In vitro Antioxidant Activities of Extract and Oil from Roselle (Hibiscus sabdariffa L.) Seed against Sunflower Oil Autoxidation

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ABSTRACT

Introduction: In order to overcome the stability problems of oils and fats, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) have widespread use as food additives in many countries. Recent reports reveal that these compounds may be implicated in many health risks, including cancer and carcinogenesis. Hence, there is a move towards the use of natural antioxidants of plant origin to replace these synthetic antioxidants. Methods: In this study, roselle seed oil (RSO) and extract (RSE) were mixed with sunflower oil, respectively to monitor degradation rate and investigate antioxidant activity during accelerated storage. Results: The antioxidant activity was found to stabilise sunflower oil of various samples and in the order of RSE>RSO>tocopherol>sunflower oil. The total percentage increased after 5 days of storage period in free fatty acid (FFA), peroxide value (PV) and anisidine value (AV). Total oxidation value (TOx) of sunflower oil supplemented with 1500 ppm RSE was 33.3%, 47.7%, 14.5%, and 45.5%, respectively. While the total percentage increased under different analysis methods, sunflower oil supplemented with 5% RSO was 17.2%, 60.4%, 36.2% and 59.0% in the order of FFA, PV, AV and TOTOX. Both RSO and RSE were found to be more effective in stabilisation of sunflower oil compared to tocopherol. Total phenolic content of RSE was 46.40 ± 1.51 mg GAE/100g of oil while RSO was 12.51 ± 0.15 mg GAE/100g of oil. Conclusion: The data indicates that roselle seed oil and seed extract are rich in phenolics and antioxidant activities and may be a potential source of natural antioxidants.

Keywords: Antioxidant activity, total phenolic content (TPC), roselle seed oil and extract

INTRODUCTION

Roselle (Hibiscus sabdariffa L.) is a medicinal plant found in Asia. Roselle originated from India and was brought to Malaysia. It has been used in different countries around the world as a culinary and therapeutic resource for many years. Roselle calyces have...
repeatedly been shown to have positive health effects (Faraji & Tarkhani, 1999). About 50% of the velvety capsules containing the seeds are usually discarded as waste. The nutritional values of roselle seeds has been rarely studied as compared with the calyces (Mohd Esa et al., 2010). Roselle seeds may be further processed into nutrients for food, feed or fertiliser to maximise the utilisation of the plant (Nyam et al., 2009). In some parts of Africa, roselle seeds are consumed grounded and added to meals although it tastes bitter (Duke & DuCellier, 1993). The seeds are high in protein and have been roasted as a substitute for coffee (Morton, 1987) and is said to have aphrodisiac properties (Duke & DuCellier, 1993). According to Omabuwajo, Sanni & Balami (2000), in northern Nigeria, the seeds are fermented into a condiment known as mungza ntusa. Besides, roselle seeds are used for medical purposes in some communities. For instance, in Myanmar, the seeds are used to treat debility (Perry, 1980). Meanwhile, the Taiwanese regard the seeds as a diuretic, laxative and tonic (Duke & DuCellier, 1993).

Roselle seeds are a good source of culinary oil (Ahmed & Hudson, 1979). According to Nyam et al. (2009) roselle seeds contain approximately 15 wt% on a dry weight basis (dwb) of highly unsaturated triacylglycerols and small amounts of other lipid components. The major unsaturated fatty acids found in roselle seed oil are oleic and linoleic acid. The presence of high linoleic acid shows that roselle seed oil could be a good source of essential fatty acids (Nyam et al., 2009). Furthermore, roselle seed oil is a rich source of α-tocopherol. α-tocopherol is the second major component in roselle seed oil (Nyam et al., 2009). Tocopherols are well known as biological antioxidants that can prevent or retard the oxidation of body lipids, which include polyunsaturated fatty acids and lipid components of cells and organelle membranes. High levels of vitamin E detected in the oils, may contribute to greater stability in oxidation (Nyam et al., 2009). In addition, the seeds also contain phytosterol compounds such as desmethylerolesterol (Nyam et al., 2009), which is known for its ability to reduce the absorption of dietary cholesterol when included in the human diet (Jones et al., 2000).

Numerous studies report that roselle seed oil and seed extract have high antioxidant activities (Mohd-Esa et al., 2010). However, comparative studies on antioxidant and antimicrobial activity of roselle seed oil or extract are still limited. In this study, roselle seed oil and extract were mixed with sunflower oil, respectively to monitor its degradation rate and compared with commercial standard (α-tocopherol) to investigate antioxidant activity.

METHODS

Dried roselle seeds were obtained from Malaysian Agricultural Research and Development Institute (MARDI) (Selangor, Malaysia). The roselle seeds were ground into powder using a grinder (Model EM-11; Sharp, Japan). The powder was sieved using a 1 mm sieve. The roselle seed powder was then vacuum-packaged into a nylon-linear low density polyethylene film (nylon-LDPE) (Flexoprint, Malaysia). The vacuum-packaged samples were wrapped with aluminium foil and stored in a freezer until further use.

Solvent extraction

The oils were extracted from the seeds with a soxhlet extractor for 8 hours (Nyam et al., 2009). Hexane was chosen as the extraction solvent (sample-to-solvent ratio: 1:5, w/v). The oil was then recovered by evaporating off the solvent using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikai Co., Ltd., Japan). An amount of 50 g of defatted roselle seed powder was then weighed using analytical balance (AB204-S, Mettler Toledo, Switzerland) and mixed with 250 mL of
ethanol (sample-to-solvent ratio: 1:5, w/v) in a 500 ml conical flask which was covered and sealed by aluminium foil (Diamond, USA) and parafilm to avoid light exposure and prevent spilling of the mixture. The mixture was then shaken with a shaker (Green Seriker, Vision, Korea) at 150 rpm for 2 hours. After extraction, the mixture was filtered with Whatman No. 1 filter paper (Whatman International, England) to obtain a clear solution of the crude extract. The clear solution was then transferred into several round bottom flasks and evaporated using a rotary evaporator (Rotavapor R-200, BUCHI, Switzerland) at 40°C for 20 minutes until a sticky extract was obtained. The sticky extract was transferred into a universal bottle wrapped with aluminium foil and oven dried for 4 hours to eliminate the presence of ethanol residue. The roselle seed contained 14.6 g/100 g crude oil.

**Accelerated storage**

Five percent of roselle seed oil and 1500 ppm of roselle seed extract were weighed and mixed with 100 mL sunflower oil, respectively in a 250 mL Scott bottle, which was wrapped with aluminium foil. The control was 100 mL sunflower oil while 200 ppm of tocopherol was mixed with 100 mL sunflower oil and used as standard in this study. The oils were then stored in an oven at 65°C for 5 days to accelerate the deterioration of the oil. From this, 25 mL of the oils was withdrawn and transferred into a 50 mL Scott bottle at day 0, day 1, day 3 and day 5 after storage in oven, for further analysis.

**Chemical analyses**

Iodine value (IV), free fatty acid content (% FFA), peroxide value (PV) and p-anisidine value (p-AV) were determined according to AOAC (2005). Meanwhile total oxidation value was determined according to the following equation:

\[
\text{Total oxidation value (TOTOX)} = 2 \times \text{PV} + p-AV
\]

**Total phenolic content (TPC)**

Phenolic compounds were extracted from oil according to Rotondi et al. (2004) with slight modifications. Then, 3.0 ± 0.01 g of oil sample was weighed into 50 mL Falcon tube, to which was added 5 mL of hexane and vortexed using a vortex mixer (VTX-3000L, Copens Scientific, Germany). This was followed by the addition of 10 mL of methanol:water (60:40, v/v) to extract the phenolic compounds and the mixture was vortexed for 5 minutes followed by centrifugation at 4500 rpm for 5 minutes. The upper layer, methanolic phase was collected and the lower layer, hexane phase was extracted twice with the previous step. The methanolic phase from the three extractions were then transferred into a Falcon tube and mixed with 10 mL of hexane to eliminate residual oil samples in a separating funnel. The methanolic fraction was transferred into a round bottom flask which was subjected to rotary evaporation by using a rotary evaporator (Rotavapor R-200, BUCHI, Switzerland) at 40°C until dried. It was then reconstituted with 10 mL of methanol:water (60: 40, v/v). All steps were repeated for control and standard.

Total phenolic content was determined using Folin-Ciocalteu assay, based on the method described by Li et al. (2008) with slight modifications. Exactly 500 µL of methanolic extract was added to 1.5 mL of 10-fold diluted Folin-Ciocalteu reagent (FCR) in an aluminium-foil wrapped 15 mL test tube. After 3 minutes, 800 µL of 7.5 % (w/v) sodium carbonate solution was added and mixed thoroughly by using a vortex mixer (VTX-3000L, Copens Scientific, Germany) for 5s. The solution was then stored at room temperature in the dark. A blank was prepared by replacing 0.5 µL of methanolic extract with 0.5 µL of deionised water. The absorbance was measured at 765 nm against blank using UV-Vis spectrophotometer.
Measurements were carried out in duplicate and mean values were calculated. Gallic acid was used for determination of calibration curve. The calibration equation for gallic acid was $y = 19.27x + 0.01$ ($R^2 = 0.997$). The results were expressed as milligram gallic acid equivalent per 100 grams of oil sample (mg GAE / 100 g of oil) and calculated based on Equation 2 below:

$$\text{Total phenolic content (TPC)} = \frac{(Ab+0.01) \times V \times 100}{19.27 \times Wt} \quad (2)$$

Statistical analysis

All results were analysed using Minitab software (Minitab Version 15.1.1.0.). All assays were replicated and every measurement was duplicated. All values are expressed as mean of duplicate ± standard deviation (SD). One-way analysis of variance was used to determine significant difference ($p<0.05$) between the means.

RESULTS AND DISCUSSION

Free fatty acid (FFA) content

The FFA of SFO (sunflower oil), SFO-α tocopherol (sunflower oil supplemented with 200 ppm tocopherol), SFO- 5% RSO (sunflower oil supplemented with 5% roselle seed oil) and SFO-1500 ppm RSE (sunflower oil supplemented 1500 ppm roselle seed extract) are shown in Table 1. The FFA of SFO and SFO-α tocopherol before accelerated storage were significantly different from SFO– 5% RSO and SFO- 1500 ppm extract, in which SFO had the lowest FFA (0.10% oleic acid equivalent) while SFO– 5% RSO had the highest FFA (0.29% oleic acid equivalent). SFO- 5% RSO and SFO-1500 ppm RSE exhibited the highest FFA content due to the formation of free fatty acids during heat treatment (60°C for 8 hours) for oil extraction. According to Iqbal & Bhanger (2007), hydrolysis of triglycerides produces FFA which may get promoted by reaction of oil with moisture.

Table 1. Effects of accelerated storage (65°C for 5 days) on free fatty acid content for SFO, SFO-α tocopherol, SFO- 5% RSO and SFO- 1500 ppm RSE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Free fatty acid content (% oleic acid equivalent)</th>
<th>Total increase in FFA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>SFO</td>
<td>0.10 ± 0.02bc</td>
<td>0.11 ± 0.02bC</td>
</tr>
<tr>
<td>SFO-α tocopherol</td>
<td>0.11 ± 0.03bB</td>
<td>0.11 ± 0.01SC</td>
</tr>
<tr>
<td>SFO- 5% RSO</td>
<td>0.29 ± 0.04aA</td>
<td>0.30 ± 0.01aA</td>
</tr>
<tr>
<td>SFO- 1500 ppm RSE</td>
<td>0.24 ± 0.04bA</td>
<td>0.27 ± 0.05bb</td>
</tr>
</tbody>
</table>

* Values are represented in means ± standard deviation (n=4); Mean values in the same row with different superscript a are significantly different ($p<0.05$); Mean values in the same column with different superscript A are significantly different ($p<0.05$).
Antioxidant Activities of Extract & Oil from H. sabdariffa L. against Sunflower Oil Autoxidation

Generally, FFA content was increased during the storage period for all the samples. The % FFA of sunflower oil slightly increased during day 1 and day 3, followed by a considerable increase during day 5. Similar trends were observed in SFO-α tocopherol, SFO-5% RSO and SFO-1500 ppm RSE. After 5 days of storage at 65°C, the FFA of SFO reached a maximum of 0.16 ± 0.01% oleic acid equivalent. Addition of tocopherol, RSO and RSE caused a significant reduction in the formation of FFA during 5 days of storage. FFA of sunflower oil treated with tocopherol, RSO and RSE increased 27.3%, 17.2% and 33.3% of initial value, respectively, while the total increase in FFA for SFO was 60%. Zhang et al. (2010) reported that %FFA of sunflower oil after 21 days of storage at 60°C reached 0.71 ± 0.05 mg/g.

The SFO exhibited the highest increment in FFA content after 5 days, followed by SFO-1500 ppm RSE, SFO-α tocopherol and SFO-5% RSO. Total increment in FFA of SFO was nearly double of that SFO-1500 ppm RSE and SFO-α tocopherol and 3.5 times of SFO-5% RSO. These trends indicate that 5% RSO had the best antioxidant properties against the formation of free fatty acid. Although RSE had the highest phenolic content, the increase in FFA was still higher than in RSO. The only explanation to this circumstance is the presence of impurities, such as water presented in the RSE. Water molecules might be found as residue in the extract during the rotary evaporation at 40°C. Zhang et al. (2010) reported that FFA is one of the products from hydrolysis of triglycerides and this reaction could be accelerated by the presence of water.

**Peroxide value (PV)**

The changes in PV of different samples during accelerated storage are shown in Table 2. In general, a regular increase in PV during storage period was observed for all samples at all intervals. At day 0, the lowest PV was observed for SFO (11.20 meq O₂ / kg oil), followed by SFO-α tocopherol, SFO-5%

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peroxide value (milliequivalent O₂ / kg of oil)</th>
<th>Total increase in PV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>SFO</td>
<td>11.22 ± 0.33</td>
<td>13.60 ± 0.78</td>
</tr>
<tr>
<td>SFO-α tocopherol</td>
<td>11.80 ± 0.42</td>
<td>12.52 ± 0.75</td>
</tr>
<tr>
<td>SFO-5% RSO</td>
<td>12.40 ± 0.41</td>
<td>13.48 ± 0.65</td>
</tr>
<tr>
<td>SFO-1500 ppm RSE</td>
<td>13.27 ± 0.44</td>
<td>14.31 ± 0.06</td>
</tr>
</tbody>
</table>

* Values are represented in means ± standard deviation (n=4); Mean values in the same row with different superscript * are significantly different (p<0.05); Mean values in the same column with different superscript ^ are significantly different (p<0.05).

SFO (sunflower oil),
SFO-α tocopherol (sunflower oil supplemented with 200 ppm tocopherol)
SFO-5% RSO (sunflower oil supplemented with 5% roselle seed oil)
SFO-1500 ppm RSE (sunflower oil supplemented 1500 ppm roselle seed extract)
RSO and SFO- 1500 ppm RSE. Initially, the PV of SFO was significantly lower than SFO- 5% RSO and SFO- 1500 ppm RSE. After day 1, the peroxide content of SFO rose rapidly until the end of analysis. According to Iqbal and Bhanger (2007), the PV of sunflower oil increased to 170 meq O₂ / kg oil after 20 days of storage in an oven at 65°C.

The PV of SFO rose more sharply during accelerated storage compared with other test materials. During the storage period of 5 days, PV of SFO increased from 11.20 meq O₂ / kg oil to 22.20 meq O₂ / kg oil, which is significantly higher than other sunflower oil supplemented with RSO, RSE and tocopherol, respectively. Both SFO- 5% RSO showed good antioxidant efficiency by slowing down the degradation of sunflower oil. In addition, the maximum PV of SFO- 5% RSO and SFO- 1500 ppm RSE were lower than 200 ppm tocopherol. These data suggest the superiority of SFO- 5% RSO and SFO- 1500 ppm RSE over tocopherol because of their antioxidant activity and efficiency.

The PV of SFO- 5% RSO was comparable to that of SFO- 1500 ppm RSE at all stages initially but increased after day 5 revealing better antioxidant efficiency of SFO- 1500 ppm RSE compared to SFO- 5% RSO. Maximum PV for SFO- 5% RSO and SFO- 1500 ppm RSE were 19.90 meq O₂ / kg oil and 19.60 meq O₂ / kg oil, respectively. Abramovic et al. (2007) found that there was a very good correlation between PV and total phenolic compounds (TPC) in camelina oil during storage. The TPC was negatively and linearly correlated with PV (r = -0.977). Looking at the total phenolic content (TPC) of different samples as shown in Table 5, the initial phenolic content of SFO- 5% RSO and SFO- 1500 ppm RSE were 12.50 and 42.40 mg GAE / 100 g of oil. Therefore, during the accelerated storage, SFO- 1500 ppm RSE might experience a lower rate of lipid oxidation than SFO- 5% RSO.

Different samples inhibited formation of primary oxidation product to various extent. As expected, control exhibited the highest increase in PV while SFO- 1500 ppm RSE exhibited the least. After 5 days of accelerated storage, the PV of SFO rose up to 97.80% of its initial PV. In contrast, the PV of SFO- 1500 ppm RSE only increased 47.70% of its initial value. SFO supplemented with RSO and RSE showed better antioxidant activity in comparison with standard, SFO-α tocopherol. Antioxidant activity of RSO and RSE could be attributed to the distinctly high amounts of phenolic compounds.

p-Anisidine value (AV)

The increasing AV value of all oil samples during storage are shown in Table 3. The AV of all oil samples before exposure to heat treatment was in the range of 1.58 and 1.86, while SFO only had the lowest AV value (1.58 ± 0.05) and SFO- 1500 ppm RSE had the highest AV value (1.86 ± 0.01). All oil samples were significantly different from each other except for SFO and SFO-α tocopherol.

As the storage period was prolonged, the p-AV of different samples were increased. SFO (control) experienced the highest increment in AV. The antioxidant effect of RSO and RSE were obvious in the inhibition of secondary oxidation. The AV of SFO-α tocopherol, SFO- 5% RSO and SFO- 1500 ppm RSE were significantly lower than SFO. SFO experienced the highest increase in AV (77.20 %), while SFO- 1500 ppm RSE experienced the least (14.50 %). Total increase in SFO-α tocopherol and SFO- 5% RSO was 37.10 % and 36.20 %, respectively, suggesting that the antioxidant activity of 1500 ppm RSE was much better than 200 ppm α-tocopherol. Compared to control, 1500 ppm RSE was found to be even more effective in slowing down the formation of secondary oxidation products. The trend obtained for a total increase in AV for all samples after 5 days of accelerated storage was similar when compared to the total increase in PV, but the total increase in AV for all samples after 5 days of accelerated storage was lower than PV.
Antioxidant Activities of Extract & Oil from H. sabdariffa L. against Sunflower Oil Autoxidation

Table 3. Effects of accelerated storage (65°C for 5 days) on p-anisidine value for SFO, SFO-α tocopherol, SFO- 5% RSO and SFO- 1500 ppm RSE

<table>
<thead>
<tr>
<th>Sample</th>
<th>p-Anisidine value</th>
<th>Total increase in AV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>SFO</td>
<td>1.58 ± 0.05&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>1.85 ± 0.09&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFO- α tocopherol</td>
<td>1.59 ± 0.03&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>1.69 ± 0.05&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFO- 5% RSO</td>
<td>1.77 ± 0.06&lt;sup&gt;dB&lt;/sup&gt;</td>
<td>1.91 ± 0.02&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFO- 1500 ppm RSE</td>
<td>1.86 ± 0.01&lt;sup&gt;DA&lt;/sup&gt;</td>
<td>1.92 ± 0.03&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values are represented in means ± standard deviation (n=4); Mean values in the same row with different superscript * are significantly different (p<0.05); Mean values in the same column with different superscript ^ are significantly different (p<0.05).

SFO (sunflower oil).
SFO-α tocopherol (sunflower oil supplemented with 200 ppm tocopherol)
SFO- 5% RSO (sunflower oil supplemented with 5% roselle seed oil)
SFO- 1500 ppm RSE (sunflower oil supplemented 1500 ppm roselle seed extract)

Table 4. Effects of accelerated storage (65°C for 5 days) on total oxidation value for SFO, SFO-α tocopherol, SFO- 5% RSO and SFO- 1500 ppm RSE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total oxidation value</th>
<th>Total increase in TOx value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>SFO</td>
<td>24.02 ± 0.68&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>29.04 ± 1.61&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFO- α tocopherol</td>
<td>25.19 ± 0.86&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>26.73 ± 1.54&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFO- 5% RSO</td>
<td>26.58 ± 0.84&lt;sup&gt;dB&lt;/sup&gt;</td>
<td>28.86 ± 1.28&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFO- 1500 ppm RSE</td>
<td>28.40 ± 0.88&lt;sup&gt;DA&lt;/sup&gt;</td>
<td>30.53 ± 0.11&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values are represented in means ± standard deviation (n=4); Mean values in the same row with different superscript * are significantly different (p<0.05); Mean values in the same column with different superscript ^ are significantly different (p<0.05).

SFO (sunflower oil).
SFO-α tocopherol (sunflower oil supplemented with 200 ppm tocopherol)
SFO- 5% RSO (sunflower oil supplemented with 5% roselle seed oil)
SFO- 1500 ppm RSE (sunflower oil supplemented 1500 ppm roselle seed extract)

Total oxidation (TOx) value

SFO- 1500 ppm RSE had the highest TOx value, followed by SFO- 5% RSO, SFO-α tocopherol and SFO before accelerated storage (Table 4). TOx values of SFO-5% RSO and SFO- 1500 ppm RSE were significantly higher than that of SFO. During RSO extraction, roselle seed powder was subjected to a heat treatment at 60°C for 8 hours. Furthermore, the defatted roselle seed
powder was treated at 40°C for 1-2 hours in order to obtain RSE. These steps could lead to a higher degree of primary and secondary oxidation products in the oil.

Generally, the TOx value of all samples increased during accelerated storage at 60°C. During day 1, TOx value of SFO increased rapidly. The TOx value of SFO continued to rise rapidly until it reached a maximum value (47.20) at day 5 of analysis. The antioxidant effects of tocopherol, RSO and RSE were significant in inhibiting the formation of primary and secondary oxidation products.

After day 5 of accelerated storage, SFO-1500 ppm RSE had the lowest TOx value while SFO had the highest TOx value. The mean difference in TOx value between SFO and SFO-1500 ppm RSE was 5.90. However, no significant difference could be observed between SFO-α-tocopherol, SFO-5% RSO and SFO-1500 ppm RSE.

The percentage of total increase in TOx for all samples was in the range of 45.50% - 96.40%. SFO-1500 ppm RSE was observed to have the least increase in TOx, being 45.50% of its initial value. On the other hand, SFO was observed to have the highest increase in TOx, being 96.40% of its initial value. SFO-α-tocopherol and SFO-5% RSO were observed to have the second highest increase (86.90%) and second lowest (59.00%) increase in total TOx value of its initial value. This could be due to the presence of other antioxidants besides polar phenolic compounds such as phytosterols and tocopherols in RSO that provide protection against oil oxidation (Mohd-Esa et al., 2010). According to Mohd Nor et al. (2008), a combination of different antioxidants might provide synergistic effects that further enhance the oxidative stability of oils. The results also indicate that SFO-1500 ppm RSE experienced the least degree of oxidation, while SFO experienced the most.

According to the polar paradox theory by Frankel (1993), the lipophilic antioxidants are more effective in inhibiting oxidation in oil-in-water emulsions, whereas hydrophilic antioxidants are more effective in bulk oil systems. Since α-tocopherol is a lipophilic antioxidant, it is dissolved in the oil but not positioned between the oil-air interfaces (Huber et al., 2009). As the oxidation of oil was mainly initiated on the oil surface which is in contact with air, α-tocopherol was definitely unable to provide sufficient protection to the oils preventing it from oxidation by donating hydrogen atoms to free radicals (Laguerre, Lecomte & Villeneuve, 2007).

**Total phenolic content (TPC)**

The amounts of phenolic content (mg GAE/100 g of oil) of different oil samples were determined in this study in order to determine the phenolic compound losses during accelerated storage. TPC of SFO, SFO-5% RSO, SFO-1500 ppm RSE and SFO-α-tocopherol are shown in Table 5. The TPC for all fresh samples (day 0) were significantly different except for SFO and SFO-α-tocopherol, in which, SFO-1500 ppm RSE had the highest TPC (46.40 mg GAE/100 g of oil), followed by SFO-5% RSO (12.51 mg GAE/100 g of oil), SFO-α-tocopherol (11.87 mg GAE/100 g of oil) and SFO (11.65 mg GAE/100 g of oil). Phenolic content in sunflower oil supplemented with RSO and RSE was significant higher (P< 0.05) than those of sunflower oil supplemented with tocopherol and sunflower oil itself. The results also indicate that phenolic compounds from roselle seeds are better extracted with ethanol than with hexane. In other words, most of the phenolic compounds in roselle seed were hydrophilic. According to Nyam et al. (2009), phenolic acids found in roselle seed include vanillic acid, caffeic acid, gallic acid, ferulic acid and p-hydroxybenzoic acid. These phenolic acids are believed to protect polyunsaturated fatty acids in oil against autoxidation.

Phenolic compounds of SFO-α-tocopherol was slightly decreased (P > 0.05)
after day 1 of storage, while there was a significant decrease at days 3 and 5. After 5 days of storage, the phenolic content decreased to 9.15 ± 0.35 mg GAE/100 g of oil, 77.1 % reduction of initial phenolic content. On the other hand, the phenolic content of SFO, SFO- 5% RSO and SFO- 1500 ppm RSE decreased to 53.40 %, 70.00 % and 73.80 % of its respective initial phenolic content after 5 days of storage, respectively.

SFO had the lowest phenolic content, while SFO- 1500 ppm RSE had the highest phenolic content. Before exposure to accelerated storage, there was no significant difference between the phenolic content of SFO and SFO-α tocopherol. However, after 1 day of storage at 65°C, the TPC of SFO decreased rapidly and significant difference was observed between the TPC of SFO and SFO-α tocopherol. This indicates that the sunflower oil started to degrade. On the other hand, the phenolic content of SFO- 5% RSO was similar to SFO-α tocopherol after exposure to accelerated storage conditions. A good correlation between the TPC and antioxidant activity showed that the TPC contributed to delaying lipid oxidation.

A comparison of the percentage of total losses in the TPC of different samples showed that SFO had the highest decrease in TPC with a value of 46.60% of its initial value; followed by SFO- 5%, (30%), SFO-α tocopherol (22.9%) and SFO- 1500 ppm RSE (26.2%) (Table 5).

CONCLUSION

Roselle seed is a byproduct of the roselle processing industry. This unwanted byproduct can be recycled as value added food supplement, as it provide advantageous bioactive compounds. It can be utilised for the preparation of food products with improved nutritional properties as well as enhance the stability of food products.

REFERENCES


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**Table 5.** Effects of accelerated storage (65°C for 5 days) on total phenolic content for SFO, SFO-α tocopherol, SFO- 5% RSO and SFO- 1500 ppm RSE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic content (mg GAE / 100g of oil)</th>
<th>Total loss in TPC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>SFO</td>
<td>11.65±0.31aC</td>
<td>10.51±0.31bC</td>
</tr>
<tr>
<td>SFO-α tocopherol</td>
<td>11.87±0.28aC</td>
<td>11.56±0.31ab</td>
</tr>
<tr>
<td>SFO- 5% RSO</td>
<td>12.51±0.15ab</td>
<td>11.62±0.38ab</td>
</tr>
<tr>
<td>SFO- 1500 ppm RSE</td>
<td>46.40±1.51aaa</td>
<td>43.53±0.77ab</td>
</tr>
</tbody>
</table>

* Values are represented in means ± standard deviation (n=4); Mean values in the same row with different superscript a are significantly different (p<0.05); Mean values in the same column with different superscript A are significantly different (p<0.05).
SFO (sunflower oil).
SFO-α tocopherol (sunflower oil supplemented with 200 ppm tocopherol)
SFO- 5% RSO (sunflower oil supplemented with 5% roselle seed oil)
SFO- 1500 ppm RSE (sunflower oil supplemented 1500 ppm roselle seed extract)


