

## Bioactive Compounds and Antioxidant Activities of Thyme-Enriched Refined Corn Oil

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### ABSTRACT

This study was designed to examine bioactive compounds of the thyme-enriched corn oil and to determine the antioxidant activity of its methanolic extract. Volatile compounds composition was investigated by Gas Chromatography-Mass Spectrometry (GC-MS), whereas phenolic compounds analysis was performed by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). Antioxidant activities of the flavoured oil methanolic extract were evaluated using DPPH radical scavenging,  $\beta$ -carotene-linoleic acid bleaching, reducing power and total antioxidant assays. Flavoured oil major volatiles were *p*-cymene (26.47%),  $\alpha$ -thujene (24.06%) and  $\gamma$ -terpinene (14.30%). Its methanolic extract had higher Total Phenol Contents (TPCs) (53.99 mg 100 g<sup>-1</sup>) than that of the crude oil (23.63 mg 100 g<sup>-1</sup>). Thymol was the main phenolic compound in the flavoured oil (55.84%). Flavoured oil methanolic extract showed higher total antioxidant activity (185.22 mg Gallic Acid Equivalent (GAE) 100 g<sup>-1</sup>) than that of the crude one (100.66 mg GAE 100 g<sup>-1</sup>). In  $\beta$ -carotene-linoleic acid bleaching and DPPH radical scavenging assays, flavoured oil methanolic extract showed higher activities than that of the crude oil based on  $IC_{50}$  values. Flavoured and crude oils were characterized by a close  $CE_{50}$  values (1.81 and 1.85 mg mL<sup>-1</sup>, respectively). Antioxidant activities of the thyme-enriched oil were mainly due to the presence of phenolic compounds such as thymol and hydrocarbons such as  $\gamma$ -terpinene and *p*-cymene. The thyme-enriched oil could be considered as a new and natural source of antioxidant.

**Keywords:** Antioxidant activity, Corn oil, Essential oil, Polyphenols, *Thymus capitatus*.

### INTRODUCTION

In order to overcome oils stability problems, synthetic antioxidants, such as Butylated HydroxyAnisole (BHA), Butylated HydroxyToluene (BHT), Ter-Butyl HydroQuinone (TBHQ) have been used as food additives. However, recent reports reveal that these compounds may be implicated in many health risks, including cancer. Therefore, there is an increasing trend to replace these synthetic antioxidants with natural ones, which are supposed to be safer (Bouaziz *et al.*, 2008). The importance of aromatic plants as natural antioxidants has been well established. In fact, plant

natural antioxidants used as preventative components towards diseases, also prevent oxidative deterioration of vegetable oils and fats during processing, distribution, and storage (Vagi *et al.*, 2005). Among plant extracts, *Thymus* species are widely used in the food industry as herbal teas, flavouring agents (condiment and spice), aromatic, and medicinal plants (Tepe *et al.*, 2005). Thymes were well known for their antispasmodic, sedative, antioxidant (Safaei-Ghomi *et al.*, 2009), antimicrobial (Hazzit *et al.*, 2009), antifungal (Giordani *et al.*, 2008) and antibacterial (Bounatirou *et al.*, 2007) properties. Moreover, thyme was used as alternative to antibiotic on growth

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performance, immune system, blood factors and intestinal microflora in broiler chickens for example (Rahimi *et al.*, 2011).

Effectiveness of plant extracts in stabilizing vegetable oils has been previously studied (Sultana *et al.*, 2007). In fact, Moldao-Martins *et al.* (2004) studied volatile compounds and sensory characteristics of the olive oil flavoured by *Mentha piperita* and *Thymus mastichina* L. In addition, Bensmira *et al.* (2007) showed the effect of lavender and thyme incorporation in sunflower seed oil on its resistance to frying temperatures. Moreover, Ali (2010) study revealed the improvement of the stability of fried sunflower oil by using different levels of pomposia (*Syzygium Cumini*). Abramovic and Abram (2006) also studied the effect of added rosemary extract on oxidative stability of Camelina sativa oil. Furthermore, Bede and Chigbu (2007) proved the effectiveness of some spices on palm oil stability and acceptability. Few studies focused on the oxidative stability of corn oil enriched with antioxidants (Naz *et al.*, 2004). However, there is no information regarding bioactive compounds and antioxidant activity of thyme-enriched corn oil.

The first aim of the present study was to evaluate volatiles of corn oil supplemented with *Thymus capitatus* flowers and the second one was to investigate its phenolics and antioxidant activities compared to the crude and refined oils. Such information is valuable as corn oil containing high antioxidant activity can have an extended shelf life and the presence of bioactive compounds can prevent it from oxidation.

## MATERIALS AND METHODS

All solvents used in the experiments (diethyl ether, chloroform, hexane, acetonitrile and methanol) were purchased from Merck (Darmstadt, Germany). Sodium methylate ( $\text{CH}_3\text{ONa}$ ), sodium chloride ( $\text{NaCl}$ ), sulphuric acid ( $\text{H}_2\text{SO}_4$ ), Folin-Ciocalteu reagent,  $\beta$ -carotene, linoleic acid,

ethylenediaminetetraacetic acid (EDTA), sodium carbonate anhydrous ( $\text{Na}_2\text{CO}_3$ ), gallic acid, sodium nitrite ( $\text{NaNO}_2$ ), aluminum chloride hexahydrate ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), 2,2-DiPhenyl-1-PicrylHydrazyl (DPPH), trichloroacetic acid, iron (II) chloride ( $\text{FeCl}_2$ ), iron (III) chloride anhydrous ( $\text{FeCl}_3$ ), ascorbic acid, Calcium hexacyanoferrat (III), iron (II) chloride tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] were obtained from Sigma-Aldrich (Steinheim, Germany). Essential oil standards were purchased from Fluka and Sigma-Aldrich (Steinheim, Germany). Homologous series of  $\text{C}_8$ – $\text{C}_{22}$  *n*-alkanes used for identification were obtained from Sigma-Aldrich (Steinheim, Germany). Authentic standards of phenolic compounds were purchased from Sigma and Fluka. Stock solutions of these compounds were prepared in HPLC-grade methanol. These solutions were wrapped in aluminum foil and stored at 4°C. All other chemicals used were of analytical grade.

Refined corn (*Zea mays* L.) oil was purchased from a local refinery and stored in the cold (4°C) in the dark. Fresh thyme flowers were collected at full flowering stage from the Mornag Mountain (North of Tunisia). They were air-dried and stored at room temperature away from humidity.

### Oil Treatment with Natural Herbs

Thyme dried flowers were incorporated into refined corn oil in a mixing agitator at a rate of 5 g in 40 mL of oil, for 25 minutes, after optimizing conditions. The mixture was centrifuged in order to remove solid residue. Flavoured oil was stored at 4°C in amber flasks until volatile extraction.

### Volatile Compounds Extraction

Three lots of 100 g air-dried flowers were separately hydrodistilled for 90 minutes.

Volatile compounds were collected in diethyl ether using liquid-liquid isolation. All experiments were done in triplicates and results were expressed in percentages.

Flavoured oil volatile compounds were extracted by dynamic headspace method with splashed gas N<sub>2</sub> (Strip-trap) according to published data (Dhifi *et al.*, 2002). All experiments were made in triplicates.

### Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS analyses were carried out on a gas chromatograph HP 5890 series (II) coupled to a HP 5972 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization (70eV). A HP-5MS capillary column (30 m×0.25mm, 0.25 μm film thickness Agilent Technologies, Hewlett–Packard, CA, USA) was used. Column temperature was programmed to rise from 50 to 240°C at a rate of 5 °C min<sup>-1</sup>. The carrier gas was helium with a flow rate of 1.2 mL min<sup>-1</sup> and a split ratio of 60:1. Scan time and mass range were 1 second and 40–300 m z<sup>-1</sup>, respectively. Oil volatiles identification was based on the comparison of their retention indices relative to (C<sub>8</sub>–C<sub>22</sub>) *n*-alkanes with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC–MS data system and other published mass spectra (Adams, 2001).

### Polyphenol Extraction

The air-dried flowers were finely ground with a blade-carbide grinding (IKA-WERK Type: A: 10) system. Triplicate sub-samples of 1 g of ground flowers were extracted by stirring with 10 mL of pure methanol for 30 minutes at room temperature (25°C). The extract was then kept for 24 hours at 4°C, filtered through a

Whatman No. 4 filter paper, evaporated under vacuum to dryness and stored at 4°C until analysis (Mau *et al.*, 2001).

Oil phenolics extraction was carried out with the slightly modified method of Baccouri *et al.* (2008). Briefly, 8 g of oil was added to 8 mL of *n*-hexane and 8 mL of a methanol/water (90:10, v/v) solution in a 20 mL centrifuge tube. The mixture was centrifuged for 3 minutes at 1,490 g. The hydroalcoholic phase was collected, and the hexanic phase was re-extracted twice with 8 mL of methanol/water (90:10, v/v) solution.

### Reversed-Phase (RP-HPLC) Analysis and Identification of Phenolic Compounds

Phenolic compounds analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with an UV–vis multiwavelength detector. The separation was carried out on a 250×4.6-mm, 4-μm Hypersil ODS C<sub>18</sub> reversed phase column (Pore size: 100 Å; Carbon load: 10%) at ambient temperature. This encapped column adds a monolayer of octadecyl silane (C18) to the Hypersil spherical porous Silica support. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 mL min<sup>-1</sup>. The gradient program was as follows: 15% A/85% B 0–12 minutes, 40% A/60% B 12–14 minutes, 60% A/40% B 14–18 minutes, 80% A/20% B 18–20 minutes, 90% A/10% B 20–24 minutes, 100% A 24–28 minutes (Bourgou *et al.*, 2008). The injected volume was 20 μl, and peaks were monitored at 280 nm. Samples were filtered through a 0.45 μm membrane filter before injection. Phenolic compounds were identified according to their retention times and spectral characteristics of their peaks against those of standards. Analyses were performed in triplicate.



### Total Antioxidant Capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by oil extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto *et al.*, 1999). An aliquot of oil or flower methanolic extract was combined in an eppendorf tube with 1 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 minutes. After, the mixture had cooled to room temperature and the absorbance of each solution was measured at 695 nm (Anthelie Advanced 2, SECOMAN) against a blank. The antioxidant capacity was expressed as mg gallic acid equivalents per 100 gram of oil (mg GAE 100 g<sup>-1</sup>). All samples were analyzed in three replications.

### DPPH Assay

The ability to scavenge DPPH radical was measured by bleaching of the purple-coloured solution of 1,1-DiPhenyl-2-PicrylHydrazyl radical (DPPH) according to the method of Hanato *et al.* (1988). Samples (1 mL) with different concentrations (0.1, 0.5, 1 and 2 mg mL<sup>-1</sup>) of flowers or oils extracts in methanol/water (90:10, v/v) were added to 0.5 mL of a DPPH methanolic solution (0.2 mmol L<sup>-1</sup>). After shaking vigorously, the mixture was left standing for 30 minutes at room temperature. The mixture absorbance was measured at 517 nm. The antiradical activity was expressed as IC<sub>50</sub> (mg mL<sup>-1</sup>): efficient concentration corresponding to 50% DPPH inhibition. The capacity to scavenge the DPPH radical was expressed as:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where,  $A_0$  is the absorbance of the control at 30 minutes, and  $A_1$  is the absorbance of

the sample at 30 minutes. All samples were analyzed in triplicate.

### Reducing Power

The reducing power of flowers or oils methanolic extracts was carried out using the method of Oyaizu (1986). Samples (1 mL) with different concentrations (0.1, 0.5, 1, 2, 4 and 5 mg mL<sup>-1</sup>) of oils extracts in methanol/water (90:10, v/v) were added to 2.5 mL of 1% potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] and 2.5 mL of sodium phosphate buffer (0.2M, pH 6.6). After incubation at 50°C for 20 minutes, the mixture was added to 2.5 mL of 10% trichloroacetic acid, then centrifuged for 10 minutes at 650 g. After that, 2.5 mL of the upper layer fraction was supplemented with 0.5 mL of ferric chloride (0.1%) and deionised water. The absorbance of the mixture was measured at 700 nm and ascorbic acid was considered as positive control. EC<sub>50</sub> value (mg mL<sup>-1</sup>) is the effective concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm. Ascorbic acid was used as a positive control. Tests were carried out in triplicate.

### β-Carotene Bleaching Test

According to the slightly modified method of Koleva *et al.* (2002), β-carotene (2 mg) was dissolved in 20 mL chloroform, then, linoleic acid (40 mg) and Tween 40 (400 mg) were added to 4 mL of this mixture. After, the chloroform was evaporated at 40°C under vacuum. The mixture was supplemented with 100 mL of oxygenated water and then shaken vigorously. Samples (10 μL) with different concentrations (0.1, 0.5, 1, 2, 4 and 5 mg mL<sup>-1</sup>) of flowers or oils extracts in methanol/water (90:10, v/v) were added to an aliquot (150 μL) of the β-carotene/linoleic acid emulsion. The mixture was stored for 120 minutes at 50°C, and the absorbance was measured at 470 nm by a

microtitre reader (model EAR 400, Labsystems Multiskan MS). Readings of all samples were performed immediately ( $t=0$  min) and after 30 or 120 minutes of incubation. The antioxidant activity (%) of oils methanolic extracts was evaluated in terms of  $\beta$ -carotene bleaching inhibition using the following formula:

$$\% \text{ Inhibition} = [(A_t - C_t) / (C_0 - C_t)] \times 100$$

Where,  $A_t$  and  $C_t$  are the absorbance values measured for the test sample and control, respectively, after incubation for 120 minutes, and  $C_0$  is the absorbance values for the control measured at zero time during the incubation. The results are expressed as  $IC_{50}$  values ( $\text{mg mL}^{-1}$ ): efficient concentration corresponding to 50%  $\beta$ -carotene bleaching inhibition. All experiments were carried out in triplicate.

### Oil Oxidative Stability Index

A Metrohm 743 Rancimat (Metrohm AG, Herisau, Switzerland) was used for analysis of the Oxidative Stability Index (OSI). The tests were done with 3 g oil samples at a temperature of 120°C and an airflow rate of 15 L h<sup>-1</sup> (Farhoosh *et al.*, 2009).

### Statistical Analysis

All data were subjected to statistical analysis using STATISTICA–“Tulsa, USA”. Values obtained were the mean of three replicates  $\pm$  SD (Statsoft, 1998). Differences were tested for significance by ANOVA procedure performed to test differences between samples, using a significance level of  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Volatile Compounds Contents

Thirty-one components were identified in the *Thymus capitatus* essential oil, amounting for 99.02% of the total oil (Table

1). Phenols (67.30%) mainly represented by carvacrol (67%) were the major components, followed by monoterpene hydrocarbons, which attained 28.23% mainly represented by  $\gamma$ -terpinene (7.83%) and *p*-cymene (5.88%). The monoterpene alcohol, terpinene-4-ol, was also found at high proportion of 4.46%. Other compounds are present with relatively low levels of between 1 and 2%: *Alpha*-thujene (1.23%), myrcene (1.68%), *alpha*-terpinene (1.55%), 1,8 cineole (1.95%), linalool (1.12%) and  $\beta$ -caryophyllene (1.46%).

Twenty components were identified in the flavoured oil representing 99.75% of the total volatiles (Figure 1). However, no compound was detected in the refined oil. Flavoured oil was dominated by monoterpene hydrocarbons (92.59% of the total volatiles) and *p*-cymene (26.47%), *alpha*-thujene (24.06%) and  $\gamma$ -terpinene (14.30%) were the major compounds (Table 1). To the best of our knowledge there is no published data on thyme-flavoured oil volatiles to compare with. However, several studies confirmed that thyme essential oil was characterized by its richness in phenols (67.30%), followed by monoterpene hydrocarbons mainly represented by  $\gamma$ -terpinene and *p*-cymene (Bounatirou *et al.*, 2007; Hazzit *et al.*, 2009; Jabri Karoui *et al.*, 2013). The low retention of phenols such as carvacrol in the enriched oil could be due to the high hydrophilic character of these compounds versus monoterpene hydrocarbons which had high hydrophobic character and important affinity to corn oil (Jabri Karoui *et al.*, 2012).

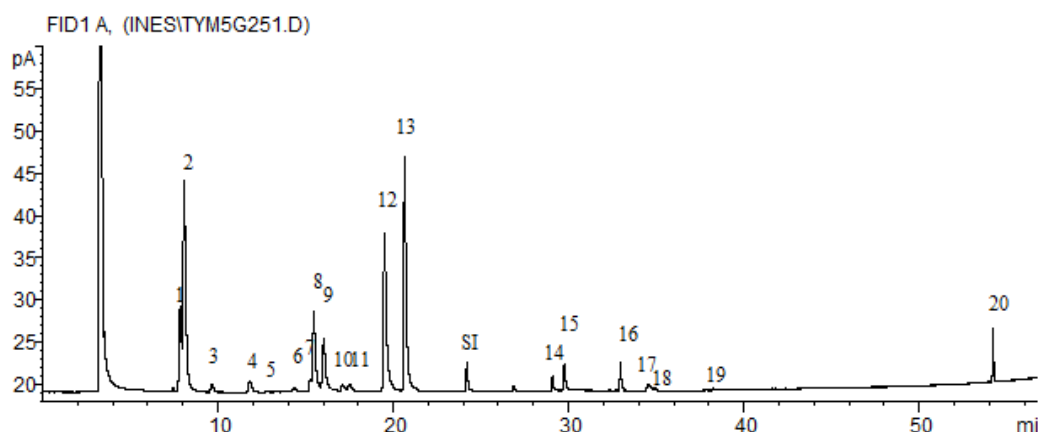
### Phenolic Identification by RP-HPLC

Sixteen phenolic compounds were identified in the flowers methanolic extract, including ten phenolic acids (gallic, vanillic, caffeic, chlorogenic, rosmarinic, o-coumaric, ferulic, p-coumaric and cinnamic acids), five flavonoids (gallate epicatechin, quercetin, quercitrin, apigenin and luteolin) and one phenolic monoterpene (thymol) (Table 2).

**Table 1.** Volatile compounds composition (%) of the flavoured oil.

Compounds	RI <sup>a</sup>	RI <sup>b</sup>	Thyme flavoured oil	Thyme flowers
$\alpha$ -Thujene	928	1035	24.06 $\pm$ 0.03 <sup>a</sup>	1.23 $\pm$ 0.11 <sup>b</sup>
$\alpha$ -Pinene	939	1032	8.16 $\pm$ 0.01 <sup>a</sup>	0.50 $\pm$ 0.01 <sup>b</sup>
Camphene	954	1076	1.10 $\pm$ 0.00 <sup>a</sup>	0.12 $\pm$ 0.02 <sup>b</sup>
1 Octen-3ol	961	-	0.27 $\pm$ 0.00 <sup>a</sup>	0.07 $\pm$ 0.00 <sup>b</sup>
Sabinene	975	1132	-	0.57 $\pm$ 0.07 <sup>b</sup>
$\beta$ -Pinene	980	1118	1.65 $\pm$ 0.00 <sup>a</sup>	0.18 $\pm$ 0.00 <sup>b</sup>
Myrcene	991	1174	8.68 $\pm$ 0.01 <sup>a</sup>	1.68 $\pm$ 0.03 <sup>b</sup>
$\alpha$ -Phellandrene	1006	1176	0.75 $\pm$ 0.01 <sup>a</sup>	0.14 $\pm$ 0.01 <sup>b</sup>
$\delta$ -3-Carene	1011	1159	0.5 $\pm$ 0.01 <sup>a</sup>	0.49 $\pm$ 0.01 <sup>a</sup>
$\alpha$ -Terpinene	1018	1188	6.28 $\pm$ 0.02 <sup>a</sup>	1.55 $\pm$ 0.03 <sup>b</sup>
<i>p</i> -Cymene	1026	1280	26.47 $\pm$ 0.03 <sup>a</sup>	5.88 $\pm$ 0.02 <sup>b</sup>
Limonene	1030	1203	0.63 $\pm$ 0.00 <sup>a</sup>	0.18 $\pm$ 0.01
1-8 Cineole	1033	1213	0.78 $\pm$ 0.01 <sup>b</sup>	1.95 $\pm$ 0.17 <sup>a</sup>
<i>E</i> - $\beta$ -Ocimene	1050	1266	-	0.14 $\pm$ 0.04 <sup>b</sup>
<i>Trans</i> -sabinene hydrate	1053	1474	0.74 $\pm$ 0.00 <sup>a</sup>	0.24 $\pm$ 0.01 <sup>b</sup>
$\gamma$ -Terpinene	1062	1266	14.30 $\pm$ 0.02 <sup>a</sup>	7.83 $\pm$ 0.06 <sup>b</sup>
Linalol	1098	1553	1.38 $\pm$ 0.02 <sup>a</sup>	1.12 $\pm$ 0.03 <sup>b</sup>
Camphor	1143	1532	1.58 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>b</sup>
Borneol	1165	1719	0.09 $\pm$ 0.00 <sup>b</sup>	0.37 $\pm$ 0.02 <sup>a</sup>
Terpinen-4-ol	1178	1611	0.59 $\pm$ 0.01 <sup>b</sup>	4.46 $\pm$ 0.02 <sup>a</sup>
Teroinolene	1088	1290	-	0.18 $\pm$ 0.01 <sup>a</sup>
Geraniol	1255	1857	-	0.25 $\pm$ 0.02 <sup>a</sup>
Acetate de linalyle	1257	1556	-	0.04 $\pm$ 0.00 <sup>a</sup>
Thymol	1290	2198	-	0.31 $\pm$ 0.03 <sup>a</sup>
Carvacrol	1292	2239	1.56 $\pm$ 0.01 <sup>b</sup>	66.99 $\pm$ 0.01 <sup>a</sup>
Eugenol	1356	2192	-	0.09 $\pm$ 0.00 <sup>a</sup>
Acetate de geranyle	1383	1765	-	0.26 $\pm$ 0.01 <sup>a</sup>
Acetate de neryle	1385	1733	-	0.15 $\pm$ 0.01 <sup>a</sup>
$\beta$ -Caryophyllene	1419	1612	0.16 $\pm$ 0.01 <sup>b</sup>	1.46 $\pm$ 0.04 <sup>a</sup>
Germacrene D	1480	1726	-	0.22 $\pm$ 0.01 <sup>a</sup>
Caryophyllene oxide	1581	2008	-	0.32 $\pm$ 0.02 <sup>a</sup>
<i>Chemical classes</i>				
Monoterpene hydrocarbons			92.59 $\pm$ 0.04 <sup>a</sup>	20.67 $\pm$ 0.05
Monoterpene phenols			1.56 $\pm$ 0.01 <sup>b</sup>	67.30 $\pm$ 0.04 <sup>a</sup>
Monoterpene alcohols			3.07 $\pm$ 0.03 <sup>b</sup>	6.61 $\pm$ 0.09 <sup>a</sup>
Monoterpene esters			0.79 $\pm$ 0.01 <sup>b</sup>	0.45 $\pm$ 0.00 <sup>b</sup>
Monoterpene ketones			1.59 $\pm$ 0.0 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>b</sup>
Sesquiterpene hydrocarbons			0.16 $\pm$ 0.01 <sup>b</sup>	1.68 $\pm$ 0.05 <sup>a</sup>
Oxygenated sesquiterpenes			-	0.32 $\pm$ 0.02 <sup>a</sup>
Total			99.75 $\pm$ 0.00 <sup>a</sup>	99.02 $\pm$ 0.01 <sup>b</sup>

<sup>a</sup> Values are given as mean  $\pm$  SD (n = 3). Means with different small letter are significantly different ( $P < 0.05$ ). <sup>b</sup> Components are listed in order of elution in apolar column (HP-5). Retention indices calculated using respectively an apolar column (HP-5) and polar column (HP Innowax). Volatile compound proportions were calculated from the chromatograms obtained on the HP Innowax column. MS: Mass Spectrometry; GC: Gas Chromatography; Co-C: Co-Chromatography; - = not detected.



**Figure 1.** GC Chromatographic profile of thyme-enriched corn oil volatiles. 1.  $\alpha$ -Pinene, 2.  $\alpha$ -Thujene, 3. Camphene, 4.  $\beta$ -Pinene, 5. 1 Octen-3ol, 6.  $\delta$ -3-Carene, 7.  $\alpha$ -Phellandrene, 8. Myrcene, 9.  $\alpha$ -Terpinene, 10. Limonene, 11. 1,8-Cineole, 12.  $\gamma$ -Terpinene, 13. *p*-Cymene, 14. Trans-sabinene hydrate, 15. Camphor, 16. Linalol, 17. Terpinene-4-ol, 18.  $\beta$ -Caryophyllene, 19. Borneol, 20. Carvacrol, SI. Internal Standard (6-methyl-5-hepten-2-one).

**Table 2.** Percentages (%) of phenolic compounds of crude and flavoured corn oils measured by RP-HPLC.<sup>a</sup>

	Crude oil	Thyme flavoured oil	Thyme flowers
Phenolic acids	51.07 ± 0.62 <sup>a</sup>	18.58 ± 0.18 <sup>b</sup>	56.86 ± 0.31
Caffeic acid	1.53 ± 0.02 <sup>a</sup>	0.35 ± 0.03 <sup>b</sup>	0.82 ± 0.02
Gallic acid	6.02 ± 0.08 <sup>a</sup>	1.17 ± 0.08 <sup>b</sup>	14.25 ± 0.31
Dihydroxycinnamic acid	6.21 ± 0.02 <sup>a</sup>	-	-
Chlorogenic acid	10.14 ± 0.12 <sup>a</sup>	4.63 ± 0.03 <sup>b</sup>	2.41 ± 0.05
Syringic acid	1.21 ± 0.01 <sup>a</sup>	-	0.93 ± 0.03
Vanillic acid	1.22 ± 0.01 <sup>a</sup>	0.63 ± 0.05 <sup>a</sup>	1.26 ± 0.24
<i>p</i> -coumaric acid	6.46 ± 0.08 <sup>a</sup>	0.69 ± 0.00 <sup>b</sup>	3.35 ± 0.01
Ferulic acid	7.38 ± 0.09 <sup>a</sup>	8.04 ± 0.17 <sup>a</sup>	14.59 ± 0.13
<i>Trans</i> -2-hydroxycinnamic acid	-	0.36 ± 0.00 <sup>a</sup>	6.17 ± 0.13
Cinnamic acid	1.12 ± 0.01 <sup>a</sup>	-	-
<i>trans</i> -cinnamic acid	2.75 ± 0.03 <sup>a</sup>	-	-
Rosmarinic acid	1.49 ± 0.02 <sup>a</sup>	0.46 ± 0.00 <sup>b</sup>	9.90 ± 0.21
<i>O</i> -Coumaric acid	5.49 ± 0.07 <sup>a</sup>	2.24 ± 0.02 <sup>b</sup>	3.17 ± 0.41
Flavonoids	45.09 ± 1.12 <sup>a</sup>	17.52 ± 0.14 <sup>b</sup>	12.54 ± 0.31
Gallate epicatechin	-	-	0.62 ± 0.01
Quercetrin	2.84 ± 0.03 <sup>a</sup>	3.24 ± 0.02 <sup>a</sup>	5.84 ± 0.12
Luteolin	2.98 ± 0.04 <sup>a</sup>	0.83 ± 0.01 <sup>b</sup>	2.25 ± 0.29
Quercetin	2.53 ± 0.03 <sup>b</sup>	6.27 ± 0.04 <sup>a</sup>	2.29 ± 0.26
Apigenin	1.31 ± 0.02 <sup>b</sup>	7.18 ± 0.20 <sup>a</sup>	1.55 ± 0.04
Amentoflavone	11.75 ± 0.14 <sup>a</sup>	-	-
Flavone	23.68 ± 1.36 <sup>a</sup>	-	-
Phenolic monoterpenes	-	55.84 ± 0.28 <sup>a</sup>	27.93 ± 0.13
Thymol	-	55.84 ± 0.28 <sup>a</sup>	27.93 ± 0.13
NI	3.84 ± 0.54 <sup>b</sup>	8.06 ± 0.06 <sup>a</sup>	2.67 ± 0.26
Total	100	100	100

<sup>a</sup> Values are given as mean ± SD (n = 3). Means with different small letter are significantly different ( $P < 0.05$ ). - = Not detected, NI = Non Identified.



The main phenolic class was phenolic acids (56.86%) followed by phenolic monoterpenes ( $27.93 \pm 0.13\%$ ) and flavonoids (12.54%). Thymol, ferulic and gallic acids were the most abundant phenolic compounds of flower extract with proportions of (27.93, 14.59, and 14.25%, respectively).

A total of fourteen phenolic compounds were successfully identified in the flavoured oil (91.94% of the TPCs) including eight phenolic acids (caffeic, gallic, chlorogenic, trans-2-hydroxycinnamic, vanillic, *p*-coumaric, rosmarinic and *o*-coumaric acids), four flavonoids (quercetrin, luteolin, quercetin and apigenin) and one phenolic monoterpene (thymol) (Figure 2). The presence of such compounds in the polar subfraction of oil flavoured with *Thymus capitatus* flowers is the main cause of its high TPCs and TFCs. The main phenolic class was phenolic monoterpenes (55.84%) followed by phenolic acids and flavonoids, which had a close similar importance in this oil and formed 18.58 and 17.52%, respectively. However no compound was detected in refined oil.

HPLC analysis of refined corn oil methanolic extract was performed and showed the absence of any phenolic compound. Our data confirm those found previously by Ortega-García *et al.* (2006). Indeed these authors mentioned that in particular, the total polyphenol content of oil determined colorimetrically is reduced almost to zero with refining. Flavoured oil had far richer total phenol content than crude oil.

This is the first report showing the presence of an array of phenolic compounds in corn oil flavoured with thyme flowers. The presence of flavonoids and phenolic acids in the flavoured oil could be related to the phenolic compound migration from thyme flowers to oil. In fact, *Thymus* species Essential Oils (EOs) were reported to be rich sources of phenolic monoterpenes such as carvacrol followed by monoterpene hydrocarbons (Jabri Karoui *et al.*, 2012). Additionally, thyme is described as rich in

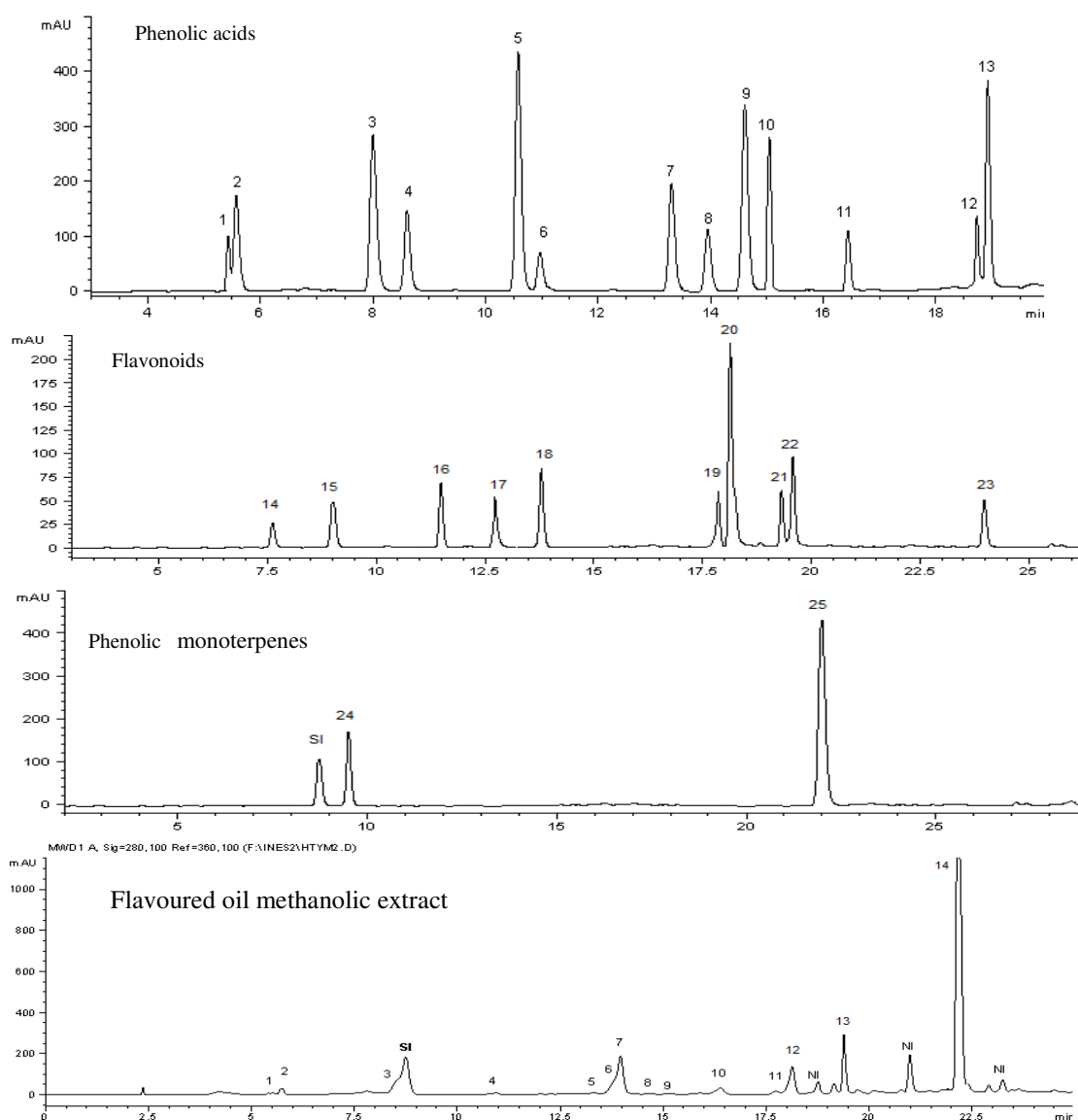
apigenin, luteolin, caffeic acid, ferulic acid, rosmarinic acid and quercetin (Jordán *et al.*, 2009).

### Antioxidant Activity

Total antioxidant capacity (mg GAE 100 g<sup>-1</sup>) of oils methanolic extracts are presented in Table 3. Our study reveals that the antioxidant activity of flavoured oil methanolic extract was 0.54-fold higher than that of the crude one (Table 3). This stronger antioxidant activity (185.22 mg GAE 100 g<sup>-1</sup> oil) might be attributed to the presence of phytochemicals such as phenolic compounds in the thyme-enriched oil as found previously by HPLC. In addition, our study revealed a total antioxidant capacity of 12.42 mg GAE g<sup>-1</sup> dried flowers (Table 3). Our data thus support conclusions of others who attributed antioxidant activities to the presence of phenolic compounds in thyme (Miguel *et al.*, 2004; Bounatirou *et al.*, 2007; Jabri Karoui *et al.*, 2012).

Free radical scavenging properties of flowers and oil methanolic extracts were presented in Table 3. Oil methanolic extracts were characterized by statistically significant ( $P < 0.05$ ) differences in their antioxidant activity measured by the DPPH method. As for the activity previously described, Table 3 depicts that methanolic extract of thyme-enriched oil was the most powerful sample in DPPH scavenging ( $IC_{50} = 0.103$  mg mL<sup>-1</sup>), followed by the crude oil one ( $IC_{50} = 0.213$  mg mL<sup>-1</sup>). However, refined oil methanolic extract showed a low antioxidant property against DPPH ( $IC_{50} > 2$  mg mL<sup>-1</sup>). The antiradical activity characterizing thyme-enriched oil may be connected to its composition, dominated by monoterpene hydrocarbons. In fact, according to results presented in Table 3 flower extracts were characterized by a higher free radical scavenging property ( $IC_{50}$  of 12 µg mL<sup>-1</sup>) in comparison to that of synthetic antioxidant BHT ( $IC_{50} = 25$  µg mL<sup>-1</sup>). Moreover, according to Kumaran and Karunakaran (2007), antioxidant molecules





**Figure 2.** RP-HPLC Chromatographic profiles of phenolic acids (A), flavonoids (B), phenolic monoterpenes (C) standards, and flavoured oil (D) methanolic extract monitored at 280 nm.

\*The peak numbers correspond to: 1. Caffeic acid; 2. Gallic acid; 3. *p*-Hydroxybenzoic acid; 4. Chlorogenic acid; 5. syringic acid; 6. vanillic acid; 7. *p*-coumaric acid; 8. ferulic acid; 9. *trans*-2-hydroxycinnamic acid; 10. rosmarinic acid; 11. *O*-Coumaric acid; 12. Cinnamic acid; 13. *Trans*-cinnamic acid; 14. Galate epicatechin; 15. Catechin; 16. Rutin; 17. Naringin; 18. Quercetrin; 19. Luteolin; 20. Quercetin; 21. Apigenin; 22. Amentoflavone; 23. Flavone; 24. Tyrosol; 25. Thymol, SI. Resorcinol.

such as polyphenols, flavonoids, and tannins reduce and decolourize DPPH due to their hydrogen donating ability.

Beta-carotene bleaching test measures the sample's potential for inhibiting conjugated diene hydroperoxides formation from linoleic acid oxidation (Tepe *et al.*, 2005).

Based on a 120 minutes reaction time (Table 3), the inhibition percentage ranged from 45.96% (refined oil) to 82.65% (oil with thyme) at the highest concentration used (4 mg mL<sup>-1</sup>). Oils methanolic extracts concentrations providing 50% inhibition (IC<sub>50</sub>) were 1.2 and 0.93 mg mL<sup>-1</sup> in the

**Table 3.** Total antioxidant capacity (mg GAE 100 g<sup>-1</sup> of oil) and antioxidant activities of methanolic extracts from crude, refined and flavoured oils.<sup>a</sup>

	Total antioxidant capacity (mg GAE 100 g <sup>-1</sup> of oil) (mg GAE g <sup>-1</sup> DW flowers)	DPPH (IC <sub>50</sub> , mg mL <sup>-1</sup> oil) (IC <sub>50</sub> , μg mL <sup>-1</sup> flowers)		β-Carotene bleaching (IC <sub>50</sub> , mg mL <sup>-1</sup> )	Reducing power (EC <sub>50</sub> <sup>b</sup> , mg mL <sup>-1</sup> )
Flavoured oil	185.22 ± 3.27 <sup>a</sup>	0.10 ± 0.03 <sup>b</sup>		0.93 ± 0.04 <sup>a</sup>	1.81 ± 0.19 <sup>a</sup>
Crude oil	100.66 ± 7.46 <sup>b</sup>	0.21 ± 0.06 <sup>a</sup>		1.20 ± 0.2 <sup>a</sup>	1.85 ± 0.14 <sup>a</sup>
Refined oil	0.00 <sup>c</sup>	> 2		> 5	> 5
Thyme flowers	12.42 ± 0.55	12 ± 0.06		0.9 ± 0.02	0.38 ± 0.06

<sup>a</sup> Values are given as mean ± SD (n = 3). Means with different small letter are significantly different (P < 0.05), <sup>b</sup> EC<sub>50</sub> value: the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; 1,1-DiPhenyl-2-PicrylHydrazyl (DPPH) radicals were scavenged by 50%. The EC<sub>50</sub> value was obtained by interpolation from linear regression analysis.

crude and the enriched oils, respectively. As for the activities previously studied, methanolic extract of thyme-enriched oil remained the powerful sample although it presented an IC<sub>50</sub> value close to that of the crude oil. This antioxidant capacity could be attributed to the presence of phenolic compounds, at high proportion. In fact, thyme flowers methanolic extract was characterized by an IC<sub>50</sub> of 0.9 mg mL<sup>-1</sup> (Table 3). Furthermore, Undeger *et al.* (2009) evaluated the antioxidant capacity of the main constituents of thyme: thymol, carvacrol and γ-terpinene and demonstrated that thymol and carvacrol are endowed with significant antioxidant activity at different concentrations tested, while γ-terpinene, which does not possess a phenolic group has no antioxidant activity.

Another reaction pathway in electron donation is the reduction of an oxidized antioxidant molecule to regenerate the “active” reduced antioxidant (Sbih *et al.*, 2010). As for β-carotene assay, Table 3 showed that the Fe<sup>3+</sup> reducing power ability of enriched oil methanolic extract was quite similar than that of the crude oil one (1.81 and 1.85 mg/mL, respectively) and was clearly more important than that of refined oil (EC<sub>50</sub> > 5 mg mL<sup>-1</sup>). This low activity characterizing the flavoured oil could be due to the little Fe<sup>3+</sup> reducing power ability of the flowers methanolic extract (EC<sub>50</sub> = 0.38 mg mL<sup>-1</sup>) by comparison with that of ascorbic acid (40 μg mL<sup>-1</sup>). The reductive

potential of flavoured oil may be due to the di- and monohydroxyl substitutions in the aromatic ring which possesses potent hydrogen donating abilities as described by Shimada *et al.* (1992).

#### Oil Oxidative Stability (OSI)

The OSI observed in the flavoured oil was higher (6.48 hours) than that of the refined oil (4.36 hours). This could be attributed to the polyphenols and other antioxidant compounds of thyme EO that cause an extension of oil thermal stability. Indeed, thyme essential oil was reported to have antioxidant activity that is mediated mainly by thymol and carvacrol, their phenolic components (Jabri Karoui *et al.*, 2012).

Based on the analysis performed in this study, it can be concluded that thyme-enriched oil volatiles were dominated by monoterpene hydrocarbons mainly represented by *p*-cymene, α-thujene and γ-terpinene. Phenolic monoterpenes were dominant in the flavoured oil and thymol was the only compound identified. This flavoured oil exhibited important antioxidant activities determined by radical scavenging activity, reducing power, β-carotene assay and total antioxidant activity. These activities were high enough compared to those of the crude and the refined corn oils. Thus, flavoured oil can be considered as a new source of antioxidant since the

nutritional value of refined corn oil and its oxidative stability were improved and its chemical composition was suitable. Indeed, thyme phenolics were considered as one of the most important primary antioxidants, and during aromatization process they migrate in oil and protect it from oxidation besides giving it nice aroma.

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## ترکیبات فعال زیستی و فعالیت آنتی اکسیدانی روغن ذرت تصفیه شده غنی شده با آویشن

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### چکیده

این مطالعه به منظور بررسی روغن ذرت تصفیه شده غنی شده با آویشن و تعیین فعالیت آنتی اکسیدانی عصاره متانولی آن طراحی شده است. ترکیب ترکیبات فرار توسط کروماتوگرافی گازی طیف سنجی جرمی (GC-MS) مورد بررسی قرار گرفت، در حالی که تجزیه و تحلیل ترکیبات فنلی با استفاده از کروماتوگرافی مایع عملکرد بالا با فاز معکوس (RP-HPLC) انجام شد. فعالیت آنتی اکسیدانی عصاره متانولی روغن طعم دار شده با استفاده از مهار رادیکال DPPH، سفیدسازی اسید  $\beta$  کاروتن-لینولئیک، کاهش قدرت و سنجش آنتی اکسیدانی کل مورد بررسی قرار گرفت. ترکیبات فرار عمده روغن طعم دار شده عبارت بودند از  $\alpha$ -thujene (26.47%)، P-cymene (24.06%) و  $\gamma$ -terpinene (14.30%). عصاره متانولی فنل کل (TPCs) (53.99 میلی گرم / 100g) بیشتری نسبت به روغن خام (23.63 میلی گرم / 100g مدت) بود. تیمول ترکیب فنولی اصلی در روغن طعم دار (55.84%) بود. عصاره متانولی روغن طعم دار فعالیت آنتی اکسیدانی کل بیشتری (185.22 میلی گرم اسید گالیک معادل / 100 گرم) نسبت به روغن خام (100.66 میلی گرم / 100g) را نشان داد. در سفیدسازی  $\beta$  کاروتن-لینولئیک اسید و سنجش مهار رادیکال DPPH، عصاره متانولی روغن طعم دار فعالیت بالاتری نسبت به روغن خام بر اساس ارزش  $IC_{50}$  نشان داد. روغن خام و طعم دار ارزش  $CE_{50}$  نزدیکی (181 و 185 میلی گرم / میلی لیتر، به ترتیب) نشان دادند. فعالیت آنتی اکسیدانی روغن غنی شده با آویشن عمدتاً به دلیل حضور ترکیبات فنلی مانند تیمول و هیدروکربن هایی مانند  $\gamma$ -terpinene و p-cymene بودند. روغن غنی شده با آویشن می تواند به عنوان یک منبع جدید و طبیعی آنتی اکسیدان محسوب شود.