Effects of natural phenolics and synthetic antioxidants on the oxidative thermal stability of refined and purified sunflower oils

Seyma YILDIZ[1]
Seyma DILMEN[1]
Semra TURAN[1]
Mustafa KIRALAN[2]
Mohamed Fawzy RAMADAN[3,4*]

[1] Bolu Abant Izzet Baysal University Engineering Faculty Food Engineering Department, Bolu, Turkey

[2] Balıkesir University Engineering Faculty Food Engineering Department Balıkesir, Turkey

[3] Agricultural Biochemistry Department Faculty of Agriculture Zagazig University Zagazig, Egypt

[4] Umm Al-Qura University Deanship of Scientific Research Makkah, Kingdom of Saudi Arabia

(*) CORRESPONDING AUTHOR:
Prof. Dr. Mohamed Fawzy Ramadan
Deanship of Scientific Research
Umm Al-Qura University
Makkah, Kingdom of Saudi Arabia
E-mail: mhassanien@uqu.edu.sa
E-mail: hassanienmohamed@yahoo.com

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The antioxidant activity of phenolic compounds [thymol (T), carvacrol (C), and thymoquinone (TQ)] was compared with commercial antioxidants [butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol] using the β-carotene bleaching and ABTS assays. At 1000 ppm concentration, commercial antioxidants showed better antioxidative properties (94.9-95.9%) than phenolic compounds (64.9-85.7%) according to β-carotene bleaching assay. In the ABTS assay, except for BHT, BHA and α-tocopherol exhibited stronger radical scavenging activity than phenolic compounds. Regarding the Rancimat method, BHT-enriched stripped sunflower oil (SSO) had the longest induction period. At the same time, phenolic compounds caused a slightly higher increase in the induction periods than the control sample. Refined sunflower oil (RSO) enriched with BHT at 100 ppm was the most stabile during storage at 60°C. Phenolic compounds added to RSO at 250, and 500 ppm exhibited less protection to lipid oxidation than BHT, while phenolic compounds, notably TQ, improved the RSO's oxidative stability. BHT at 100 ppm showed a strong antioxidant effect on SSO during storage at 60°C. The effect of phenolic compounds, especially TQ on SSO, were lower than BHT, but higher than the control sample at 60°C. The results are essential to applying new natural compounds as antioxidant agents in oils, fats, and lipids-rich foodstuffs.

Keywords: Phenolics; BHT; BHA; Thymol; Carvacrol; Thymoquinone; Refined sunflower oil

1. INTRODUCTION

Lipid oxidation is the main deterioration problem in oils, fats, and lipids-containing foodstuffs. This deterioration affects quality parameters such as colour, flavour, texture, and the nutritional value of foods. Besides, lipid oxidation generates some toxic products with health risks such as inflammatory diseases, cancer, atherosclerosis, and aging [1-4].

The fatty acid composition is a significant factor in determining the oxidation rate of oils/fats. The consumption of polyunsaturated fatty acids (PUFA), mainly omega fatty acids, provides several health benefits to humans, while the PUFA are prone to oxidation [5]. Antioxidants could delay the lipid oxidation process *via* delaying the initiation of oxidation chain cades, the demand for natural antioxidants has increased. Research intensively examined natural antioxidants as safe alternatives to synthetic compounds [9-11]. The most active natural antioxidants belong to the family of phenolic and polyphenolic compounds [12]. Most natural phenolics have been reported to possess a powerful antioxidant activity. Phenolics could be classified into a hydrophilic group (simple phenolics, anthocyanins, phenolic acids, flavonoids, and tannins) and lipophilic groups (tocopherols) [13]. Besides preventing lipid oxidation, phenolics exhibit a wide range of health-promoting traits, including an-

ti-aging, anti-inflammatory, anti-atherosclerosis, and anticancer [14].
Sunflower (*Helianthus annuus*) is primarily harvested for oil production, and its oil is rich in PUFA. Linoleic acid is a major fatty acid found in sunflower oil (55-70%), followed by a monounsaturated fatty acid, oleic acid (20-25%) [15]. Due to the higher content of PUFA, sunflower oil is prone to lipid oxidation [16]. Thymol (T) and carvacrol (C) are terpenoids found as major constituents of oregano and thyme essential oils [17]. Thymol and carvacrol had potent antioxidant and biological activities [18]. Thymoquinone (TQ) is the main bioactive constituent in the black cumin (*Nigella sativa*) essential oil. Thymoquinone possesses antioxidant activity and some biological activity such as anti-inflammatory, antineoplastic, neuro- and hepatoprotective properties [19]. Thymol and carvacrol were used as an active natural antioxidant against the oxidation of sunflower oil [20, 21]. Although there is no literature using TQ directly on vegetable oils, it was reported that black cumin oil rich in TQ increased the oxidative stability of sunflower oil [21].
In the current study, phenolic compounds including T, C, and TQ compared with commercial antioxidants were tested as antioxidant agents to improve the oxidative stability of refined sunflower oil (RSO) and stripped sunflower oil (SSO). β-carotene bleaching and ABTS tests were used to evaluate the antioxidant and antiradical activity of phenolic compounds and commercial antioxidants. The effects of phenolic compounds and commercial antioxidants on the oxidative stability of RSO and SSO under Schaal oven test conditions (60°C) were investigated and compared with synthetic antioxidants (BHT).

2. MATERIALS AND METHODS

2.1. MATERIALS

Commercial RSO was purchased from the market (Bolu, Turkey) and stored at -18°C. BHA, BHT, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemicals and solvents used in this study were of analytical grade and obtained from Sigma-Aldrich (Buchs, Switzerland) and Merck (Merck KGaA, Darmstadt, Germany).

2.2. METHODS

2.2.1. Analytical determinations of RSO

The initial quality parameters of RSO were tested by the determination of free fatty acid content (FFA) (Ca 5a-40), *p*-anisidine value (AV) (AOCS Cd 18-90), peroxide value (PV) (Cd 8-53), specific absorbance values (K_{232} and $K_{268)}$ (Ch 5-91) according to AOCS [22] Official methods.
Total tocopherols content in oil samples was analysed according to Wong et al. [23]. The oil sample (200 mg) was weighed in a 10-mL flask. Toluene (5 mL) were added then 3.5 mL 2,2'-bipyridine (0.07% w/v in ethanol 95%) and 0.5 mL of $FeCl_3 \cdot 6H_20$ (0.2% w/v in ethanol 95%) were added. The solution was made up to 10 mL using ethanol 95%. After 1 min, the absorption (520 nm) was recorded using a blank solution (without oil). The test was calibrated using standards containing 0-250 µg α-tocopherol in toluene. Total tocopherols in the oil was calculated as follow:

$$\text{Total tocopherols (ppm)} = (A-B)/M.W.$$

Where:
A = sample absorption in 10 mm-cell,
B = blank absorption in 10 mm-cell,
M = gradient of absorbance *vs*. weight graph for α-tocopherol calibration,
W = weight of the sample (g).
The fatty acid profile was determined by GLC after methylation, using an Agilent 7890A fused silica capillary column (J & W Scientific, USA). The column is 100 m long, 0.2 µm film thickness, and 0.25 mm inner diameter. The injector temperature was maintained at 250°C and the detector temperature at 260°C. Helium was the carrier gas at 1 mL/min flow rate. One µL sample was injected into the column, and the split ratio was 1:30. The column temperature was set at 140°C for 5 min, then programmed to 240°C at 4°C/min and held at 240°C for 10 min. Fatty acid methyl ester (FAME) standard solution (37 FAME mix, Sigma, St. Louis, USA) was used to identify the peaks. The fatty acid composition of oils was given in percentage proportions of FAME using the peak areas.

2.2.2. Antioxidant properties of phenolics and commercial antioxidants

2.2.2.1. β-Carotene bleaching test

The antioxidant activity was evaluated using the β-carotene/linoleic acid test, according to Kulisic et al. [24] and Cheung et al. [25]. β-Carotene (1 mL, 0.06 g/10 mL chloroform), linoleic acid (60 mg), and Tween 40 (600 mg) were mixed, and the solvent was evaporated in a rotary evaporator under vacuum. Distilled water (150 mL) was added to the dried mixture and homogenised at 30.000 rpm at 5 min to form a β-carotene/linoleic acid emulsion. Samples (0.05 mL) with different concentrations was added to 4 ml of the resulting emulsion. A solution with 0.05 mL methanol and 4 ml of the emulsion was used as control. Absorbance readings at 470 nm using a spectrophotometer (Shimadzu, Kyoto, Japan) were carried out at 40 min intervals during 120 min, keeping the cuvettes in a water bath at 50°C. The antioxidant activity (%) of the sample was calculated using the

following formula:

$$\%AA = 100\ (DRControl-DRSample)/DRControl$$

Whereby:
DR = degradation rate, $ln(a/b)/t$; a = absorbance$_{470\ nm}$ of the sample before incubation; b = absorbance$_{470\ nm}$ of the sample after incubation at t time; t: incubation time, 40, 80 and 120 min;
$DR_{control}$ = degradation rate of the control sample;
DR_{sample} = degradation rate of the tested sample.

2.2.2.2. ABTS assay

The ABTS test was performed according to Re et al. [26]. ABTS radical cation was prepared by mixing 7 mM ABTS stock solution with 4.90 mM potassium persulfate. The mixture was kept in the dark for 24 h at room temperature until the reaction was completed, and the absorbance was stabled. ABTS solution was mixed with ethanol to obtain an absorbance of 0.700 units at 734 nm using the spectrophotometer. Phenolic solutions with different concentrations (10 µL) were placed to react with 1 mL of ABTS solution in the dark for 6 min. The absorbance was recorded at 734 nm using the spectrophotometer (Shimadzu, Kyoto, Japan). The control sample was prepared with absolute ethanol (10 µL). The % inhibition was calculated as below:

$$\%\ Inhibition = [(A\ control - A\ sample) \times 100] / A\ control$$

A control and A sample: Absorbance at 734 nm for control and sample

2.2.3. Determination of sunflower oil stability

2.2.3.1. Stripping of RSO and preparation of experimental samples

The RSO was purified using the method described by Karabulut et al. [27] using activated carbon and alumina column chromatography treatments. In the Schaal oven test, 8 RSO and 8 SSO experimental designs were studied.

(a) Refined sunflower oil (RSO)
(b) RSO supplemented with 100 ppm of BHT
(c) RSO supplemented with 250 ppm of thymol (T)
(d) RSO supplemented with 500 ppm of thymol (T)
(e) RSO supplemented with 250 ppm of carvacrol (C)
(f) RSO supplemented with 500 ppm of carvacrol (C)
(g) RSO supplemented with 250 ppm of thymoquinone (TQ)
(h) RSO supplemented with 500 ppm of thymoquinone (TQ)
(i) Stripped sunflower oil (SSO)
(j) SSO supplemented with 100 ppm of BHT
(k) SSO supplemented with 250 ppm of thymol (T)
(l) SSO supplemented with 500 ppm of thymol (T)
(m) SSO supplemented with 250 ppm of carvacrol (C)
(n) SSO supplemented with 500 ppm of carvacrol (C)
(o) SSO supplemented with 250 ppm of thymoquinone (TQ)
(p) SSO supplemented with 500 ppm of thymoquinone (TQ)

2.2.3.2. Rancimat test

The induction periods of RSO and SSO mixed with phenolic compounds, and commercial antioxidants were carried out with the Rancimat apparatus (Metrohm, Herisau, Switzerland). The oil sample (3 g) was placed in the Rancimat apparatus at 90°C in the airflow rate of 10 L h^{-1}.

2.2.3.3. Schaal oven test

Fifty grams of RSO were weighted in 50 mL glass bottles and kept 21 days at 60°C in an oven. Samples were examined at 3-day intervals by collecting them from the same bottles at specific periods. Besides, 10 g of SSO were tested under the same storage conditions for 7 days. Samples were examined at daily intervals by collecting samples from the same bottles at specific periods. The stability of the samples was tested by determining PV, AV, and K_{232}. Experiments were set up in two repetitions for each sample.

2.2.4. Statistical analysis

Oxidation experiments were carried out in two replicates. The results were given as mean ± standard deviation. The results were statistically evaluated using the Minitab 17 Statistical Software (v17.3.1) package program. The difference between the group means was determined according to the variance analysis technique (ANOVA) (p <0.05).

3. RESULTS AND DISCUSSION

3.1. COMPOSITION AND CHEMICAL CHARACTERISTICS OF RSO AND SSO

Table I summarises the FFA content, PV, K_{232}, K_{268} p-AV, total tocopherols, induction period, and fatty acid composition of sunflower oil. The initial value for FFA, PV, K_{232}, K_{268}, p-AV, and total tocopherols was found to 0.10%, 4.90 meq O_2/kg, 3.28, 1.20, 11.00 and 656.5 mg/kg, respectively. These values agree with those in literature [28-31]. The PV, K_{232}, K_{268}, and p-AV of SSO decreased to 1.60 meq O_2/kg, 1.43, 0.54, and 0.08, respectively. Besides, total tocopherols were absent in SSO. The induction period of RSO (22.78 h) was higher than that of SSO (4.66 h). Linoleic acid was the predominant fatty acid in RSO (59.4%), followed by oleic acid (30.86%). Palmitic acid was the main saturated fatty acid in RSO (6.23%). The fatty acid composition reported here agrees with those reported in literature [30].

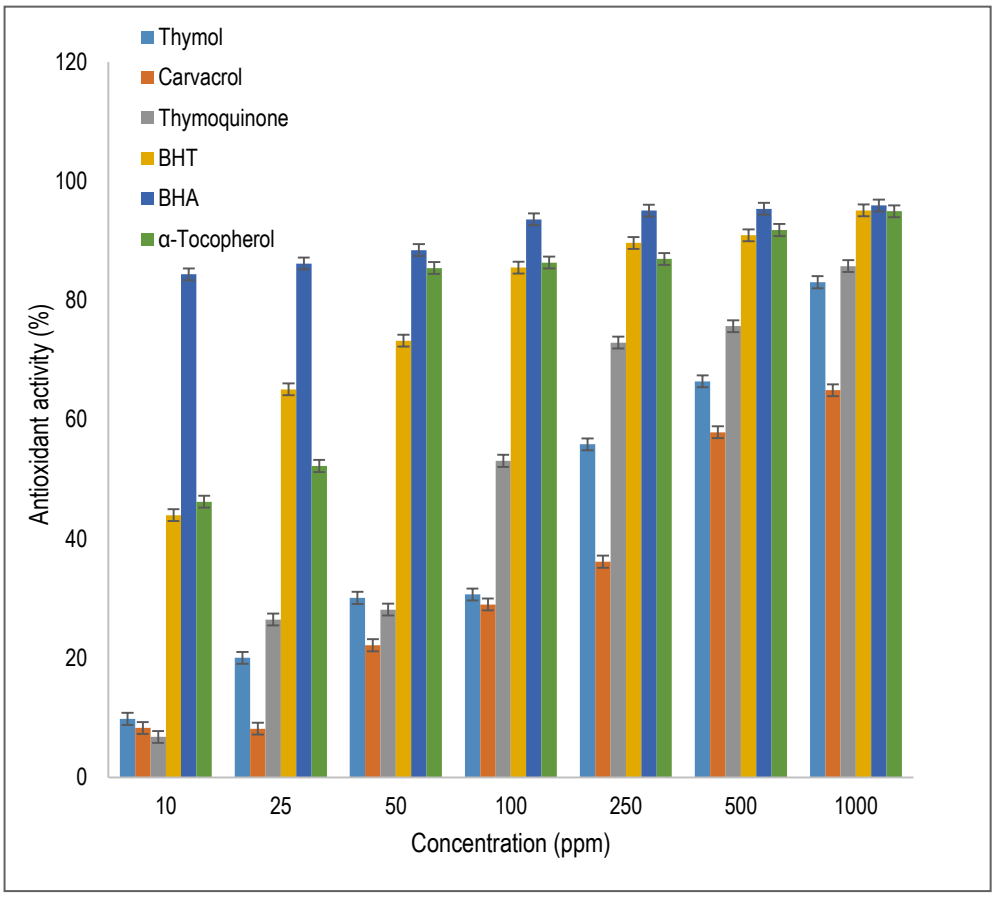

Figure 1 - Antixoidant activity (%) of phenolic compounds, α-tocopherol, BHA, and BHT in β-carotene system. Values reported are the mean of replicates. Error bars show the variations of three determinations in terms of standard deviation.

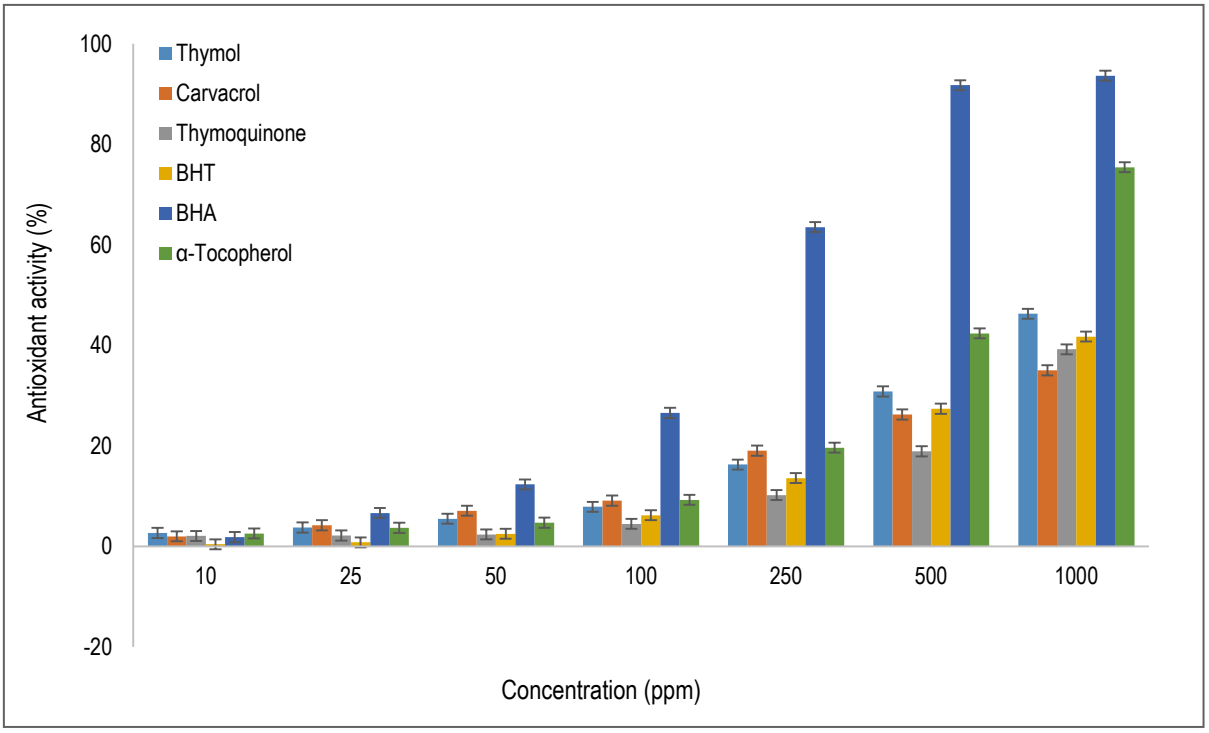

Figure 2 - Antoxidant activity (%) of phenolic compounds, α-tocopherol, BHA, BHT BHT in ABTS test. Values reported are the mean of replicates. Error bars show the variations of three determinations in terms of standard deviation.

3.2. ANTIOXIDANT ACTIVITIES OF THYMOL, CARVACROL, TQ AND COMMERCIAL ANTIOXIDANTS

Different methods based on different mechanisms should be tested to screen the antioxidant potential of phytochemicals or plant extracts since different methods could yield different results [1, 32, 33].

3.2.1. β-Carotene bleaching test

Figure 1 presents the antioxidant activities of the phenolic compounds, BHT, BHA, and α-tocopherol estimated by β-carotene bleaching assay. The antioxidant activity of all samples increased with increasing concentration. Above 500 ppm, BHA, BHT, and α-tocopherol showed more powerful antiradical activity (above 90%). However, the antioxidant activities of phenolic compounds were lower than those of synthetic antioxidants. Among the phenolic compounds, carvacrol had lower antioxidant capacity at 1000 ppm concentration, while the antioxidant activity of thymol and TQ was similar.

3.2.2. ABTS test

In the ABTS assay, all tested samples showed an increase with an increasing concentration (Figure 2). Among synthetic antioxidants, BHA possessed high antioxidant capacity (93.7% of ABTS inhibition), followed by α-tocopherol (75.4%) at 1000 ppm concentration. Koksal et al. [34] found similar results for synthetic antioxidants and demonstrated that BHA's radical scavenging activity was stronger than BHT and α-tocopherol. Regarding phenolic compounds, the antioxidant activity was lower than synthetic antioxidants, while thymol and TQ showed similar values with BHT. IC_{50} values were also calculated and exhibited in Figure 3. The antiradical activity of phenolics and synthetic antioxidants according to IC_{50} values increased in the following order: BHA>α-tocopherol>thymol>BHT>thymoquinone>carvacrol.

3.3. EFFICIENCY OF PHENOLICS AND COMMERCIAL ANTIOXIDANTS ON THE STABILITY OF RSO AND SSO

The stability of oil and fats is usually tested under heat conditions. To test the antioxidant effects of phenolics and commercial antioxidants in RSO and SSO, several tests were used to monitor the oxidation [22, 35, 36].

3.3.1. Induction periods of SSO

The oxidative stability index values of SSO samples enriched with phenolics and synthetic antioxidants are presented in Table II. Regarding commercial antioxidants, BHT enriched SSO showed a higher induction period (15.61 h) than other samples. Phenolic compounds showed lower induction periods for SSO compared with BHT. The antioxidant potential of phenolic compounds increased significantly ($p<0.05$) by increasing concentration. Slight differences in induction period values were observed between samples enriched with 250 ppm and 500 ppm concentration of phenolic compounds.

3.3.2. Schaal oven test for RSO

Table III presents the PV of RSO samples containing phenolics and BHT during storage at 60°C. At the end

Table I - Chemical parameters of RSO and SSO

Chemical parameter	RSO	SSO
Free fatty acid (oleic acid, %)	0.10 ± 0.00*	0.10 ± 0.00
Peroxide value (meq O_2 /kg)	4.90 ± 1.40	1.60 ± 0.60
K_{232}	3.28 ± 0.22	1.43 ± 0.19
K_{268}	1.20 ± 0.09	0.54 ± 0.06
p-Anisidine value	11.00 ± 0.11	0.08 ± 0.09
Total tocopherols (mg/kg)	656.50±1.80	ND**
Induction period (h)	22.78 ± 0.00	4.66 ± 0.16
Fatty acid (%)		
C14:0	0.07±0.00	
C16:0	6.23±0.00	-
C16:1	0.11±0.00	-
C18:0	3.25±0.02	-
C18:1	30.86±0.04	-
C18:2	59.4±0.01	-
C18:3	0.08±0.01	-

*Mean±standard deviation ** ND: not detected.

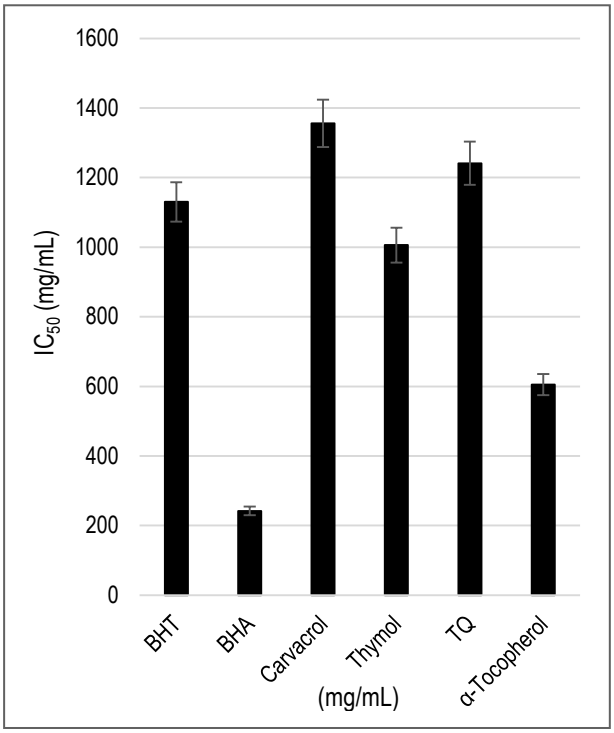

Figure 3 - IC_{50} values of phenolic compounds, α-tocopherol, BHA, BHT in ABTS assay.

of the storage test (21 days), the control sample oxidised rapidly and had the highest PV (196.1 meq O_2/kg oil). Carvacrol had a prooxidant effect on RSO and resulted in a marked increase in their PV during storage. The addition of thymol at 500 ppm concentration and TQ (250 ppm and 500 ppm) to RSO leads to lower PV induction than the control sample, thus enhancing the oxidative stability of RSO. Besides, only one sample (TQ at 250 ppm) showed a stronger antioxidant activity similar to BHT at the end of the storage period. The similar trend exhibited in RSO blended with black cumin oil rich in TQ [21].

Table IV presents the impacts of natural and synthetic antioxidants on the formation of conjugated dienes (CD) in RSO. At the end of storage, K_{232} value of RSO increased from 3.54 to 44.37. BHT, thymol (250 and 500 ppm), TQ (250 ppm and 500 ppm) were equivalent or slightly better in preventing the formation of CD compared with the control sample at the end of storage. After 21 days of storage, only carvacrol addition reduced the formation of CD in RSO.

During 21 days of storage at 60°C, BHT had lower *p*-AV than that of the control (Table V). The *p*-AV in the control sample reached the maximum value of 22.2 after 21 days of storage from an initial value of 12.3. At the end of the storage experiment (21 days), BHT and TQ had lower *p*-AV than control and oils enriched with thymol and carvacrol.

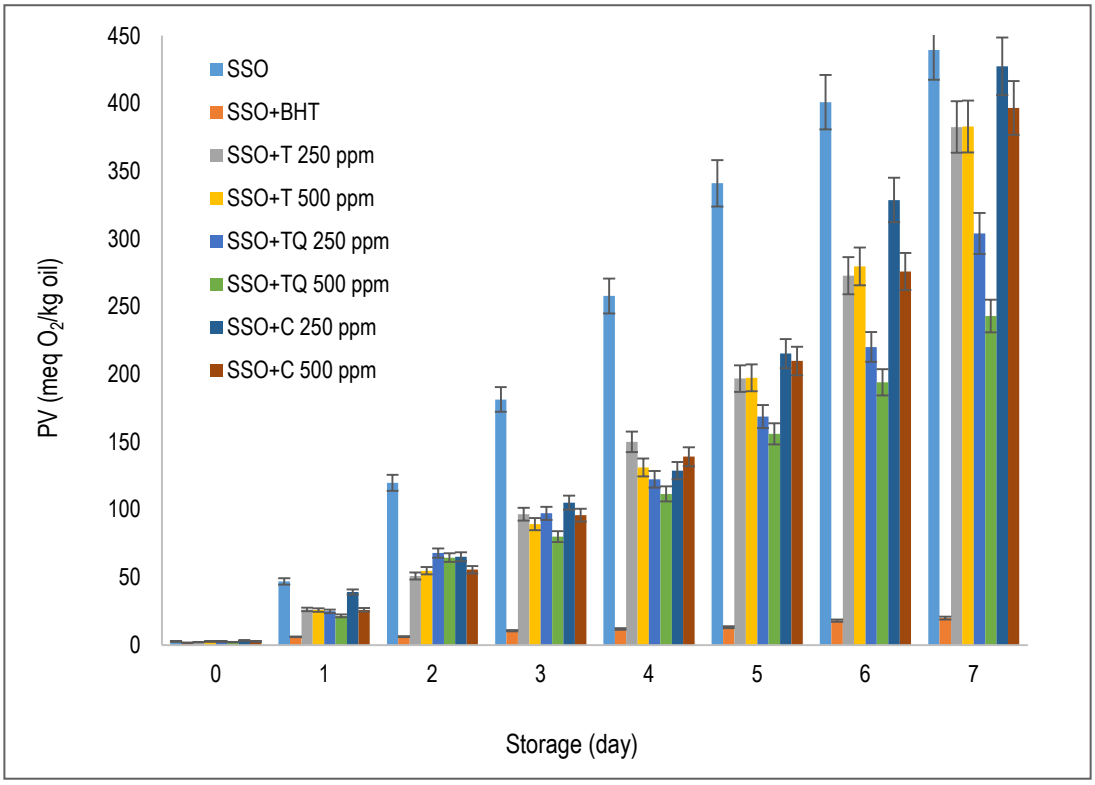

Figure 4 - Effect of phenolic compounds and BHT on the PV (meq O_2/kg oil) of SSO during storage at 60°C. Values reported are the mean of replicates. Error bars show the variations of three determinations in terms of standard deviation.

Table II - Induction periods of SSO with phenolic compounds and synthetic antioxidants

Oil	Induction period (h)	Protection factor
SSO	4.66 ± 0.16d*	1.0
SSO+BHT (100 ppm)	15.61 ± 0.29a	3.4
SSO+T (250 ppm)	6.46 ± 0.00b	1.4
SSO + T (500 ppm)	6.67 ± 0.11b	1.4
SSO + TQ (250 ppm)	5.78 ± 0.16c	1.2
SSO + TQ (500 ppm)	6.79 ± 0.16b	1.5
SSO + C (250 ppm)	5.67 ± 0.17c	1.2
SSO + C (500 ppm)	6.77 ± 0.03b	1.5

*Mean±standard deviation of three determinations. SSO: Stripped sunflower oil, T: Thymol, C: Carvacrol, TQ: Thymoquinone. Different letters for IP mean significant differences between samples ($P < 0.05$).

Table III - Effect of phenolic compounds and BHT on PV (meq O_2/kg oil) of RSO during storage at 60°C

Storage (day)	RSO	RSO + BHT (100 ppm)	RSO + T (250 ppm)	RSO + T (500 ppm)	RSO + TQ (250 ppm)	RSO + TQ (500 ppm)	RSO + C (250 ppm)	RSO + C (500 ppm)
0	5.4±0.7hBC*	3.0±0.0gD	4.0±0.0gCD	4.5±0.7hCD	6.9±0.1fB	10.0±0.0fA	4.5±0.7gCD	3.9±0.0hCD
3	26.0±0.0gABC	22.7±0.0fE	26.9±0.1fAB	27.3±0.9gA	23.8±0.0fDE	25.1±0.3fCD	26.8±0.1fAB	25.7±0.2gBC
6	56.0±0.7fA	47.2±0.8eB	54.7±2.3eA	51.7±2.7fAB	51.3±0.6eAB	51.4±0.8eAB	55.6±0.2eA	54.0±0.4fA
9	82.7±0.2eAB	70.5±0.9dC	79.8±1.5dAB	77.6±0.0eB	80.2±0.4dAB	82.9±3.9dAB	85.7±0.4dA	85.8±0.0eA
12	117.4±2.8dA	102.9±0.5cE	111.4±2.6cBC	103.3±0.4dDE	108.0±0.1cCDE	109.0±0.3cBCD	114.4±1.4cAB	113.4±0.7dABC
15	154.3±7.3cA	157.5±8.6bA	143.2±2.8bA	145.7±0.3cA	142.5±4.2bA	149.0±6.3bA	153.9±7.1bA	152.8±5.6cA
18	171.6±4.6bA	161.9±7.0abA	180.3±10.0aA	169.1±0.6bA	167.9±5.8aA	165.5±5.6bA	167.7±9.8bA	167.5±2.9bA
21	196.1±2.1aABC	173.8±3.1aCD	194.0±4.8aABC	184.0±1.3aBCD	164.9±9.8aD	186.0±8.6aBCD	216.5±0.5aA	206.6±7.7aAB

*Mean±standard deviation of two determinations. BHT: butylated hydroxy toluene, RSO: Refined sunflower oil, T: Thymol, C: Carvacrol, TQ: Thymoquinone.
[a-h]The values having different superscripts in the same column are significantly different at $p<0.05$
[A-E]The values having different superscripts in the same row are significantly different at $p<0.05$
Small letters: show the difference in storage days ($p<0.05$). Capital letters: show the difference in oils ($p<0.05$).

Table IV - Effect of phenolic compounds and BHT on K_{232} of RSO during storage at 60°C

Storage (day)	RSO	RSO + BHT (100 ppm)	RSO + T (250 ppm)	RSO + T (500 ppm)	RSO + TQ (250 ppm)	RSO + TQ (500 ppm)	RSO + C (250 ppm)	RSO + C (500 ppm)
0	3.54±0.25gA*	3.47±0.09fA	3.57±0.09eA	3.44±0.01fA	3.51±0.09dA	3.59±0.11fA	3.47±0.14fA	3.37±0.21dA
3	6.11±0.00fgA	5.39±0.01efC	6.03±0.02eAB	6.00±0.01efAB	5.61±0.03dBC	5.17±0.24fC	6.25±0.05efA	5.99±0.21dAB
6	12.68±0.72efA	10.40±0.42deB	11.46±0.11dAB	11.72±0.62deAB	11.24±0.98cAB	10.33±0.47eB	12.00±0.04deAB	11.40±0.09cdAB
9	15.31±0.10deA	13.30±0.56cdB	14.67±0.50dAB	14.76±0.16cdAB	14.62±0.65cAB	14.21±0.67dAB	15.26±0.04cdA	14.54±0.15bcAB
12	20.50±0.60cdA	18.25±0.87cB	21.04±0.52cA	20.69±0.53bcA	19.68±0.17bAB	19.26±0.24cAB	19.70±0.75bcAB	19.65±0.29bAB
15	22.47±1.39cA	19.40±1.05 cA	21.91±1.78cA	22.43±4.44bA	21.36±2.39bA	21.15±2.52cA	23.26±3.77bA	20.47±0.87bA
18	36.01±0.30bAB	30.89±4.09bB	33.64±1.22bAB	37.71±1.26aA	35.99±1.16aAB	35.50±0.31bAB	36.50±0.74aAB	37.01±0.21aAB
21	44.37±4.52aA	39.32±1.76aA	39.51±0.5aA	40.30±0.13aA	39.96±0.01aA	44.49±0.24aA	36.21±2.65aA	36.53±5.69aA

*Mean±standard deviation of two determinations. BHT: butylated hydroxy toluene, RSO: Refined sunflower oil, T: Thymol, C: Carvacrol, TQ: Thymoquinone.
[a-g]The values having different superscripts in the same column are significantly different at $p<0.05$
[A-C]The values having different superscripts in the same row are significantly different at $p<0.05$
Small letters: show the difference in storage days ($p<0.05$). Capital letters: show the difference in oils ($p<0.05$).

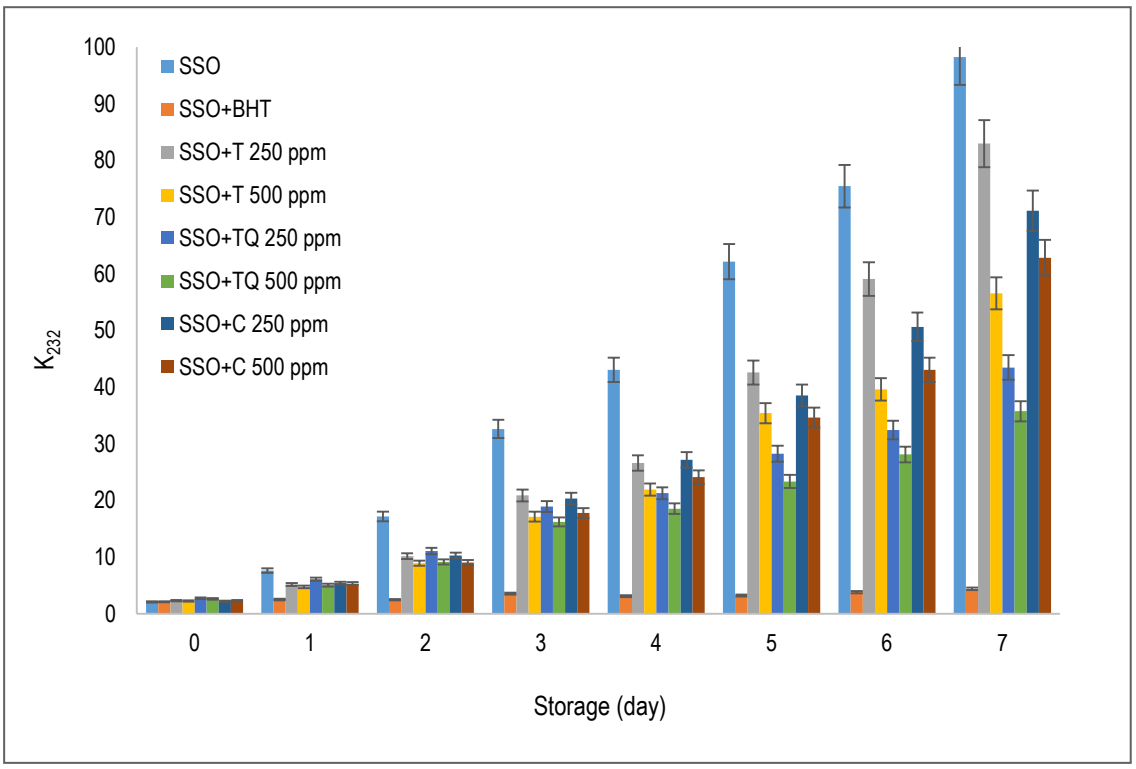

Figure 5 - Effect of phenolic compounds and BHT on K_{232} of SSO during storage at 60°C. Values reported are the mean of replicates. Error bars show the variations of three determinations in terms of standard deviation.

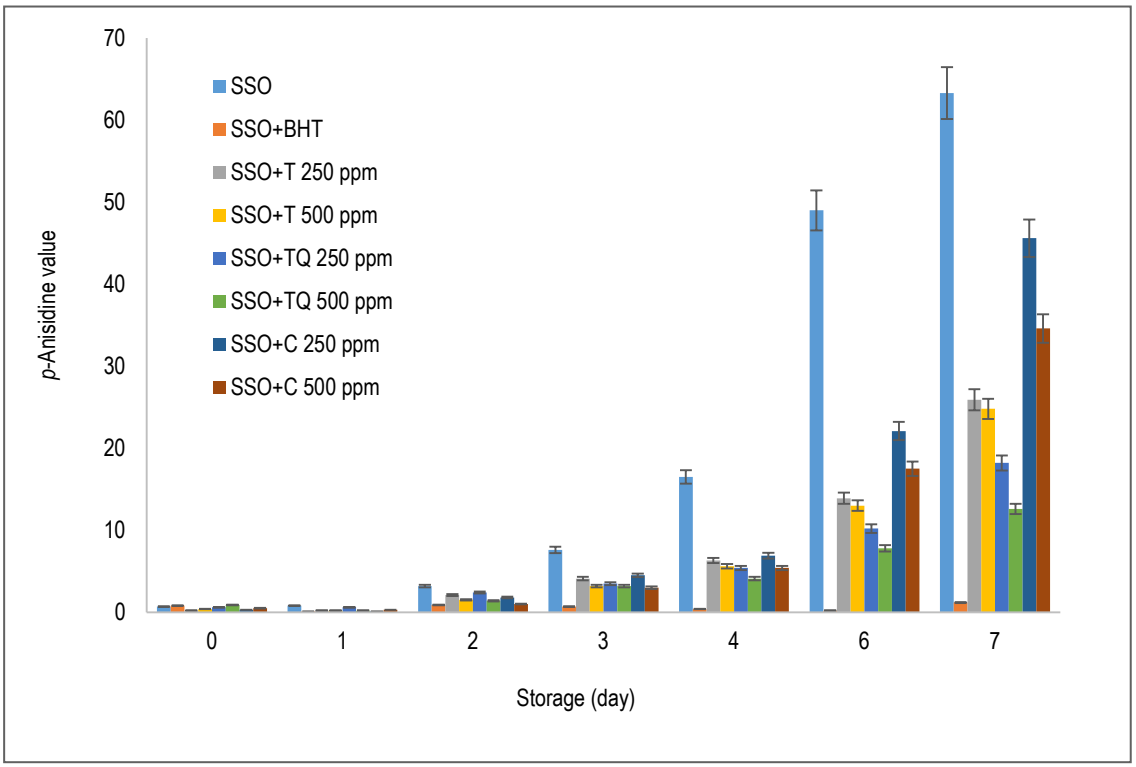

Figure 6 - Effect of phenolic compounds and BHT on the *p*-AV of SSO during storage at 60°C. Values reported are the mean of replicates. Error bars show the variations of three determinations in terms of standard deviation.

Table V - Effect of phenolic compounds and BHT on the *p*-AV of RSO during storage at 60°C

Storage (day)	RSO	RSO + BHT (100 ppm)	RSO + T (250 ppm)	RSO + T (500 ppm)	RSO + TQ (250 ppm)	RSO + TQ (500 ppm)	RSO + C (250 ppm)	RSO + C (500 ppm)
0	12.3±0.1bcA*	10.1±1.7abcA	12.2±1.0bcA	12.5±0.5cA	12.6±1.2bA	14.4±1.0aA	11.6±2.0cA	11.2±1.5bcA
3	8.8±0.4cdA	7.6±0.6bcA	8.4±1.5cA	9.3±0.4eA	7.9±0.7cA	9.8±0.4bA	8.9±0.5cA	8.0±0.8bcA
6	9.7±0.4cdB	10.5±0.3abcAB	11.0±0.2cAB	10.7±0.5deAB	11.0±0.2bAB	11.1±0.5bAB	11.5±0.4cAB	12.0±1.2bA
9	10.0±1.0cdA	11.0±0.3abcA	11.1±0.1cA	11.4±0.4cdA	11.4±0.6bA	10.8±0.1bA	10.9±1.8cA	10.2±1.1bcA
12	6.6±2.4dA	6.2±0.3cA	8.4±0.2cA	4.8±0.0fA	4.7±0.2dA	5.3±0.0cA	7.7±1.8cA	5.8±0.3cA
15	11.4±2.0cA	10.3±0.4abcA	12.7±1.5bcA	12.3±0.2cA	11.9±0.4bA	11.5±1.0bA	13.1±1.3bcA	12.0±0.4bA
18	16.6±0.7bA	15.1±5.3abA	16.8±2.4abA	15.5±0.4bA	15.7±0.1aA	16.1±1.3aA	17.6±1.7abA	21.1±3.6aA
21	22.2±0.5aA	15.6±0.5aDE	17.9±0.3aCD	18.8±0.5aBC	16.1±0.4aDE	15.3±0.2aE	20.7±1.3aAB	19.2±0.7aBC

*Mean±standard deviation of two determinations. BHT: butylated hydroxy toluene, RSO: Refined sunflower oil, T: Thymol, C: Carvacrol, TQ: Thymoquinone.
[a-e]The values having different superscripts in the same column are significantly different at $p<0.05$
[A-E]The values having different superscripts in the same row are significantly different at $p<0.05$
Small letters: show the difference in storage days ($p<0.05$). Capital letters: show the difference in oils ($p<0.05$).

Table VI - Effect of phenolic compounds and BHT on the total tocopherols (mg/kg) of RSO during storage at 60°C

Storage (day)	RSO	RSO + BHT (100 ppm)	RSO + T (250 ppm)	RSO + T (500 ppm)	RSO + TQ (250 ppm)	RSO + TQ (500 ppm)	RSO + C (250 ppm)	RSO + C (500 ppm)
0	656.5±1.8aA*	661.3±9.3aA	657.6±3.0aA	659.3±11.7aA	660.0±4.2aA	666.9±4.7aA	671.0±3.7aA	671.0±16.8aA
3	418.6±15.8bC	461.3±12.6bB	420.6±6.7bC	416.0±9.1bC	466.5±7.8bAB	501.4±1.5bA	442.4±1.1bBC	440.3±8.5bBC
6	270.7±8.1cC	314.0±9.0cAB	276.1±8.9cC	273.8±4.7cC	312.0±9.9cAB	322.0±10.3cA	282.7±10.7cBC	279.4±6.6cBC
9	164.6±5.9dC	209.8±0.9dA	166.4±10.0dC	174.2±7.1dBC	209.0±11.2dA	197.6±2.4dAB	174.9±4.7dBC	174.6±0.4dBC
12	101.9±2.1eB	138.0±0.0eAB	119.2±22.6deAB	118.1±0.2eAB	127.6±8.8eAB	143.5±8.3eA	113.8±3.8eAB	120.2±8.2eAB
15	74.0±8.5eAB	111.1±7.5eA	74.4±21.6efAB	73.4±1.4fAB	90.9±5.3eAB	104.5±5.2fA	78.3±2.4fAB	60.3±8.8fB
18	30.3±1.2fB	68.7±1.5fA	34.6±16.8fAB	37.8±11.3gAB	51.3±16.8fAB	55.6±8.6gAB	30.8±8.1gAB	27.1±13.6fgAB
21	29.6±1.7fAB	33.4±3.4gA	20.1±10.4fABC	16.5±1.8hABC	25.9±1.6fAB	21.3±1.9hABC	13.4±4.3gBC	8.0±0.0gC

*Mean±standard deviation of two determinations. BHT: butylated hydroxy toluene, RSO: Refined sunflower oil, T: Thymol, C: Carvacrol, TQ: Thymoquinone.
[a-g]The values having different superscripts in the same column are significantly different at $p<0.05$
[A-C]The values having different superscripts in the same row are significantly different at $p<0.05$
Small letters: show the difference in storage days ($p<0.05$). Capital letters: show the difference in oils ($p<0.05$).

The change in total tocopherols in RSO during storage is given in Table VI. The total tocopherol content decreased to 29.6 mg/kg from the initial value of 656.5 mg/kg after 21 days of storage. The RSO enriched with BHT had higher total tocopherols among all analysed oils after 21 days of storage. At the end of the storage experiment, the remaining tocopherols of enriched oils with phenolic compounds were lower than that of the control sample and also BHT-enriched RSO sample. The dramatic decrease in total tocopherols was observed in RSO samples enriched with carvacrol at 250 and 500 ppm concentration.

3.3.3. Schaal oven test for SSO

The PV of SSO and enriched SSO stored at 60°C are presented in Figure 4. At the end of 7 days of storage, PV in the control sample increased to 439.5 meq O_2/kg from the initial value of 2.7 meq O_2/kg. During storage, the PV of SSO enriched 100 ppm BHT was the lowest and reached 19.9 meq O_2/kg at the end of the storage experiment. However, the increase in PV of SSO samples enriched with phenolic compounds was higher than those of BHT-enriched SSO. Besides, as compared with the control sample, TQ enriched oil had a lower PV among samples treated with phenolic compounds.

Regarding thymol and carvacrol enriched SSO, PV was slightly lower than the control sample. Similar results were noted in the report of Yanishlieva et al. [20]. They demonstrated that thymol is a more active antioxidant than carvacrol on oxidation in purified triacylglycerols of sunflower oil during storage at ambient conditions.

The formation of CD in SSO samples during the 7-day oxidation test is summarised in Figure 5. The CD value of control reached 98.23 from the initial value of 2.06 after 7 days of storage. Absorptivity at 232 nm in SSO and SSO enriched with phenolic compounds, due to the induction of primary oxides, showed a pattern like that of PV. BHT showed the highest inhibition activity on lowering the formation of CD in SSO during storage. However, the effect of phenolic compounds was lower on CD formation of SSO compared with BHT. It was also noted that TQ exhibited better preventive effects on diene formation among phenolics and also when compared to the control sample.

Figure 6 shows the changes in *p*-AV of SSO samples during storage. The *p*-anisidine value of the control sample reached 63.3 at the end of storage from an initial value of 0.7. *p*-AV results showed similarity with the results of PV and K_{232}. Among the SSO samples analysed, the lowest *p*-AV was recorded for BHT-enriched SSO with a mean of 1.2 at the end of storage. However, phenolic compounds exhibited lower inhibition on aldehyde formation during storage compared with BHT-0enriched SSO. Among phenolic compounds, the highest inhibition activity was exhibited by TQ.

4. CONCLUSION

The results of the current study revealed that commercial antioxidants had higher radical scavenging activity than phenolic compounds. BHT at 100 ppm was more active on the oxidative thermal stability of RSO during storage at 60°C compared to phenolic compounds at 250 and 500 ppm. Besides, BHT showed a similar pattern in the oxidation of SSO at 60°C. Moreover, BHT showed a more stable behaviour in the oxidation of SSO than in the oxidation of RSO. This could be related to tocopherols, carotenoids in RSO. Among phenolic compounds, TQ at 250 and 500 ppm inhibited sunflower oil oxidation during the accelerated thermal storage.

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Compliance with ethical standards

Conflict of interest: none.

Compliance with ethics requirements: this article does not contain any studies using human or animal beings.

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