha$ -thujene,  $\alpha$ -terpinene, and  $\gamma$ -terpinene (HARDER & FOSS, 1999). In fact, a decrease of  $\alpha$ -terpinene (from 2% in the beginning to zero at the end of the assay) and  $\alpha$ -thujene (from 1 to 0.1%) was also registered (Table 1). In the present work, the temperature assayed might be responsible for the isomerisation and aromatisation processes. The aromatisation of  $\gamma$ -terpinene to *p*-cymene is a normal biosynthetic pathway in plants (POULOUSE & CROTEAU, 1978). In essential oils submitted to different temperatures

(from room temperature to 180 °C, for 3 h), TOMAINO and co-workers (2005) reported an increase of some components. For oregano and thyme oils, *p*-cymene amounts were higher at 180 °C than at room temperature, nevertheless without statistical significance.

Table 1. Percentage composition of the essential oils isolated from the aerial parts of *T. capitatus*, maintained at 60 °C for 37 days

IP*	Components	RI	<i>Thymus capitatus</i>					
			Time (days)					
			0	4	8	11	28	37
RO 1, RO 2	$\alpha$ -Thujene	924	1.3	0.8	0.7	0.6	0.1	0.5
CAS 1, RO 1	$\alpha$ -Pinene	930	0.8	0.6	0.6	0.6	0.5	0.2
CAS 1, RO 1	Camphene	938	0.3	0.2	0.2	0.2	0.2	0.1
CAS 2	1-Octen-3-ol	961	0.1	0.1	0.1	t	0.1	0.1
CAS 1, RO 1, RO 2	$\beta$ -Pinene	963	0.1	0.1	0.1	t	0.2	0.1
CAS 2, RO 1, RO 2	Myrcene	975	2.2	1.6	1.7	1.6	1.2	1.3
CAS 2, RO 1, RO 2	$\alpha$ -Phellandrene	995	0.3	0.3	0.2	0.3	0.1	t
CAS 2, RO 1, RO 2	$\delta$ -3-Carene	1000	0.1	0.1	0.1	0.1	0.1	t
CAS 2, RO 1, RO 2	$\alpha$ -Terpinene	1002	1.9	1.4	1.3	1.2	0.2	t
CAS 2, RO 1, RO 2	<i>p</i> -Cymene	1003	9.2	9.0	11.3	12.7	16.0	17.0
CAS 1, RO 1, RO 2	$\beta$ -Phellandrene	1005	0.3	0.3	0.3	0.3	0.2	0.2
CAS 3, RO 1, RO 2	Limonene	1009	0.4	0.4	0.4	0.4	0.4	0.4
CAS 3, RO 1, RO 2	<i>cis</i> - $\beta$ -Ocimene	1017	t	t	t	t	t	t
CAS 3, RO 1, RO 2	<i>trans</i> - $\beta$ -Ocimene	1027	t	t	t	t	t	t
CAS 2, RO 1, RO 2	$\gamma$ -Terpinene	1035	9.2	6.5	5.2	4.3	0.2	0.3
CAS 3	<i>trans</i> -Sabinene hydrate	1037	0.1	0.2	0.2	0.1	t	t
CAS 2, RO 1, RO 2	Terpinolene	1064	0.2	0.1	0.1	0.1	0.1	t
CAS 3	<i>cis</i> -Sabinene hydrate	1066	0.1	t	t	t	t	t
CAS 2	Linalool	1074	0.8	0.9	0.9	0.8	1.4	1.2
CAS	Borneol	1134	0.4	0.5	0.5	0.5	0.6	0.6
CAS 2, RO 1, RO 2	Terpinen-4-ol	1148	0.5	0.6	0.6	0.6	1.0	0.9
CAS 4, RO 1	Thymol	1275	0.4	0.4	0.4	0.4	0.7	0.6
CAS 3, RO 1	Carvacrol	1286	68.3	72.6	70.4	71.8	73.6	73.7
CAS 2, RO 3	Eugenol	1327	t	t	t	t	t	t
RO 1	Carvacrol acetate	1348	t	0.2	0.1	0.2	0.1	t
CAS 2, RO 1, RO 2	$\beta$ -Caryophyllene	1414	1.6	2.0	1.8	1.8	0.6	1.3
RO 2	Aromadendrene	1428	0.2	0.1	0.2	0.1	0.5	t

Table 1. continued

IP*	Components	RI	<i>Thymus capitatus</i>						
			Time (days)						
			0	4	8	11	28	37	
CAS 3, RO 1, RO 2	$\alpha$ -Humulene	1447	0.1	t	t	t	t	t	t
RO 1	Viridiflorene	1487	t	t	t	t	t	t	t
RO 1, RO 2	$\beta$ -Bisabolene	1494	0.1	0.1	0.1	0.2	0.2	0.3	
RO 1	Spathulenol	1551	t	t	t	t	t	t	t
CAS 3, RO 1, RO 2	$\beta$ -Caryophyllene oxide	1561	0.2	0.6	0.6	0.8	1.1	0.7	
	% Identification		99.2	99.4	98.0	99.6	99.3	99.7	
	Grouped components								
	Monoterpene hydrocarbons		26.3	21.3	22.3	22.4	19.4	20.2	
	Oxygen-containing monoterpenes		70.6	75.2	73.0	74.3	77.3	77.1	
	Sesquiterpene hydrocarbons		1.9	2.3	2.0	2.1	1.4	1.6	
	Oxygen-containing sesquiterpenes		0.3	0.6	0.6	0.8	1.1	0.7	
	Phenylpropanoids		t	t	t	t	t	t	
	Others		0.1	0.1	0.1	t	0.1	0.1	

\*IP: Identification procedure. All components were identified based on a home-made library created with reference oils (RO), laboratory-synthesized components (LSC) and commercial available standards (CAS). RO 1. *Thymus caespitius* oils, RO 2. *Achillea millefolium* oils, RO 3. *Cinnamomum zeylanicum* oil. CAS 1. Extrasynthese (Cymit Química, S.L.), CAS 2. Sigma-Aldrich, CAS 3. Fluka, CAS 4. Riedel-de Haën.

RI: Retention Index relative to C<sub>9</sub>-C<sub>16</sub> n-alkanes on the DB-1 column, t: traces (<0.05%)

## 2.2. Antioxidant activity

Although differences were detected on the relative amounts of some components of *T. capitatus* oil, maintained at 60 °C over 37 days, they were not sufficiently important to modifying the ability to prevent lipid peroxidation or the free radical-scavenger effectiveness (Table 2), particularly at the highest concentrations tested (500 and 1000 mg l<sup>-1</sup>).

From the thermal stability point of view, and with the exception of the fourth day of study, heating the essential oil of *T. capitatus* did not greatly influence its antioxidant ability when evaluated by the TBARS method at the highest concentrations tested (500 and 1000 mg l<sup>-1</sup>) (Table 2). At the lowest concentration (100 mg l<sup>-1</sup>), the antioxidant activity improved with the time of storage at 60 °C, which remains difficult to explain. Some new compounds formed during storage and detected by GC analysis might be responsible for these results.

Table 2. Antioxidant index and scavenging effect (%) of *Thymus capitatus* essential oils during storage at 60 °C using TBARS and DPPH scavenging activity assays

Time (days)	Antioxidant index (%)					
	TBARS assay Concentrations (mg l <sup>-1</sup> )			DPPH scavenging activity Concentrations (mg l <sup>-1</sup> )		
	100	500	1000	100	500	1000
0	17.3±3.6a	90.8±0.9cd	88.2±1.4b	11.0±2.3a	69.3±4.4a	90.5±1.4a
4	53.4±3.6b	66.5±0.9a	81.8±1.4a	31.9±2.3bc	76.9±4.4ab	92.0±1.4a
8	75.2±3.6c	85.9±0.9b	89.1±1.4b	29.6±2.3b	82.8±4.4abc	91.2±1.4a
11	79.3±3.6cd	89.1±0.9c	90.2±1.4b	35.3±2.3bc	82.5±4.4abc	90.4±1.4a
28	88.6±3.6d	92.7±0.9d	93.0±1.4b	38.8±2.3c	92.7±4.4c	93.0±1.4a
37	89.5±3.6d	92.5±0.9d	92.8±1.4b	38.5±2.3c	92.5±4.4c	92.8±1.4a

Values represent mean ±standard deviation of three replicates.

Values followed by the same letter under the same column are not significantly different (P>0.05)

The dominance of carvacrol in *T. capitatus* oil can be partly responsible for the antioxidant ability detected. The antioxidant activity ability of carvacrol and thymol was already mentioned by many authors (DEIGHTON et al., 1993; MADSEN & BERTELSEN, 1995; BARATTA et al., 1998; YANISHLIEVA et al., 1999; RUBERTO & BARATTA, 2000). The decrease of  $\alpha$ -terpinene and  $\gamma$ -terpinene over time did not decrease the antioxidant capacity of *T. capitatus* essential oil, in contrast to that reported by RUBERTO and BARATTA (2000) who found a good antioxidant ability of these pure components, even comparable to that of  $\alpha$ -tocopherol. The fact that *T. capitatus* oil is a complex mixture makes it difficult to establish a correlation between its antioxidant activity and each of the oil components.

When DPPH radical scavenging activity of *T. capitatus* oil stored at 60 °C for 37 days was evaluated, all the samples showed comparable scavenging activity, particularly for the concentrations of 500 and 1000 mg l<sup>-1</sup> (Table 2). The free radical-scavenger effectiveness of the oil at 100 mg l<sup>-1</sup> was not as high as the antioxidant indices measured by the TBARS method, mainly at the end of the assay. As it was found for the TBARS method, the radical scavenging activity of the oil was not much influenced by the time of storage at 60 °C.

TOMAINO and co-workers (2005) using similar concentrations (0.026–1.32  $\mu$ l ml<sup>-1</sup>) of diverse essential oils to those assayed in our work in order to investigate the influence of thermal treatment on their free radical-scavenger activity found that temperature did not greatly influence antioxidant activity of the oils.

### 2.3. Antimicrobial activity

Data on antibacterial activity of the essential oil maintained at 60 °C for 37 days indicate stability of essential oil properties (Table 3). The tested *Staphylococcus aureus* strains demonstrated a significantly different (P<0.05) susceptibility to the essential oil during the assay period. The most susceptible strain was ATCC 6538 and no significant differences (P>0.05) between the susceptibilities of strains C15 and ATCC 25923 was found (Table 3).

Table 3. Susceptibility of *Staphylococcus aureus* strains (ATCC 6538, C15 and ATCC 25923) to essential oil mixture maintained at 60 °C during 37 days, expressed by diameter of inhibition zone (including the disc diameter, 6 mm) at concentration 0.8 µl/disc

Time (days)	<i>Staphylococcus aureus</i>		
	ATCC 6538	C15	ATCC 25923
0	41.3±1.2a	13.0±1.0a	12.7±1.2a
4	40.7±1.2a	13.7±0.6a	13.7±1.2a
8	40.7±0.6a	15.7±0.6a	12.0±0.0a
11	42.0±1.7a	15.7±2.1a	12.3±0.6a
16	41.0±2.0a	13.0±1.0a	12.0±1.0a
23	42.3±2.5a	14.0±1.7a	12.3±0.6a
28	41.0±3.5a	12.0±0.0a	12.7±0.6a
37	40.0±2.0a	12.7±1.7a	11.7±1.5a
Antibiotic	28.0±1.7a	23.7±6a	27.0±1.0a

Values represent mean ± standard deviation of three replicates.

Values of the samples followed by the same letter under the same column are not significantly different ( $P>0.05$ ).

Values of the antibiotic susceptibility are the mean of the determinations along the assay. Data followed by the same letter under the same row are not significantly different ( $P>0.05$ ).

The three strains were equally ( $P>0.05$ ) susceptible to the antibiotic chloramphenicol (Table 3).

### 3. Conclusion

Deterioration of foodstuffs by oxidation during storage, processing, or heat treatment is a great concern of food industry. Generally, antioxidants added to food are a way to prevent fat oxidation. For its application in foodstuffs, it is necessary to be sure of the chemical and biological stability over time. In the present case, thermal stability of the essential oils isolated from *T. capitatus* of Tunisia was evaluated.

The present results indicate the possible use of *T. capitatus* essential oil in food industry, since it does not lose either antioxidant or antibacterial ability, even when stored at relative high temperature (60 °C) for a period higher than a month (37 days). However, since some quantitative changes occur in the essential oil composition, the extent of these changes in the organoleptic properties of the oil and agreeability to the consumers should be evaluated.

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