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

I. Jabri Karoui¹,², K. Msaada¹, M. Abderrabba², and B. Marzouk¹

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, β-carotene-linoleic acid bleaching, reducing power and total antioxidant assays. Flavoured oil major volatiles were p-cymene (26.47%), α-thujene (24.06%) and γ-terpinene (14.30%). Its methanolic extract had higher Total Phenol Contents (TPCs) (53.99 mg 100 g⁻¹) than that of the crude oil (23.63 mg 100 g⁻¹). 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⁻¹) than that of the crude one (100.66 mg GAE 100 g⁻¹). In β-carotene-linoleic acid bleaching and DPPH radical scavenging assays, flavoured oil methanolic extract showed higher activities than that of the crude oil based on IC50 values. Flavoured and crude oils were characterized by a close CE50 values (1.81 and 1.85 mg mL⁻¹, respectively). Antioxidant activities of the thyme-enriched oil were mainly due to the presence of phenolic compounds such as thymol and hydrocarbons such as γ-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), TerButyl 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, Thymes 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
(*Syzygyium 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
methyleate (*CH2ONa*), sodium chloride
(*NaCl*), sulphuric acid (*H2SO4*), Folin–
Ciocalteu reagent, β-carotene, linoleic acid,
ethylenediaminetetraacetic acid (EDTA),
sodium carbonate anhydrous (*Na2CO3*),
gallic acid, sodium nitrite (*NaN3O2*),
alanium chloride hexahydrate (*AlCl3*
*6H2O*), 2,2-DiPhenyl-1-PicrylHydrazyl
(DPPH), trichloroacetic acid, iron (II)
chloride (*FeCl3*), iron (III) chloride
anhydrous (*FeCl3*), ascorbic acid, Calcium
hexacyanoferrat (III), iron (II) chloride
tetrahydrate (*FeCl2*•4H2O), 2,2-DiPhenyl-1-
PicrylHydrazyl (DPPH), trichloroacetic acid,
iron (II) chloride
anhydrous (*FeCl3*), ascorbic acid, Calcium
hexacyanoferrat (III), iron (II) chloride
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anhydrous (*FeCl3*), ascorbic acid, Calcium
hexacyanoferrat (III), iron (II) chloride
tetrahydrate (*FeCl2*•4H2O), 2,2-DiPhenyl-1-
PicrylHydrazyl (DPPH), trichloroacetic acid,
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\textsubscript{2} (Strip-trap) according to published data (Dhifi \textit{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\textsuperscript{-1}. The carrier gas was helium with a flow rate of 1.2 mL min\textsuperscript{-1} and a split ratio of 60:1. Scan time and mass range were 1 second and 40-300 m z\textsuperscript{-1}, respectively. Oil volatiles identification was based on the comparison of their retention indices relative to (C\textsubscript{8}–C\textsubscript{22}) \textit{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 \textit{et al.}, 2001).

Oil phenolics extraction was carried out with the slightly modified method of Baccouri \textit{et al.} (2008). Briefly, 8 g of oil was added to 8 mL of \textit{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–\textit{vis} multiwavelength detector. The separation was carried out on a 250×4.6-mm, 4-µm Hypersil ODS C\textsubscript{18} 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\textsuperscript{-1}. 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 \textit{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⁻¹). 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, 2, 4 and 5 mg mL⁻¹) 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⁻¹). 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 mg gallic acid equivalents per 100 gram of oil (mg GAE 100 g⁻¹). 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⁻¹) of oils extracts in methanol/water (90:10, v/v) were added to 2.5 mL of 1% potassium ferricyanide [K₃Fe(CN)₆] 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. EC₅₀ value (mg mL⁻¹) 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⁻¹) 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
Antioxidant Activities of Thyme-Enriched Corn Oil

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 β-carotene bleaching inhibition using the following formula:

% Inhibition\(=\frac{(A_t-C_t)/(C_0-C_t)}{C_0-C_t}\)×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 (mg mL\(^{-1}\)): efficient concentration corresponding to 50% β-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\(^{-1}\) (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±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 monoterpane hydrocarbons, which attained 28.23% mainly represented by γ-terpinene (7.83%) and p-cymene (5.88%). The monoterpane 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%), myrcone (1.68%), α-terpinene (1.55%), 1.8 cineole (1.95%), linalool (1.12%) and β-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 monoterpane hydrocarbons (92.59% of the total volatiles) and p-cymene (26.47%), α-thujene (24.06%) and γ-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 monoterpane hydrocarbons mainly represented by γ-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 monoterpane 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, quercitin, quercitrin, apigenin and luteolin) and one phenolic monoterpane (thymol) (Table 2).
Table 1. Volatile compounds composition (%) of the flavoured oil.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RI</th>
<th>RI</th>
<th>Thyme flavoured oil</th>
<th>Thyme flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Thujene</td>
<td>928</td>
<td>1035</td>
<td>24.06 ± 0.03</td>
<td>1.23 ± 0.11</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>939</td>
<td>1032</td>
<td>8.16 ± 0.01</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>Camphene</td>
<td>954</td>
<td>1076</td>
<td>1.10 ± 0.00</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>1 Octen-3ol</td>
<td>961</td>
<td>-</td>
<td>0.27 ± 0.00</td>
<td>0.07 ± 0.00</td>
</tr>
<tr>
<td>Sabinene</td>
<td>975</td>
<td>1132</td>
<td>0.57 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>β-Pinene</td>
<td>980</td>
<td>1118</td>
<td>1.65 ± 0.00</td>
<td>0.18 ± 0.00</td>
</tr>
<tr>
<td>Myrcene</td>
<td>991</td>
<td>1174</td>
<td>8.68 ± 0.01</td>
<td>1.68 ± 0.03</td>
</tr>
<tr>
<td>α-Phellandrene</td>
<td>1006</td>
<td>1176</td>
<td>0.75 ± 0.01</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>δ-3-Carene</td>
<td>1011</td>
<td>1159</td>
<td>0.5 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>α-Terpinene</td>
<td>1018</td>
<td>1188</td>
<td>6.28 ± 0.02</td>
<td>1.55 ± 0.03</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1026</td>
<td>1280</td>
<td>26.47 ± 0.03</td>
<td>5.88 ± 0.02</td>
</tr>
<tr>
<td>Limonene</td>
<td>1030</td>
<td>1203</td>
<td>0.63 ± 0.00</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>1-8 Cineole</td>
<td>1033</td>
<td>1213</td>
<td>0.78 ± 0.01</td>
<td>1.95 ± 0.17</td>
</tr>
<tr>
<td>E-β-Ocimene</td>
<td>1050</td>
<td>1266</td>
<td>0.14 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Trans-sabinene hydrate</td>
<td>1053</td>
<td>1474</td>
<td>0.74 ± 0.00</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>1062</td>
<td>1266</td>
<td>14.30 ± 0.02</td>
<td>7.83 ± 0.06</td>
</tr>
<tr>
<td>Linalool</td>
<td>1098</td>
<td>1553</td>
<td>1.38 ± 0.02</td>
<td>1.12 ± 0.03</td>
</tr>
<tr>
<td>Camphor</td>
<td>1143</td>
<td>1532</td>
<td>1.58 ± 0.01</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>Borneol</td>
<td>1165</td>
<td>1719</td>
<td>0.09 ± 0.00</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>Trans-1,4-Cineole</td>
<td>1178</td>
<td>1611</td>
<td>0.59 ± 0.01</td>
<td>4.46 ± 0.02</td>
</tr>
<tr>
<td>Teroinolene</td>
<td>1088</td>
<td>1290</td>
<td>-</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Geraniol</td>
<td>1255</td>
<td>1857</td>
<td>-</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Acetate de linalyle</td>
<td>1257</td>
<td>1556</td>
<td>-</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Thymol</td>
<td>1290</td>
<td>2198</td>
<td>-</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>1292</td>
<td>2239</td>
<td>1.56 ± 0.01</td>
<td>66.99 ± 0.01</td>
</tr>
<tr>
<td>Eugenol</td>
<td>1356</td>
<td>2192</td>
<td>0.09 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Acetate de geranyle</td>
<td>1383</td>
<td>1765</td>
<td>-</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>Acetate de neryle</td>
<td>1385</td>
<td>1733</td>
<td>-</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>1419</td>
<td>1612</td>
<td>0.16 ± 0.01</td>
<td>1.46 ± 0.04</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1480</td>
<td>1726</td>
<td>-</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>1581</td>
<td>2008</td>
<td>-</td>
<td>0.32 ± 0.02</td>
</tr>
</tbody>
</table>

Chemical classes

| Monoterpene hydrocarbons | 92.59±0.04° | 20.67 ± 0.05   |
| Monoterpene phenols      | 1.56±0.01°  | 67.30 ± 0.04   |
| Monoterpene alcohols     | 3.07±0.03°  | 6.61 ± 0.09    |
| Monoterpene esters       | 0.79±0.01°  | 0.45 ±0.00     |
| Monoterpene ketones      | 1.59±0.0°   | 0.05 ± 0.00    |
| Sesquiterpene hydrocarbons | 0.16±0.01° | 1.68 ± 0.05    |
| Oxygenated sesquiterpenes| -            | 0.32 ± 0.02    |

Total: 99.75 ± 0.00° 99.02 ± 0.01°

° Values are given as mean ± SD (n = 3). Means with different small letter are significantly different (P < 0.05). 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.

Table 2. Percentages (%) of phenolic compounds of crude and flavoured corn oils measured by RP-HPLC.\textsuperscript{a}

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

\textsuperscript{a} 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±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 monoterpen (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-Garcìa 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 monoterpen 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⁻¹) 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⁻¹ 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⁻¹ 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 (IC₅₀ = 0.103 mg mL⁻¹), followed by the crude oil one (IC₅₀ = 0.213 mg mL⁻¹). However, refined oil methanolic extract showed a low antioxidant property against DPPH (IC₅₀> 2 mg mL⁻¹). The antiradical activity characterizing thyme-enriched oil may be connected to its composition, dominated by monoterpen hydrocarbons. In fact, according to results presented in Table 3 flower extracts were characterized by a higher free radical scavenging property (IC₅₀ of 12 µg mL⁻¹) in comparison to that of synthetic antioxidant BHT (IC₅₀= 25 µg mL⁻¹). Moreover, according to Kumaran and Karunakaran (2007), antioxidant molecules...
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\(^{-1}\)). Oils methanolic extracts concentrations providing 50% inhibition (IC\(_{50}\)) were 1.2 and 0.93 mg mL\(^{-1}\) in the

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**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.

Table 3. Total antioxidant capacity (mg GAE 100 g⁻¹ of oil) and antioxidant activities of methanolic extracts from crude, refined and flavoured oils.a

<table>
<thead>
<tr>
<th></th>
<th>Total antioxidant capacity (mg GAE 100 g⁻¹ of oil)</th>
<th>DPPH (IC₅₀, mg mL⁻¹ oil)</th>
<th>β-Carotene bleaching (IC₅₀, mg mL⁻¹)</th>
<th>Reducing power (EC₅₀, mg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg GAE g⁻¹ DW flowers)</td>
<td>µg</td>
<td>mL⁻¹</td>
<td></td>
</tr>
<tr>
<td>Flavoured oil</td>
<td>185.22 ± 3.27</td>
<td>0.10 ± 0.03</td>
<td>0.93 ± 0.04</td>
<td>1.81 ± 0.19</td>
</tr>
<tr>
<td>Crude oil</td>
<td>100.66 ± 7.46</td>
<td>0.21 ± 0.06</td>
<td>1.20 ± 0.2</td>
<td>1.85 ± 0.14</td>
</tr>
<tr>
<td>Refined oil</td>
<td>0.00c</td>
<td>&gt; 2</td>
<td>&gt; 5</td>
<td>&gt; 5</td>
</tr>
<tr>
<td>Thyme flowers</td>
<td>12.42 ± 0.55</td>
<td>12 ± 0.06</td>
<td>0.9 ± 0.02</td>
<td>0.38 ± 0.06</td>
</tr>
</tbody>
</table>

a Values are given as mean±SD (n= 3). Means with different small letter are significantly different (P< 0.05). b EC₅₀ value: the effective concentration at which the antioxidant activity was 50%; the absorbance 0.5 for reducing power; 1,1-DiPhenyl-2-PicrylHydrazyl (DPPH) radicals were scavenged by 50%. The EC₅₀ value was obtained by interpolation from linear regression analysis.

Another reaction pathway in electron donation is the reduction of an oxidized antioxidant molecule to regenerate the “active” reduced antioxidant (Shih et al., 2010). As for β-carotene assay, Table 3 showed that the Fe³⁺ 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 (CE₅₀> 5 mg mL⁻¹). This low activity characterizing the flavoured oil could be due to the little Fe³⁺ reducing power ability of the flowers methanolic extract (EC₅₀= 0.38 mg mL⁻¹) by comparison with that of ascorbic acid (40 µg mL⁻¹). 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 monoterpane 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.

REFERENCES


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

ا. جبری کارویی، ک. مسادا، م. عبدالله‌یا، و. مروک

چکیده

این مطالعه به منظور بررسی روغن ذرت تصفیه شده غنی شده با آویشن با تغییر فعالیت آنتی اکسیدانی عصاره‌های آنتی اکسیدانی طبیعی جرمی (GC-MS) مورد بررسی قرار گرفت که همگرا که تجزیه و تحلیل ترکیبات فنی با استفاده از کرومانتوگرافی مایع عمکرک با ا فاز معموس (RP-HPLC) انجام شد. فعالیت آنتی اکسیدانی عصاره‌های روغن ذرت شده با استفاده از مهار رادیکال DPPH فیزیسوئازید اسید β-ثوئن-لیبولیک کاهش قدرت و سنجش آنتی اکسیدانی کل مورد بررسی قرار گرفت. ترکیبات فرار عمده روغن طعم دار شده عبارت بودند از (26.47٪) α-ثوئن پی‌سیمین و (14.30٪) terpinene

روغن خام (32.43٪) میلی گرم / 100 غرم (GAE) نشان داد. عصاره مناتولی روغن طعم دار فعالیت آنتی اکسیدانی کل بیشتری (22.44٪) بود. عصاره مناتولی روغن طعم دار فعالیت آنتی اکسیدانی کل بیشتری (18.51٪) بود. عصاره مناتولی روغن طعم دار فعالیت آنتی اکسیدانی کل بیشتری (16.07٪) بود.