Enhancing of oxidative stability and quality attributes of olive oil using spirulina (Arthrospira platensis) nanoparticles

Mohamed K. Morsya,∗, Osama M. Morsyb, Hend A. Elbarbaryc, Marwa A. Saadd

a Department of Food Technology, Faculty of Agriculture, Benha University, Qaluobia, Egypt
b Department of Basic and Applied Sciences, Faculty of Engineering, Arab Academy of Science, Technology, and Maritime Transport, Cairo, Egypt
c Department of Food Hygiene, Faculty of Veterinary Medicine, Benha University, Qaluobia, Egypt
d Department of Food Control, Faculty of Veterinary Medicine, Shebin Al-Kom, Menofia University, Menofia, Egypt

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ABSTRACT

This study was performed to evaluate the effect of spirulina whole cell (Sp-WC) and spirulina nanoparticles (Sp-NPs) as an antioxidant in olive oil (OO) under accelerated storage at 60 ± 1 °C up to 49 days. Sp-NPs were incorporated into freshly OO at 0.25, 0.5, and 1% (w/v), Sp-WC at 0.5% (w/v), and both of them compared with 0.01% BHT and 0.01% α-tocopherol as a reference and without antioxidant as the control sample. The kinetic rate of oxidation markers and shelf life (assuming Q10 value of 2.0 for lipid oxidation) of OO were estimated. Sp-NPs exhibited a high phenolic content and antioxidant activity. In Sp-NPs-treated samples, the contents of thiobarbituric acid (TBA), peroxide, p-anisidine, totox, K232, and K270 were significantly lower than the control. The rate constant (k) was low in OO with BHT and 1% Sp-NPs compared with other treatments. Chlorophyll content in olive oil containing Sp-NPs was improved during the storage. The antioxidant indices and sensory attributes of oil samples including Sp-NPs were significantly higher than that of the control. These results confirmed that Sp-NPs were more effective in retarding oxidation, improving oil color, and extending shelf life (up to 475 days at 25 °C).

1. Introduction

Olive oil is one of the most important edible oils in Egypt and highly consumed worldwide. It is a good source of monounsaturated fatty acids (MUFA) which has beneficial impacts on health (Caporaso et al., 2015). However, olive oil (OO) has a short shelf-life during storage (Kehili, Choura, Zammel, Allouche, & Sayadi, 2018) due to partially elimination of phenolic compounds during the refining process (Garcia, Ruiz-Méndez, Romero, & Brenes, 2006). Therefore, OO becomes more prone to oxidation and change in quality attributes, which requires the antioxidants addition to prolong its shelf life, improve the acceptability, and enhance nutritional value (Kehili et al., 2018).

Although for more than 50 years, synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been successfully used to prevent edible oils oxidation however, they are under consideration for possible health risks (Ben-Ali, Dhouib, Damak, & Allouche, 2014). Hence, natural antioxidants are receiving much attention in recent years such as potato peels (Jeddou et al., 2016), algal extracts (Alavi & Golmakan, 2017a, 2017b), tomato peels (Kehili et al., 2018), and pomegranate peels (Morsy, Mekawi, & Elsabagh, 2018). Natural antioxidants have many advantages such as safety, consumer acceptance and health-beneficial properties (Asensio, Nepote, & Grosso, 2012).

Spirulina (Arthrospira platensis) is a non-toxic blue-green alga (cyanobacterium), naturally growing in sea-water or cultivated. It has been approved as a generally recognized as safe (GRAS) as food by (FDA, 2002). The global annual production of spirulina powder around 3000 tons, but no data are available in Egypt (Alavi & Golmakani, 2017a). Spirulina (Sp) is a rich source of phycocyanin, carotenoids, biliprotein pigment, proteins, and vitamins (Ovando et al., 2018). Moreover, Sp contains potent antioxidants and superoxide radical scavengers (Shabana, Gabr, Moussa, El-Shaer, & Ismael, 2017), and possesses antimicrobial activity (Elshouny, El-Sheekh, Sabae, Khalil, & Badr, 2017); and therefore, it is used as a health ingredient (Ovando et al., 2018). One study by Wang, Pan, Sheng, Xu, and Hu (2007) reported that the antioxidant activity of Sp extract was higher than α-tocopherol, but it was lower than that of BHT. In recent years, the antioxidant effect of Sp extracts has been investigated in Kilka oil (Golmakan, Keramat, Moosavi-Nasab, & Moosavian, 2017), in beef sausage (Luo et al., 2017), in yogurt (Barkallah et al., 2017), and snack foods (Lucas, de Morais, & Elsabagh, 2018). Natural antioxidants have many advantages such as safety, consumer acceptance and health-beneficial properties (Asensio, Nepote, & Grosso, 2012).

∗ Corresponding author.
E-mail address: mohamed.abdelhafez@fagr.bu.edu.eg (M.K. Morsy).

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<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Olive oil</th>
<th>Standard, 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>12.85 ± 0.17</td>
<td>7.5–20</td>
</tr>
<tr>
<td>Palmitoleic acid (C 16:1)</td>
<td>0.43 ± 0.03</td>
<td>0.3–3.5</td>
</tr>
<tr>
<td>Margaric acid (C 17:0)</td>
<td>0.05 ± 0.01</td>
<td>≤0.40</td>
</tr>
<tr>
<td>Margaroleic (C 17:1)</td>
<td>0.06 ± 0.01</td>
<td>≤0.60</td>
</tr>
<tr>
<td>Stearic acid (C 18:0)</td>
<td>2.12 ± 0.05</td>
<td>0.5–5.0</td>
</tr>
<tr>
<td>Oleic acid (C 18:1)</td>
<td>72.65 ± 0.31</td>
<td>55–83</td>
</tr>
<tr>
<td>Linoleic acid (C 18:2)</td>
<td>10.24 ± 0.12</td>
<td>2.5–21</td>
</tr>
<tr>
<td>α-Linolenic acid (C 18:3n-3)</td>
<td>0.79 ± 0.05</td>
<td>≤1.0</td>
</tr>
<tr>
<td>Arachidic acid (C 20:0)</td>
<td>0.44 ± 0.02</td>
<td>≤0.60</td>
</tr>
<tr>
<td>Eicosanoic acid (C 20:1)</td>
<td>0.45 ± 0.02</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Saturated fatty acid ΣSFA</td>
<td>15.46 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Monounsaturated fatty acid ΣMUFA</td>
<td>73.59 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated fatty acid ΣPUFA</td>
<td>11.03 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>ΣMUFA/ΣPUFA</td>
<td>0.71 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>ΣMUFA/PUFA</td>
<td>6.67 ± 0.12</td>
<td></td>
</tr>
</tbody>
</table>

SFA, saturated fatty acid (%) ; MUFA, monounsaturated fatty acid (%) ; PUFA, polyunsaturated fatty acid (%).

### 2. Materials and methods

#### 2.1. Chemicals and reagents

Reagent grade chemicals, including ascorbic acid, Gallic acid, acetic acid, thiobarbituric acid, potassium iodide, sodium thiourea, NaOH, starch, ethanol 95%, hexane, butanol, chloroform, iso-octane, p-anisidine, Folin-Ciocalteu’s phenol reagent, phenolphthalein, butylated hydroxytoluene (BHT), α-tocopherol, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma –Aldrich (St. Louis, MO, USA).

#### 2.2. Raw materials

Fresh olive oil (OO) which contained (refined pomace olive oil: 95:5 v/v) was exported from Spain during the 2017 season and packed by Sinai Olive Oil Company (Arish, Egypt) without any additives. The oil samples were transported in dark bottles to the laboratory and kept in dark place at 25 °C until use. Food grade spirulina (Arthrospira platensis) powder was obtained from Aquaculture Research Center (ARC) at Arab Academy for Science, Technology, and Maritime Transport (Alexandria, Egypt). The spirulina powder was packed under vacuum and kept in the dark under dry conditions until further use.

#### 2.3. Preparation of spirulina nanoparticles (Sp-NPs)

The dried spirulina (Sp) was milled to nanoparticle size according to Morsy et al. (2018). Briefly, Sp was ground with a mill grinder (Moulinex; Model MC300, France) to particle size range of 140–190 μm, and then smashed by a high-energy planetary ball-mill (PM 2400 Model, Iran) to prepare Sp-NPs. The ball-milling process was done under the following conditions; ball mass: powder mass ratio (10:1), rotation speed 320 rpm for 2 h under atmospheric conditions (25°C). Sp-NPs were measured with a Zetasizer Nano ZS (Nano Sight NS300, UK) with an average size of 92 ± 6 nm. Sp-NPs were packed under vacuum in opaque packages before use. All processes were carried out under controlled conditions for maintaining the Sp-NPs antioxidant contents.

#### 2.4. Olive oil and antioxidants

The homogenous olive oil was divided into 7 groups. Sp-NPs were added to oil sample for three groups at a concentration of 0.25, 0.5, and 1% (w/v). The fourth group included Sp-WC at 0.5% (w/v). The fifth and sixth groups contained α-tocopherol and BHT at 0.01% (w/v), respectively, as reference treatments and the last one was antioxidant-free (control). The antioxidants were dispersed gently in olive oil. When the antioxidants were added, they were dissolved gently and dispersed in olive oil. All samples were placed in dark glass bottles and stored in an incubator at 60 ± 1°C for 49 days (under accelerated oxidation conditions). The samples were taken for measuring the oxidative stability at 0, 7, 14, 21, 28, 35, 42, and 49 days of storage.

#### 2.5. Total phenolic and flavonoid contents of spirulina

Total phenolic content (TPC) of spirulina was determined by the Folin-Ciocalteu colorimetric method as described by Hajimahmoodi et al. (2010) and TPC was expressed as mg of gallic acid equivalent (GAE) per g of spirulina. Total flavonoid content (TFC) of spirulina was determined according to the method of Zhishen, Mengcheng, and Jianming (1999). TFC were calculated as mg rutin equivalents per gram of spirulina (mg RE g⁻¹). Rutin was utilized to develop the standard curve.

#### 2.6. Antioxidant capacity of spirulina

The capacity of spirulina to scavenge DPPH radicals was evaluated according to the method of Brand-Williams, Cuvelier, and Berret (1995). In this experiment, BHT was also run as a positive control. The results were expressed as the IC₅₀ (µg/mL) using ascorbic acid as the standard according to Apak, Güçlü, Özyürek, and Karademir (2004).
2.7. GC-FID analysis of olive oil fatty acids profile

Fatty acids methyl esters (FAMEs) of olive oil were performed as described by Christie (1989). The FAMEs were analyzed using GC/FID (Shimadzu 17A, Kyoto, Japan). The FAME capillary column, 50 m, 0.32 and 0.20 mm of Carbowax 20M was used. The oven temperature was held at 160 °C for 20 min and then programmed at 10 °C min⁻¹ until 240 °C. Injector temperature 220 °C, detector (FID) temperature 250 °C, carrier gas was hydrogen, column flow 1.2 mL min⁻¹, make-up gas was nitrogen 40 mL min⁻¹ and split ratio was 1/100. Identification of peaks were performed by comparing the retention times of appropriate standards (Pure methyl esters of fatty acids mixture; Larodan, Malmoe, Sweden) at the same conditions.

2.8. K232 and K270 values

The extinction coefficients at 232 nm (K₂₃₂ value) and 270 nm (K₂₇₀ value) were determined by the UV–Vis spectrophotometer (CE 599 Universal, USA) with isooctane as described by AOCS (1998).

2.9. Chlorophyll content (Ch)

Chlorophyll content in olive oil was measured according to the method described by Salvador, Aranda, and Fregapane (2001) and was expressed as mg of pheophytin kg⁻¹ of olive oil.

2.10. Thiobarbituric acid (TBA)

TBA value was determined using the spectrophotometric method (CE 599 Universal, USA) at 530 nm as described by Paquot and Hautfenne (1987). TBA values were expressed in mg of malonaldehyde kg⁻¹ oil.

2.11. Peroxide value (PV)

The PV of oil samples was determined according to AOCS (1998). The PV was expressed as milliequivalent per kilogram (meq kg⁻¹) oil.

2.12. p-Anisidine value (AV) and Totox value (TV)

p-Anisidine value of oil samples was determined using the spectrophotometric method at 350 nm according to the method of Paquot and Hautfenne (1987). The results expressed as mg kg⁻¹ oil according to the following calculation.

\[ p\text{-Anisidine value (AV)} = \frac{25(1.2 \times E_b - E_a)}{W} \]

Where \( E_b\) is the net absorbance of the oil-solution, \( E_a\) is the net absorbance of the oil-anisidine–solution, and \( W\) is the weight of oil sample. Totox values were calculated as [2 X Peroxide value (PV) + p-Anisidine value (AV)] according to AOCS (1998).

2.13. Chemical kinetic data analysis

The rate constants (k) of zero order reactions were determined from the slopes of the lines generated by plotting concentration and time (Gharby, Harhar, Mamouni, Matthäus, & Charrouf, 2016). The temperature acceleration factor \((Q_{10})\) based on the increase in oxidation rate per 10 °C increase in temperature was calculated from the slopes of the lines according to Steele (2004) from the following equation;

\[ Q_{10} = e^{10b} \]

Where \( e\) is constant and \( b\) is Δ temperature.
2.14. Antioxidant indices

The induction period (IP) of olive oil was performed using rancimat apparatus (Metrohm 679 AG, Herisau, Switzerland) according to Läubli and Brutel (1986). The test was done under 110 °C with air flow rate of 10–15 L h⁻¹ in a reaction tube. The results were expressed as induction time. The Antioxidant activity (AA), antioxidant power (AOP), and the improved oxidative stability (IOS) were determined according to International Olive oil Council (IOC, 2015). The results were calculated using the following equation:

\[
\text{Antioxidant activity (AA)} = \left( \frac{\text{IP sample} - \text{IP control}}{\text{Sample concentration} \times \text{IP control}} \right) \times 100
\]

\[
\text{Antioxidant power (AOP)} = 100 - \left( \frac{\text{(IP control)}}{\text{(IP sample)}} \times 100 \right)
\]

\[
\text{% Improved oxidative stability (IOS)} = \left( \frac{\text{IP sample} - \text{IP control}}{\text{IP control}} \times 100 \right)
\]

Where, IP sample is an induction period of sample and IP control is an induction period of control.

2.15. Sensory evaluation

A trained, ten-member panel, experienced in sensory evaluation of olive oil from Food Technology Department (aged 20–45 years). Previously, the panellists were pre-screened for the basic flavors, and tastes; and they were selected based on their regular olive oil consumption. The evaluation of olive oil was performed according to the standard method of IOC (2010). A cup of water and slices of green apple were provided to panelists for rinsing the mouth between samples. The oil samples were placed in covered brown glasses (∼20 mL) with 3-digit number codes and allowed to stand at room temperature few minutes before evaluation. The panelists evaluated the samples based on positive and negative attributes as follows; positive attributes included color, fruity, bitter, pungent, and harmony, while the negative attributes included fusty, musty, vinous, metallic, and rancid. The panelists registered the intensity (score) of each attribute through a 10 cm line scale, where 0 and 10 meant the lowest and highest values, respectively (Songartz & Oberg, 2011).

2.16. Statistical analyses

The experiments and analyses were run in triplicate for the antioxidant activity, on three factors with four levels: (Sp-NPs, Sp-WC, α-tocopherol and BHT, and replication) were applied. For olive oil analysis K322, K270, FFA, Ch, TBA, PV, AV, TV, and sensory properties), factorial design ANOVA with two factors with six levels (control, α-tocopherol, BHT, 0.25% Sp-NPs, 0.5% Sp-NPs, 1% Sp-NPs and 0.5% Sp-WC) and storage time with seven levels (0, 7, 14, 21, 28, 35, 42, and 49 days) were done for each parameter using SPSS (version 18 for windows; SPSS Inc., Chicago, IL). Multiple comparisons at P < 0.05 of mean values were figured out with Tukey's multiple comparison tests (Steel & Torrie, 1980).

3. Results and discussion

3.1. Fatty acids profile and phenolic compounds of olive oil

The fatty acid composition of olive oil is shown in Table 1. The major fatty acids found in oil sample were oleic acid (72.59%), palmitic acid (12.90%), and linoleic acid (10.24%). Moreover, the olive oil is rich in monounsaturated fatty acid (MUFA) at about 73.51%. However, olive oil has a low level of polyunsaturated fatty acid (PUFA) around 11.01%. These results are in agreement with those reported by Alavi and Golmakani (2017b) and within ranges of IOC Standards (IOC, 2015). Moreover, olive oil also contains phenolic compounds at about 0.266 mg GAE g⁻¹ (data not shown). Alavi and Golmakani (2017b) reported that TPC in olive oil was 0.294 mg GAE g⁻¹. The variations in the TPC of olive oil may be olive oil cultivars and/or processing conditions. Also, Tuck and Hayball (2002) found that the main phenolic compounds in olive oil are oleuropein, tyrosol, and hydroxytyrosol.

3.2. Antioxidant capacity of Sp-WC and Sp-NPs

The phenolic and flavonoid compounds have active attributes like antioxidant capacity, and spirulina is considered a good source. As shown in Table 2 the total phenolic compounds (TPC) in Sp-WC and Sp-NPs extracts were 66.18 and 97.38 mg GAE g⁻¹ (on dry basis), respectively. Alavi and Golmakani (2017a) reported that phenolic contents about 50.56 mg GAE g⁻¹ in aqueous extract of spirulina. While, Golmakani, Moosavi-Nasab, Keramat, and Mohammad (2018) found that TPC was 38.04 mg GAE g⁻¹ in the water extract of spirulina. This variation in TPC might be due to the difference in extraction method or the solvent used. Regarding of the total flavonoid compounds (TFC), the present study shows a concentration of TFC in Sp-WC and Sp-NPs extracts were 9.35 and 13.72 mg GAE g⁻¹, respectively. These results are in agreement with the data reported by (Alavi & Golmakani, 2017a; Mory et al., 2018).

Moreover, Table 2 shows the antioxidant capacity of spirulina (DPPH radical scavenging capacity). The radical scavenging capacity of Sp-WC and Sp-NPs was comparable to that of α-tocopherol, but it was lower than that of BHT at the concentrations tested. One study by Alavi and Golmakani (2017a) found that spirulina extract showed higher free radical scavenging capacity than Chlorella. Other studies indicated that spirulina extracts had similar or stronger antioxidant activity than the

![Table 4](https://example.com/table4.png)

**Table 4**

Effect of the addition of spirulina nanoparticles on TBA (mg MDA kg⁻¹) in olive oil during storage at (60 °C) (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>k (mg MDA kg⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.06 ± 0.04a</td>
<td>0.18 ± 0.01a</td>
<td>0.6 ± 0.04c</td>
<td>0.94 ± 0.04c</td>
<td>1.35 ± 0.06c</td>
<td>1.91 ± 0.04c</td>
<td>2.38 ± 0.09a</td>
<td>3.25 ± 0.22a</td>
<td>0.06 ± 0.09</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Sp-NPs (0.25%)</td>
<td>0.06 ± 0.04a</td>
<td>0.13 ± 0.01b</td>
<td>0.25 ± 0.02a</td>
<td>0.36 ± 0.02b</td>
<td>0.55 ± 0.02c</td>
<td>0.71 ± 0.01c</td>
<td>0.84 ± 0.01d</td>
<td>1.05 ± 0.04a</td>
<td>0.02 ± 0.02</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Sp-NPs (0.5%)</td>
<td>0.06 ± 0.04a</td>
<td>0.11 ± 0.01c</td>
<td>0.19 ± 0.03c</td>
<td>0.26 ± 0.01c</td>
<td>0.45 ± 0.02d</td>
<td>0.63 ± 0.03c</td>
<td>0.76 ± 0.01e</td>
<td>0.93 ± 0.02a</td>
<td>0.02 ± 0.02</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Sp-WC (0.5%)</td>
<td>0.06 ± 0.04a</td>
<td>0.14 ± 0.01c</td>
<td>0.26 ± 0.02a</td>
<td>0.48 ± 0.01c</td>
<td>0.65 ± 0.01b</td>
<td>0.84 ± 0.02a</td>
<td>0.98 ± 0.01b</td>
<td>1.32 ± 0.02a</td>
<td>0.03 ± 0.02</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>BHT</td>
<td>0.06 ± 0.04a</td>
<td>0.13 ± 0.01c</td>
<td>0.13 ± 0.01c</td>
<td>0.17 ± 0.01c</td>
<td>0.25 ± 0.01d</td>
<td>0.29 ± 0.01c</td>
<td>0.36 ± 0.01c</td>
<td>0.57 ± 0.01b</td>
<td>0.01 ± 0.02</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>0.06 ± 0.04a</td>
<td>0.14 ± 0.01c</td>
<td>0.26 ± 0.01c</td>
<td>0.47 ± 0.01c</td>
<td>0.64 ± 0.01d</td>
<td>0.84 ± 0.02a</td>
<td>1.02 ± 0.04b</td>
<td>1.43 ± 0.02a</td>
<td>0.03 ± 0.03</td>
<td>0.97</td>
<td></td>
</tr>
</tbody>
</table>

Sp-NPs: Spirulina nanoparticles; Sp-WC: Spirulina whole cell.

- **k**: Rate constant.
- **R²**: r square.

abc no significant difference between any two means in the same column ‘have the same superscript small letter (P ≥ 0.05).

ABC no significant difference between any two means in the same row ‘have the same superscript capital letter (P ≥ 0.05).
Table 5
Effect of the addition of spirulina nanoparticles on p-anisidine value (mg kg\(^{-1}\)) in olive oil during storage at (60 °C) (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>k (mg kg(^{-1}))</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time zero</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Control</td>
<td>3.08 ± 0.1 a H</td>
<td>5.08 ± 0.17 a G</td>
<td>7.07 ± 0.13 a F</td>
</tr>
<tr>
<td>Sp-NPs (0.25%)</td>
<td>3.02 ± 0.11 a H</td>
<td>4.14 ± 0.15 a H</td>
<td>5.1 ± 0.34 a F</td>
</tr>
<tr>
<td>Sp-NPs (0.5%)</td>
<td>2.95 ± 0.08 a H</td>
<td>3.98 ± 0.17 a G</td>
<td>4.94 ± 0.34 c F</td>
</tr>
<tr>
<td>Sp-NPs (1%)</td>
<td>2.92 ± 0.1 a H</td>
<td>3.8 ± 0.1 c G</td>
<td>4.51 ± 0.16 d F</td>
</tr>
<tr>
<td>Sp-WC (0.5%)</td>
<td>3.03 ± 0.14 a H</td>
<td>4.22 ± 0.22 a G</td>
<td>5.28 ± 0.26 b F</td>
</tr>
<tr>
<td>BHT</td>
<td>2.91 ± 0.09 a H</td>
<td>3.48 ± 0.13 a G</td>
<td>3.84 ± 0.07 e F</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>3 ± 0.13 a H</td>
<td>4.16 ± 0.21 a G</td>
<td>5.16 ± 0.34 b F</td>
</tr>
</tbody>
</table>

Sp-NPs: Spirulina nanoparticles; Sp-WC: Spirulina whole cell.

k: Rate constant.

R\(^2\): r square.

abc no significant difference between any two means 'in the same column' have the same superscript small letter (P ≥ 0.05).

ABC no significant difference between any two means 'in the same row' have the same superscript capital letter (P ≥ 0.05).

Table 6
Effect of the addition of spirulina nanoparticles on totox value (meq kg\(^{-1}\)) in olive oil during storage at (60 °C) (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>k (meq kg(^{-1}))</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time zero</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Control</td>
<td>11.93 ± 0.6 a H</td>
<td>23.04 ± 0.19 a G</td>
<td>29.62 ± 0.96 a F</td>
</tr>
<tr>
<td>Sp-NPs (0.25%)</td>
<td>11.75 ± 0.41 a H</td>
<td>16.72 ± 0.94 b G</td>
<td>22.25 ± 0.39 b F</td>
</tr>
<tr>
<td>Sp-NPs (0.5%)</td>
<td>11.74 ± 0.26 a H</td>
<td>15.98 ± 0.76 c G</td>
<td>21.6 ± 0.49 d F</td>
</tr>
<tr>
<td>Sp-NPs (1%)</td>
<td>11.34 ± 0.17 a H</td>
<td>14.76 ± 0.53 d G</td>
<td>19.31 ± 0.48 e F</td>
</tr>
<tr>
<td>Sp-WC (0.5%)</td>
<td>11.73 ± 0.37 a H</td>
<td>16.94 ± 0.97 b G</td>
<td>22.92 ± 0.32 b F</td>
</tr>
<tr>
<td>BHT</td>
<td>11.23 ± 0.25 a H</td>
<td>14.41 ± 0.52 c G</td>
<td>15.16 ± 0.33 f F</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>11.81 ± 0.37 a H</td>
<td>16.74 ± 1 b G</td>
<td>22.35 ± 0.49 b H</td>
</tr>
</tbody>
</table>

Sp-NPs: Spirulina nanoparticles; Sp-WC: Spirulina whole cell.

k: Rate constant.

R\(^2\): r square.

abc no significant difference between any two means 'in the same column' have the same superscript small letter (P ≥ 0.05).

ABC no significant difference between any two means 'in the same row' have the same superscript capital letter (P ≥ 0.05).
### Table 7

Effect of the addition of spirulina nanoparticles on $K_{232}$ value in olive oil during storage at (60 °C) (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>k (at 232 nm)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.51 ± 0.01 a</td>
<td>1.59 ± 0.04 a</td>
<td>2.66 ± 0.02 a</td>
<td>2.87 ± 0.03 a</td>
<td>4.34 ± 0.25 a</td>
<td>5.49 ± 0.14 a</td>
<td>7.18 ± 0.22 a</td>
<td>0.44</td>
<td>0.12</td>
</tr>
<tr>
<td>Sp-NPs (0.25%)</td>
<td>0.43 ± 0.03 a</td>
<td>1.46 ± 0.05 a</td>
<td>1.57 ± 0.04 a</td>
<td>1.56 ± 0.04 a</td>
<td>2.08 ± 0.06 a</td>
<td>2.39 ± 0.09 a</td>
<td>3.22 ± 0.08 a</td>
<td>0.49</td>
<td>0.07</td>
</tr>
<tr>
<td>Sp-NPs (0.5%)</td>
<td>0.43 ± 0.03 a</td>
<td>1.51 ± 0.04 a</td>
<td>1.61 ± 0.04 a</td>
<td>1.66 ± 0.04 a</td>
<td>2.08 ± 0.06 a</td>
<td>2.38 ± 0.08 a</td>
<td>3.04 ± 0.08 a</td>
<td>0.50</td>
<td>0.08</td>
</tr>
<tr>
<td>Sp-NPs (1%)</td>
<td>0.41 ± 0.04 a</td>
<td>1.53 ± 0.05 a</td>
<td>1.51 ± 0.04 a</td>
<td>1.66 ± 0.04 a</td>
<td>2.08 ± 0.06 a</td>
<td>2.38 ± 0.08 a</td>
<td>3.04 ± 0.08 a</td>
<td>0.50</td>
<td>0.08</td>
</tr>
<tr>
<td>Sp-WC (0.5%)</td>
<td>0.43 ± 0.03 a</td>
<td>1.59 ± 0.04 a</td>
<td>2.66 ± 0.02 a</td>
<td>2.87 ± 0.03 a</td>
<td>4.34 ± 0.25 a</td>
<td>5.49 ± 0.14 a</td>
<td>7.18 ± 0.22 a</td>
<td>0.44</td>
<td>0.12</td>
</tr>
<tr>
<td>BHT</td>
<td>0.43 ± 0.02 a</td>
<td>1.46 ± 0.05 a</td>
<td>1.57 ± 0.04 a</td>
<td>1.56 ± 0.04 a</td>
<td>2.08 ± 0.06 a</td>
<td>2.39 ± 0.09 a</td>
<td>3.22 ± 0.08 a</td>
<td>0.49</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Sp-NPs:** Spirulina nanoparticles; **Sp-WC:** Spirulina whole cell.

k: Rate constant. R²: r square.

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ABC no significant difference between any two means 'in the same row' have the same superscript capital letter ($P \geq 0.05$).

### Table 8

Effect of the addition of spirulina nanoparticles on $K_{270}$ value in olive oil during storage at (60 °C) (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>Storage period (days)</th>
<th>k (at 270 nm)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.27 ± 0.01 a</td>
<td>0.31 ± 0.01 a</td>
<td>0.36 ± 0.02 a</td>
<td>0.46 ± 0.02 a</td>
<td>0.66 ± 0.02 a</td>
<td>0.82 ± 0.02 a</td>
<td>1.22 ± 0.03 a</td>
<td>0.54</td>
<td>0.02</td>
</tr>
<tr>
<td>Sp-NPs (0.25%)</td>
<td>0.26 ± 0.01 a</td>
<td>0.29 ± 0.01 a</td>
<td>0.3 ± 0.01 a</td>
<td>0.38 ± 0.01 a</td>
<td>0.52 ± 0.02 a</td>
<td>0.71 ± 0.02 a</td>
<td>1.22 ± 0.03 a</td>
<td>0.54</td>
<td>0.02</td>
</tr>
<tr>
<td>Sp-NPs (0.5%)</td>
<td>0.24 ± 0.01 a</td>
<td>0.28 ± 0.01 a</td>
<td>0.3 ± 0.01 a</td>
<td>0.38 ± 0.01 a</td>
<td>0.52 ± 0.02 a</td>
<td>0.71 ± 0.02 a</td>
<td>1.22 ± 0.03 a</td>
<td>0.54</td>
<td>0.02</td>
</tr>
<tr>
<td>Sp-WC (0.5%)</td>
<td>0.23 ± 0.01 a</td>
<td>0.26 ± 0.01 a</td>
<td>0.27 ± 0.01 a</td>
<td>0.31 ± 0.01 a</td>
<td>0.54 ± 0.02 a</td>
<td>0.66 ± 0.02 a</td>
<td>1.22 ± 0.03 a</td>
<td>0.54</td>
<td>0.02</td>
</tr>
<tr>
<td>BHT</td>
<td>0.26 ± 0.01 a</td>
<td>0.29 ± 0.01 a</td>
<td>0.31 ± 0.01 a</td>
<td>0.38 ± 0.01 a</td>
<td>0.52 ± 0.02 a</td>
<td>0.75 ± 0.02 a</td>
<td>1.27 ± 0.04 a</td>
<td>0.50</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Sp-NPs:** Spirulina nanoparticles; **Sp-WC:** Spirulina whole cell.

k: Rate constant. R²: r square.

abc no significant difference between any two means 'in the same column' have the same superscript small letter ($P \geq 0.05$).

ABC no significant difference between any two means 'in the same row' have the same superscript capital letter ($P \geq 0.05$).
popular antioxidants, i.e., BHT and ascorbic acid (Golmakani et al., 2018). The results suggest that Sp-NPs could be used as an alternative to synthetic antioxidant in oil and fatty products. The antioxidant action of spirulina is a multi-factorial impact and would be mainly attributed to the components such as phycobiliprotein phycocyanin (Aftari, Rezaei, Bandani, & Mortazavi, 2017).

3.3. Effect of Sp-NPs on oxidative stability of olive oil samples

3.3.1. Peroxide value (PV)

The addition of antioxidants, storage condition, and storage period on PV (Table 3) had a significant effect ($P \leq 0.05$). The olive oil naturally contained polyphenol constituents as control group (without any antioxidants addition) was evaluated. Generally, the olive oil including Sp-NPs or BHT or $\alpha$-tocopherol had $R^2 = 1$ compared with the control sample has $R^2 = 0.92$. The rate constant ($k$) of treated oil samples was the lowest in BHT sample ($0.1$ meq kg$^{-1}$) compared with others treatments. Thus, the $k$ value was ordered as following BHT $< 0.5\%$ Sp-NPs $< 0.25\%$ Sp-NPs $< 0.5\%$ Sp-WC $< \alpha$-tocopherol $< 0.5\%$ Sp-NPs. The PV in control sample was reached to the threshold limit value on 21st day, followed by a rapid increase. However, the PV in oil samples containing antioxidants such as BHT, $\alpha$-tocopherol, 0.5% Sp-WC, 0.25% Sp-NPs, 0.5% Sp-NPs, and 1% Sp-NPs were less than the limiting value (less than 20 meq kg$^{-1}$ oil) until the 49th, 28th, 28th, 28th, 42nd, and 49th day, respectively. The data referred to the control sample has a remarkable lipid-oxidation until day 21st of storage then has 74.18 meq O$_2$ kg$^{-1}$ at the end of auto-oxidation. A slight increase in PV was observed when antioxidants were added particularly Sp-NPs in olive oil after 35 days of storage. Moreover, it was noticed that PV in oil sample included $\alpha$-tocopherol was significantly higher than those of all Sp-NPs samples. However, the PV of the oil sample included BHT was significantly lower than those with spirulina (Golmakani et al., 2017).

3.3.2. Thiobarbituric acid (TBA)

As seen in Table 4, a significant difference ($P \leq 0.05$) in TBA was observed between the treated samples and the control. The lowest TBA
Influence of different concentrations of spirulina on antioxidant indices of olive oil (mean ± SD, n = 3).

Table 10

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time zero</td>
</tr>
<tr>
<td>Control</td>
<td>16.31 ± 0.33 f A</td>
</tr>
<tr>
<td>Sp-NPs (0.25%)</td>
<td>20.83 ± 0.23 a A</td>
</tr>
<tr>
<td>Sp-NPs (0.5%)</td>
<td>24.07 ± 0.29 b A</td>
</tr>
<tr>
<td>Sp-NPs (1%)</td>
<td>26.24 ± 0.49 a A</td>
</tr>
<tr>
<td>Sp-WC (0.5%)</td>
<td>15.72 ± 0.2 a A</td>
</tr>
<tr>
<td>BHT</td>
<td>16.39 ± 0.24 a A</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>20.83 ± 0.23 b A</td>
</tr>
</tbody>
</table>

Sp-NPs: Spirulina nanoparticles; Sp-WC: Spirulina whole cell.

abc no significant difference between any two means ‘in the same column’ have the same superscript small letter (P ≥ 0.05).

ABC no significant difference between any two means ‘in the same row’ have the same superscript capital letter (P ≥ 0.05).

Effect of the addition of spirulina nanoparticles on chlorophyll content (mg of pheophytin kg−1) in olive oil during storage at (60 °C) (mean ± SD, n = 3).

Table 9

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Samples</th>
<th>IP (hr)</th>
<th>AA</th>
<th>AOP (%)</th>
<th>IOS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.8 ± 0.15 f</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sp-NPs (0.25%)</td>
<td>19.7 ± 0.27 d</td>
<td>0.69 ± 0.03 c</td>
<td>14.72 ± 0.18 d</td>
<td>17.26 ± 0.28 d</td>
<td></td>
</tr>
<tr>
<td>Sp-NPs (0.5%)</td>
<td>22.5 ± 0.24 c</td>
<td>0.68 ± 0.01 c</td>
<td>25.33 ± 0.12 c</td>
<td>33.93 ± 0.43 c</td>
<td></td>
</tr>
<tr>
<td>Sp-NPs (1%)</td>
<td>24.3 ± 0.21 b</td>
<td>0.45 ± 0.02 c</td>
<td>30.86 ± 0.33 c</td>
<td>44.64 ± 0.57 c</td>
<td></td>
</tr>
<tr>
<td>Whole cell- Sp (0.5%)</td>
<td>18.8 ± 0.18 d</td>
<td>0.24 ± 0.03 c</td>
<td>10.64 ± 0.22 c</td>
<td>11.90 ± 0.21 c</td>
<td></td>
</tr>
<tr>
<td>α-tocopherol (0.01%)</td>
<td>18 ± 0.12 d</td>
<td>7.14 ± 0.09 b</td>
<td>6.67 ± 0.17 b</td>
<td>7.14 ± 0.09 b</td>
<td></td>
</tr>
<tr>
<td>BHT (0.01%)</td>
<td>28.9 ± 0.33 b</td>
<td>72.02 ± 2.11 d</td>
<td>41.87 ± 0.54 d</td>
<td>72.02 ± 2.11 d</td>
<td></td>
</tr>
</tbody>
</table>


abc no significant difference between any two means ‘in the same column’ have the same superscript small letter (P ≥ 0.05).

values were observed in oil samples containing 1% Sp-NPs and BHT, the highest value in the control sample during storage. At day zero, the TBA value in olive oil was 0.06 mg MDA kg−1, thereafter was quickly increased in the control sample to 3.25 mg MDA kg−1 after 49 days of storage; while in the treated samples with antioxidants recorded lower values. The rate constant (k) of treated oil samples was the lowest in BHT (0.01 mg MDA kg−1), while the highest value in the control sample (0.06 mg MDA kg−1). As well, most the treated samples had R2 = ∼1 compared with the control R2 = 0.97. In general, addition of antioxidants (synthetic or natural) in olive oil reduced the TBA values significantly compared to the control. Among the oil samples, the sample containing 1% Sp-NPs exhibited a higher inhibition than α-tocopherol, 0.5% Sp-WC, 0.25% Sp-NPs and 0.5% Sp-NPs incorporating samples. These results indicated that the addition of Sp-NPs can be active in preventing oil oxidation of olive oil (Asieh, Mohammad, Ali, Hossain, & Ali, 2016).

3.3.3. p-Anisidine value (AV) and Totox value (TV)

The effect of spirulina on AV and TV contents in olive oil during storage is shown in Tables 5 and 6. Antioxidants addition, storage condition, and storage time had significant effect (P ≤ 0.05) on AV and TV. The AV indicates the presence of conjugation compounds amounts, particularly 2-alkenals (Frankel, 2014). In the control sample, the AV progressively increased from 3.08 to 10.29 mg kg−1 until day 21 of the storage, and at the later stage of oxidation, it exhibited a higher increase in AV ~24.7 mg kg−1. The rate constant (k) of oil samples containing 1% Sp-NPs showed the lowest value compared with α-tocopherol, 0.25% Sp-NPs, 0.5% Sp-NPs, and 0.5% Sp-WC (Table 5). The AV threshold/limiting of (OO) is less than 2.6. Likewise, the K270 values were significantly increased (P ≤ 0.05) in the control sample compared with oil samples containing natural or synthetic antioxidants (Table 8). The rate constant (k) of oil samples including antioxidants was the lowest value in BHT and 1% Sp-NPs, while the highest in the control sample. It was noticed that oil samples contained spirulina were significantly lower in K232 than that of the control. At the same time, the oil sample containing α-tocopherol had a high K232 value. While the BHT sample recorded 1.69, which a lower value those of the Sp-NPs at different concentrations were applied. In addition, the samples included 0.5% Sp-NPs and 1% Sp-NPs had R2 = ~1 compared with the BHT sample R2 = 0.84. The K232 threshold/limiting (of OO) is less than 2.6. Likewise, the K270 values were significantly increased (P ≤ 0.05) in the control sample compared with oil samples containing natural or synthetic antioxidants (Table 8). The rate constant (k) of oil samples including antioxidants was the lowest value in BHT and 1% Sp-NPs, while the highest in the control sample. It was noticed that oil samples contained spirulina were significantly lower in K232 than that of α-tocopherol. The K270 threshold/limiting (of OO) are less than 0.9. The results are in an agreement with that reported by Alavi and Golmakani (2017a).

3.4. Kinetic rate constants of oxidation oil indicators and nanoparticles

The kinetic rate constants of some key lipid oxidation markers such as (peroxide value, p-Anisidine value, totox value, and K232) and whole group over the 49 days of storage. Therefore, the AV and TV had R2 = ~1 and the rate constant (k) of treated samples was ordered as following BHT < 1% Sp-NPs < 0.5% Sp-NPs < 0.25% Sp-NPs < 0.5% Sp-WC < α-tocopherol. These results could be attributed to spirulina phytochemicals that efficiently retarded the TV. Asieh et al. (2016) reported that the methanolic extract of spirulina (polar antioxidants) was able to protect the oils against oxidation, and attributed it to phycobilin pigments, pyrroglial, and catechin.
or nanoparticles spirulina at 0.5% were evaluated (Fig. 1). There were significant differences ($P \leq 0.05$) in oxidation indicators between samples containing 0.5% Sp-NPs and 0.5% Sp-WC. Moreover, the rate constant ($k$) of oxidation indicators in 0.5% Sp-NPs samples were lower than 0.5% Sp-WC. In Sp-NPs (0.5%), the $k$ values of peroxide, $p$-Anisidine, totox, and $K_{232}$ were 0.34 meq kg$^{-1}$, 0.24 mg kg$^{-1}$, 0.93 meq kg$^{-1}$, and 0.05 (at 232 nm), respectively. However, In Sp-WC (0.5%) were 0.50 meq kg$^{-1}$, 0.34 mg kg$^{-1}$, 1.35 meq kg$^{-1}$, and 0.08 (at 232 nm), respectively. A lower rate constant indicates a slower lipid oxidation. The data confirmed that spirulina nanoparticles enhanced

Fig. 2. Effect of the addition of spirulina nanoparticles on color (a), fruity (b), harmony (c), rancidity (d), and fusty (e) in olive oil samples during storage at (60 °C).
protection against oxidation compared to spirulina whole cell at the same concentration (0.5%) (Golmakani et al., 2018).

3.5. Chlorophyll content

Chlorophyll content is one of the most important attributes of olive oil. Table (9) illustrates that the chlorophyll content of the control sample was 16.31 mg kg\(^{-1}\) at time zero, while was progressively decreased to 6.55 and 11.75 mg kg\(^{-1}\), respectively. These results show that the oil. Table (9) illustrates that the chlorophyll content of the control samples were higher chlorophyll content than that of the control during the oxidation experiment. The chlorophyll content in samples containing α-tocopherol, 0.25% Sp-NPs, 0.5% Sp-NPs, and 0.5% Sp-WC, and BHT samples. The Sp-NPs samples were higher chlorophyll content than that of the control during the oxidation experiment. The chlorophyll content in samples containing α-tocopherol and BHT decreased from 16.75 to 16.39 mg kg\(^{-1}\) to 6.55 and 11.75 mg kg\(^{-1}\), respectively. These results show that the addition of Sp-NPs can play an important role in protecting the oil from oxidation and also improving the chlorophyll content in olive oil (Asieh et al., 2016).

3.6. Antioxidant indices

The antioxidant indices of olive oil samples were evaluated. Table (10) shows that all samples were significantly higher in IP values than that of the control. IP values in samples contained spirulina ranged from 18.8 to 24.3 days. A significant difference (P ≤ 0.05) in IP values was observed of samples containing different concentrations of Sp-NPs. Although the IP values of 1% Sp-NPs (24.3 days) were higher than that of α-tocopherol (18 days), the BHT sample showed the highest IP (28.9 days).

Furthermore, there was no significant difference (P ≥ 0.05) in antioxidant activity (AA) values between spirulina samples (Table 10). However, there were significant differences (P ≤ 0.05) in AA values between spirulina and α-tocopherol or BHT samples.

Antioxidant power (AOP) values of spirulina samples ranged from 10.64 to 30.86%. High concentrations of spirulina (1% Sp-NPs) showed that significantly active amounts of AOP. The AOP value of oil containing BHT was the highest among the samples; it was lower with α-tocopherol addition than spirulina addition; and it increased with increased addition of Sp-NPs. The improved oxidative stability (IOS) values of oil samples containing 0.25, 0.5, and 1% Sp-NPs were 17.26, 33.93, and 44.64% respectively (Table 10). Overall, the addition of spirulina in olive oil samples were significantly increased the IOS value.

A significant difference (P ≤ 0.05) in IOS values was recorded in samples containing Sp-NPs and α-tocopherol (7.14%) or BHT (72.02%). Similar results have been reported by Alavi and Golmakani (2017a).

3.7. Sensory evaluation of olive oil

The sensory scores of oil such as color, fruity, harmony, fusty, and rancidity of olive oil during storage are shown in Fig. 2. The sensory evaluation of oil samples was carried out for different storage dates due to termination of shelf life with the apparent development of off-flavour. In general, none of the treatment groups showed any changes in fusty, vinegary, metallic, bitter, and pungent attributes during storage. The color decreased in all samples with storage time; however, no significant (P ≥ 0.05) were observed during the first 35 days between BHT and Sp-NPs containing samples. However, the control sample showed the lowest color score. The addition of SP-NPs at different levels had a positive impact on the color of the oil. A significant difference (P ≤ 0.05) was noted in the fruity score between treatments and the control, although no significant differences (P ≥ 0.05) in harmony score were detected between all treatments. Odor and fusty characteristics gradually increased in all treatments during the storage, indicating oxidation of oil. The olive oil samples were quite stable for 14 days (control), while oxidation became apparent in the treated oils with storage period: day 21 (Sp-WC and α-tocopherol), on day 28th (0.25% Sp-NPs), on day 35th (0.5% Sp-NPs). However, the samples containing 1% Sp-NPs and BHT were stable until 42 days of storage. Fig. 3 illustrates the evaluation of chemical indicators and sensory attributes. In the control sample, the sensory response was rejected at day 21, in comparison when evaluating totox changes the value at which the oil are considered rancid at day 18 (threshold/limiting of totox about 35 meq kg\(^{-1}\)). While the totox value is increasing substantially from day 18–21, the intensity of sensory compounds reaches a plateau, indicating a possible for rejection. These results are in agreement with those reported by Bongartz and Oberg (2011). Based on sensory evaluation, oxidation markers and assuming Q\(_{10}\), the shelf life of olive oil under accelerated condition (60 °C) and predictable during storage at 25 °C were presented in (Fig. 4). The results demonstrated the shelf life of olive oil under accelerated condition (60 °C) was 18 days for control sample, 28 days for 0.25% Sp-NPs, 35 days for 0.5% Sp-Nps, 42 days for 1% Sp-NPs, 21 days for 0.5% Sp-WC, 49 days for BHT, and 21 days for α-tocopherol. However, the predictable shelf life of olive oil at 25 °C was ~204, 317, 396, 475, 238, 554, and 238 days, respectively. These data were estimated by assuming Q\(_{10}\) value for lipid oxidation of 2.0. Therefore, the acceleration factor was 11.31 days; this means 1 day at 60 °C would be equivalent to 11.31 days at 25 °C (Gharby et al., 2016).
4. Conclusion

In this study, phenolic content, antioxidant capacity of Sp-NPs, and chemical kinetics rate constants were evaluated. The Sp-NPs at different concentrations exhibited an effective antioxidant action. Sp-NPs improved the stability of olive oil during storage by reduction the primary and secondary oxidation products compared with the control. The levels of TBA, peroxide, \( p \)-anisidine, totox, \( K_{232} \), and \( K_{270} \) were significantly lower than the control Sp-NPs also improved the chlorophyll content and green color of olive oil. At the concentrations tested, BHT performed better than either spirulina or \( \alpha \)-tocopherol, but spirulina exhibited significantly higher antioxidant activity than that of \( \alpha \)-tocopherol. The rate constant \( (k) \) was low in OO with BHT and 1% Sp-NPs compared with other treatments. The oil samples containing Sp-NPs had acceptable sensory attributes up to 49 days. The results demonstrated that Sp-NPs are promising natural antioxidant to improve the stability and prolong the shelf life of the olive oil up to 475 days at 25 °C.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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References


IOC (2015). International Olive Oil Council: Trade standard applying to olive oil and olive-