

EFFECT OF NATURAL α -TOCOPHEROL, β -CAROTENE AND THEIR MIXTURES ON ACCELERATED OXIDATIVE STABILITY OF STRIPPED SUNFLOWER OIL

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ABSTRACT

The natural antioxidant potencies of alpha-tocopherol (α -toc) and beta-carotene (β -car) (each in 100 and 200 ppm) and their binary admixtures were studied. The autoxidation of stripped sunflower oil for 2, 4, 6, 8, 10, 12 and 14 days at 40, 60 and 100°C were evaluated by measuring peroxide value (PV), para-anisidine values (p-AV) and total oxidation (2PV+p-AV). It was found that α -toc (200 ppm) is more effective than its lower concentration (100 ppm), while β -car (100 ppm) is more effective than the higher level. The binary mixture of α -toc: β -car (200 ppm: 100 ppm) proves its performance when added to the stripped sunflower oil. Graphical representations of the results show changes of PV and p-AV during periodical heating for different periods. It was concluded that antioxidant activity of α -toc diminished when the tocopherol level exceeds its optimal concentration. It is noteworthy to mention that individual β -car acts as a prooxidant when used at a concentration equal to that of α -tocopherol, because β -car may react with oxygen to form β -carotene peroxy radicals, these peroxy radicals may enhance the propagation stage of the oxidation reaction and therefore the antioxidant capacity or "sparing efficiency" of the tocopherol decreases.

Keywords: Natural antioxidant, accelerated oxidative stability, α -tocopherol, β -carotene, sunflower oil.

INTRODUCTION

Antioxidants can minimize or prevent lipid oxidation in food products (Shahidi and Wanasundara, 1992). Consequently; the food industry is continuously exploring for natural compounds for food preservation. Carotenoids and tocopherols have antioxidant properties, providing vitamin activity, are endogenously present in plant and animal tissues and are desirable ingredients for many food products.

Synthetic antioxidants such as tert-butylhydroxytoluene (TBHT); tert-butylhydroxyanisole (TBHA), and tert-butylhydroquinone (TBHQ) have been widely used to retard lipid oxidation in foods (Ahmad, 1996). However, the synthetic antioxidants are not preferred due to toxicological concerns. Now days, attention is drawn towards using natural antioxidants such as tocopherol and β -carotene to reduce and retard lipid oxidation in foods (Ahn *et al.*, 1998). The effectiveness of different antioxidants is highly dependent on the tested system (bulk oil); the concentration, the oxidation time, and the method used to determine lipid oxidation (Huang *et al.*, 1994a).

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Information about the thermal stability of natural antioxidants and their combinations are very important, mainly because these products are designed to protect fats and oils at ambient or relatively low temperatures. Recently, it has been found that antioxidants may have different effects on the formation and the decomposition of hydroperoxides (Huang *et al.*, 1994b, Satue *et al.*, 1995). It is known that, tocopherols are phenolic antioxidants that occur naturally in vegetable oils and function to maintain oil quality by terminating free radicals. Of their advantageous, the hydrogen atom donating capacity of α -tocopherol is higher than that of γ -tocopherol (Burton, and Ingold, 1981) and α -tocopherol can reduce the decomposition of the hydroperoxides of fatty acid molecule by donating hydrogen atoms to alkoxyl radicals (Hopia *et al.*, 1996 and Lampi *et al.*, 1999).

Carotenoids have drawn considerable attention for their role as natural colorants, provitamin A activity, and ability to prevent peroxidation processes. Many studies have focused on β -carotene's ability to retard potentially harmful oxidative reactions by trapping free radicals (Kennedy, and Liebler, 1991; Miller *et al.*, 1996 and Terao, 1989) and quenching singlet oxygen (Foote, and Denny, 1968; Oliveros *et al.*, 1992 and DiMascio *et al.*, 1990). Thus, they may exert their radical trapping function by acting cooperatively with tocopherols (Palozza, and Krinsky, 1991, 1992 and Gey, 1993).

The aim of this work was to study the effect of natural antioxidants, namely, α -tocopherol and β -carotene and their binary mixtures, on sparing efficiency of stripped oil for a new variety sunflower (Miak). Also, the study shows whether β -carotene was consumed rapidly during the accelerated oxidation conditions and whether β -carotene can act cooperatively with α -tocopherol and /or may exerts trapping function to delay the onset oxidation.

MATERIALS AND METHODS

1 Materials:

Seeds of new sunflower (*Helianthus annuus*) variety (Miak) were extracted with n-hexane (iodine value = 107.0) and the oil contains oleic and linoleic acids. Their average values were 42.0 and 47.0 % respectively as determined by GLC. Natural antioxidants, namely, α -tocopherol and β -carotene were obtained from Carotech, Malaysia.

2 Methods:

2.1 Purification of sunflower oil

The oil was freed from the tocopherols as well as any other antioxidant. This was done by running the oil miscella soluble in n-hexane through silica gel (60-120 mesh) column. The stripped oil thereafter was checked by thin layer chromatography (TLC) to ensure that the oil is clear from the antioxidants.

This oil was used as control as well as in the accelerated oxidation experiments.

2.2 Antioxidant preparations:

α -Tocopherol and β -carotene were dissolved separately in n-hexane and diluted as necessary to obtain concentrations of 100 and 200 ppm.

2.3 α -Tocopherol and β -Carotene in oil

α -Tocopherol or β -Carotene in hexane (100 or 200 ppm) was added directly to petri dish glass (14.5 cm diameter) containing 50 g of stripped sunflower oil thickness, 4 ml. The solvent was evaporated under a stream of nitrogen. A control sample was prepared with 50 g stripped sunflower and n-hexane, which was evaporated under N_2 . Triplicate petri dishes were prepared for the control and each mixture.

2.4 α -Tocopherols and β -Carotene-oil mixtures:

Each α -tocopherol and β -caroten, individually, dissolved in n-hexane (100 and 200 ppm) were mixed to obtain the following ratios of α -toc / β -car 1:1, 2:1 and 1:2. The α -toc and β -car mixtures in n-hexane were added directly to petri dish glass containing 50 g of stripped sunflower oil, thickness 4 ml. The solvent was evaporated under a stream of nitrogen. A blank sample was prepared with 50 g of stripped sunflower oil and hexane which was evaporated under nitrogen. A control sample was prepared with 50 g stripped sunflower oil and n-hexane, which was evaporated under N_2 . Triplicate petri dishes were prepared for the control and each mixture.

2.5 Evaluation of the antioxidants:

Accelerated heating (at 40, 60 and 100 °C) was periodically carried out for 2, 4, 6, 8, 10, 12 and 14 days to evaluate the effect of the antioxidants.

The control sample was placed in an electric oven with mixtures of sunflower oil and individual antioxidants and their combinations.

Each sample as well as the control was exposed to heat firstly at 40 °C for the different periods (2, 4, 6, 8, 10, 12 and 14 days) and the experiment was repeated at 60 °C and 100 °C.

Thus, at each degree of temperature, four groups of tested samples, namely, two samples of oil mixed with α -toc (100 and 200 ppm), two samples of oil mixed with β -car (100 and 200 ppm) and three samples of oil mixed with mixtures of the two antioxidants at weight ratios α -toc: β -car, 1: 1, 2: 1 and 1: 2 in addition to control, were heated separately for 2, 4, 6, 8, 10, 12 and 14 days.

Each oil sample was removed from the electric oven and kept immediately in a vacuum desiccator to cool down into room temperature, then kept in dark vials in the refrigerator till analysis.

2.6 Analysis of oxidation status:

Primary oxidation was measured by peroxide value method (PV) (A.O.C.S. 1996) Secondary products were measured by para-anisidine value (ρ -AV) according to Official and Tentative Methods of the AOCS (1996). The total oxidation was calculated as follows: total oxidation = 2PV + ρ -AV.

The percentage of antioxidant effectiveness (AE) was calculated according to the following equation: $C-S / C \times 100$ where C is PV for control and S is PV for sample (Adegoke, and Gopala, 1998).

Conjugated fatty acids (conjugated dienes and trienes) were measured using Shimadzu spectrophotometer UV-240. Absorbance at 234 and 268 nm were used for determining conjugated dienes and trienes, respectively according to Official and Tentative methods of the AOCS (1996).

Expression of the Results:

All the results are calculated as the mean values of more than two experimental replications (n=2 to 4) for each experimental analysis.

RESULTS AND DISCUSSION

It has been indicated that the primary initial products of the autoxidation of fatty acid ester are the hydroperoxides. Measurement of hydroperoxides is generally carried out by determination of peroxide value (PV). Whereas secondary degradation products (mainly carbonyl compounds) are results of hydroperoxides decomposition. These secondary oxidation products are expressed as para-anisidine value (p-AV).

1- Course of primary oxidation (PV) at different temperatures

When an antioxidant is incorporated into oil, the oxidation of the oil is suppressed and accordingly the oil is protected from deterioration. Stability plots of PV versus exposure times of each heating temperature were used (fig 1-3) to express the incidence of primary oxidation.

Using α -toc (100 and 200 ppm), at the different periods (total of 14 days) at constant temperature of 40 °C, should that 200 ppm of α -toc was more efficient in suppressing the primary oxidation of oil (fig 1-a).

When β -car was added in two levels to the stripped sunflower oil, primary oxidation increased by exposure time at 40 °C approaching the control curve of the oil. It can be seen that β -car at level of 100 ppm was less effective in resisting primary oxidation (fig 1-b). Thus, the different antioxidant potencies of α -toc and β -car can be arranged in the following order: 200 ppm α -toc > 100 ppm α -toc > 200 ppm β -car > 100 ppm β -car.

Using three binary mixtures of α -toc: β -car at ratios: 1: 1, 2: 1 and 1: 2, only 2 α -toc: 1 β -car showed to improve the stability of the oil better than the two other binary mixtures as shown in figure (1-c).

Moreover the binary antioxidant mixtures were evaluated and their potencies can be arranged in the following descending order α -toc: β -car: 2: 1 >, 1: 1, and 1: 2. Generally, good results were obtained when α -toc was added to the oil.

When α -toc in two concentrations was individually incorporated into the oil at different periods at 60 °C, the two tocopherol concentrations efficiently resisted the primary oxidation (fig 2). Thus, PV curve of control was emerged far away from both PV curves of the oil treated with α -toc antioxidant. However 200 ppm of α -toc was more potent to oxidation than 100 ppm. It was found that two, four and six days heating gave reasonable results with respect of the two concentrations of α -toc (fig 2-a).

On the contrary, the course of oxidation exhibited by β -car (100 and 200 ppm), PV curves approximates the control curve indicating that the presence of β -car, gave lower resistance against oxidation (fig 2-b).

Concerning the binary mixtures of α -toc and β -car at different ratios of, 1: 1, 2: 1 and 1: 2, PV curve (1 α -toc: 2 β -car) intersected with the PV curve of control at 12 days exposure time and at 60 °C. This indicates that the antioxidant combinations loose their potencies towards primary oxidation. Meanwhile, 2 α -toc: 1 β -car was markedly more potent than 1 α -toc: 1 β -car (fig 2-c).

In conclusion, individual α -toc in two concentrations, exhibited antioxidation potency better than β -car alone. In addition, the binary mixture at ratio of 2 α -toc: 1 β -car, gave better antioxidation capacity than the other two concentrations. Generally, at 60 °C the antioxidation potency of individual and binary mixtures can be arranged in descending order as follows: 200 ppm α -toc > 100 ppm α -toc > 2 α -toc: 1 β -car > 1 α -toc: 1 β -car > 100 ppm β -car > 200 ppm β -car.

The primary oxidation was accelerated periodically at 100 °C, thus, α -toc in two concentrations (100 and 200 ppm) gave reasonable antioxidation activity Fig (3-a). However the higher concentration had better antioxidation potency than lower concentration. It is reported that the tocopherol concentration is an important factor that influences tocopherol antioxidant activity in the bulk oils. Also, it was reported that the antioxidant activity was greater at lower concentrations and decreases or may become prooxidant at higher concentrations. For example, α -toc exhibits optimal antioxidant activity at concentrations between 100 and 200 ppm (Huang *et al.*, 1994a, 1994b and Satue *et al.*, 1995). Concerning the peroxide curves in case of using two β -car concentrations (100 and 200 ppm), the peroxides were formed at higher rate as observed from approaching the peroxide curve of the control. While, 100 ppm β -car was clearly better in retarding peroxide formation (fig 3-b).

On the other side, binary mixtures of α -toc and β -car, each in two concentrations were used in resisting peroxide formation. In case of incorporating 2 α -toc: 1 β -car, the primary oxidation was comparatively occurring but at lower rate than the other two concentrations. This means that the antioxidant efficiency of the other two binary mixtures were lower (fig 3-c). The antioxidation potency of α -toc: β -car can be arranged in the following descending order: 2: 1, 1: 1 and 1: 2.

The antioxidant effectiveness (AE) was expressed using the following equation: $C-S / C \times 100$ as mentioned before (Adegoke, and Gopala, 1998). Therefore, applying this equation on a selected condition of 200 ppm α -toc at 60 and 100 °C, for two days exposure times showed percentage values of AE: 50.0 and 55.5 respectively, while in case of 100 ppm they were 41.6 and 44.4 % respectively indicating that the antioxidant at higher level was more potent than at the lower level. Meanwhile, α -toc (200 ppm) in combination with 100 ppm of β -car (2 α -toc : 1 β -car) showed AE percentage values of 50.0 % at 60 °C and 55.5 % at 100 °C indicating that α -toc decrease the prooxidant effect of β -car. On the other hand individual β -car showed AE % of 25.0 and 33.5 % for 100 ppm at 60 °C and 100 °C, respectively, and at two days exposure time. Whereas in case of 200 ppm were 17.6 and 18.5 % under the same conditions. This indicates that individual β -car at 100 ppm was more potent in delaying the autoxidation than 200ppm and this behaves inversely to α -toc (100 and 200 ppm).

2 Total Conjugated Dienes and Trienes:

Conjugation is related to the hydroperoxides formation. The formation of conjugated hydroperoxide is accepted generally as the primary products of oil oxidation. Methyl oleate yields 4 isomeric conjugated hydroperoxides,

whereas methyl linoleate and methyl linolenate give rise 2 and 4 isomeric conjugated hydroperoxides respectively (Evans, 1961) Spectrophotometric evaluation at 234 and 268 nm using iso-octane solvent was carried out (Egan *et al.*, 1981). Conjugation was followed by mixing α -toc, or β -car or their binary mixtures with the oil at different exposure periods at 40, 60 and 100 °C as shown in Table 1 (a, b, c). It was noticed that conjugated components were concomitant to the primary oxidation process of fatty acids and different levels of conjugated dienes and trienes were obtained at different exposure times and at different temperatures. It is important to mention that conjugation is a result of lipid oxidation forming conjugated hydroperoxides, through propagation step, as a primary oxidation which is subjected, after that, to splitting into aldehydes i.e. secondary oxidation products (Kim *et al.*, 2007).

Table (1): Effect of different concentrations of α -toc, β -car and their mixtures on stripped sunflower oil on the formation of conjugated components.

Sample	(a) Days of heating at 40 °C						
	2	4	6	8	10	12	14
Control	2.6	4.7	7.0	8.6	9.5	10.5	11.6
100 ppm α -toc	1.1	2.5	4.3	5.5	6.3	7.0	8.0
200 ppm α -toc	0.8	1.8	3.0	4.0	4.9	5.8	6.7
100 ppm β -car	2.0	3.5	5.9	6.8	7.5	8.0	10.0
200 ppm β -car	1.2	2.7	5.0	6.2	6.7	7.5	8.4
1 α -toc : 1 β -car	1.0	2.0	4.5	5.5	6.5	7.0	8.1
2 α -toc : 1 β -car	1.5	3.3	5.5	6.5	7.5	8.5	9.2
1 α -toc : 2 β -car	2.0	3.9	6.0	7.1	8.2	9.0	10.0
Sample	(b) Days of heating at 60 °C						
	2	4	6	8	10	12	14
Control	3.0	4.0	5.1	6.8	9.5	12.4	17.7
100 ppm α -toc	1.2	2.0	3.0	4.5	6.5	8.5	12.0
200 ppm α -toc	1.0	1.5	2.5	4.0	5.5	7.0	9.0
100 ppm β -car	1.5	2.0	2.9	5.0	7.0	10.0	12.0
200 ppm β -car	2.0	3.0	4.0	6.5	8.0	9.0	13.0
1 α -toc : 1 β -car	2.5	3.0	4.0	5.5	8.0	10.5	14.0
2 α -toc : 1 β -car	2.0	2.5	3.5	5.0	7.0	9.5	13.0
1 α -toc : 2 β -car	2.8	3.7	4.8	6.5	9.0	12.0	16.0
Sample	(c) Days of heating at 100 °C						
	2	4	6	8	10	12	14
Control	10.6	12.7	16.1	18.8	22.5	27.5	32.5
100 ppm α -toc	9.0	10.0	12.5	16.0	20.0	25.0	30.0
200 ppm α -toc	7.5	8.0	11.5	15.0	19.0	22.5	28.5
100 ppm β -car	8.7	11.5	15.0	17.5	21.5	27.0	30.0
200 ppm β -car	9.8	12.0	16.0	18.0	22.0	26.5	31.5
1 α -toc : 1- β -car	8.1	11.2	13.7	16.2	18.7	22.5	26.5
2 α -toc : 1- β -car	7.5	10.0	12.5	15.0	17.5	20.0	25.0
1 α -toc : 2- β -car	10.0	11.8	15.0	17.5	21.3	25.0	30.0

3 Course of secondary oxidation (p-AV) at different temperatures:

The major secondary oxidation products of heated oil are the aldehydes, principally 2-4-dienals and 2-alkenals, which are products of hydroperoxide splitting reaction (Kim *et al.*, 2007).

At 40 °C, the p-AV of the oil increased from 2.5 (at 2 days) to 9.5 (at 14 days) in case of 100 ppm α -toc and from 3.5 to 10.0 when using 200 ppm α -toc. In general α -toc in two concentrations was more resistant in the secondary oxidation reaction at 40 °C (fig 4-a).

Concerning the use of β -car as antioxidant, it could be noticed that levels of 100 and 200 ppm of β -car, cause an increase in p-AV from 3.0 at 2 days to 9.5 at 14 days and from 3.5 (at 2 days) to 10.0 (at 14 days) in case of 100 and 200 ppm respectively.

It is clear from the heating curves (fig 4-b) that 100 ppm β -car gave better results than that of 200 ppm.

In case of the three binary mixtures, it was noticed that 2 α -toc: 1 β -car showed lower secondary oxidation products than the other two mixtures (fig 4-c).

At 60 °C, adding 100 and 200ppm of α -toc individually to the stripped sunflower –oil, at level of 100 ppm was better with respect to the lower values of p-AV, Fig (5-a).

Using β -car at two different levels, 100 and 200 ppm, higher values of p-AV were observed approaching the values of control Fig (5-b).

Dealing with the three binary mixtures of antioxidants, namely, 1 α -toc: 1 β -car, 2 α -toc: 1 β -car and 1 α -toc: 2 β -car, it was observed that 2 α -toc: 1 β -car was better at 2, 4 and 6 days in minimizing the p-AV, whereas 1 α -toc: 1 β -car was better at 12 and 14 days Fig (5-c).

At 100 °C, individual α -toc at two concentrations of 100 and 200 ppm were used to show their periodical effects in enhancing the formation of secondary oxidation products of unsaturated hydroperoxides.

It can be seen from Fig (6-a) that positive effects of the two concentrations of α -toc were observed indicating that secondary oxidation reaction were suppressed. It can be seen that 100 ppm α -toc was more effective in suppressing the secondary products

Meanwhile periodical heating at 100 °C with the added β -car in two concentrations (100 and 200 ppm) for different periods showed enhancement of the secondary oxidation reactions. Accordingly, β -car (100 and 200 ppm) had a negative effect when added to the stripped sunflower oil giving rise to high secondary oxidation product (fig 6-b).

Concerning the p-AV of the stripped sunflower oil when binary mixtures of α -toc and β -car were added at 100 °C, it can be seen that the mixture 2 α -toc: 1 β -car was more effective in inhibiting secondary oxidation reactions. In addition, it can be noticed that the curve of 1 α -toc: 1 β -car passed parallel with that of control, however it intersected the latter curve at 8 days after which it passed parallel over the control curve (fig. 6-c).

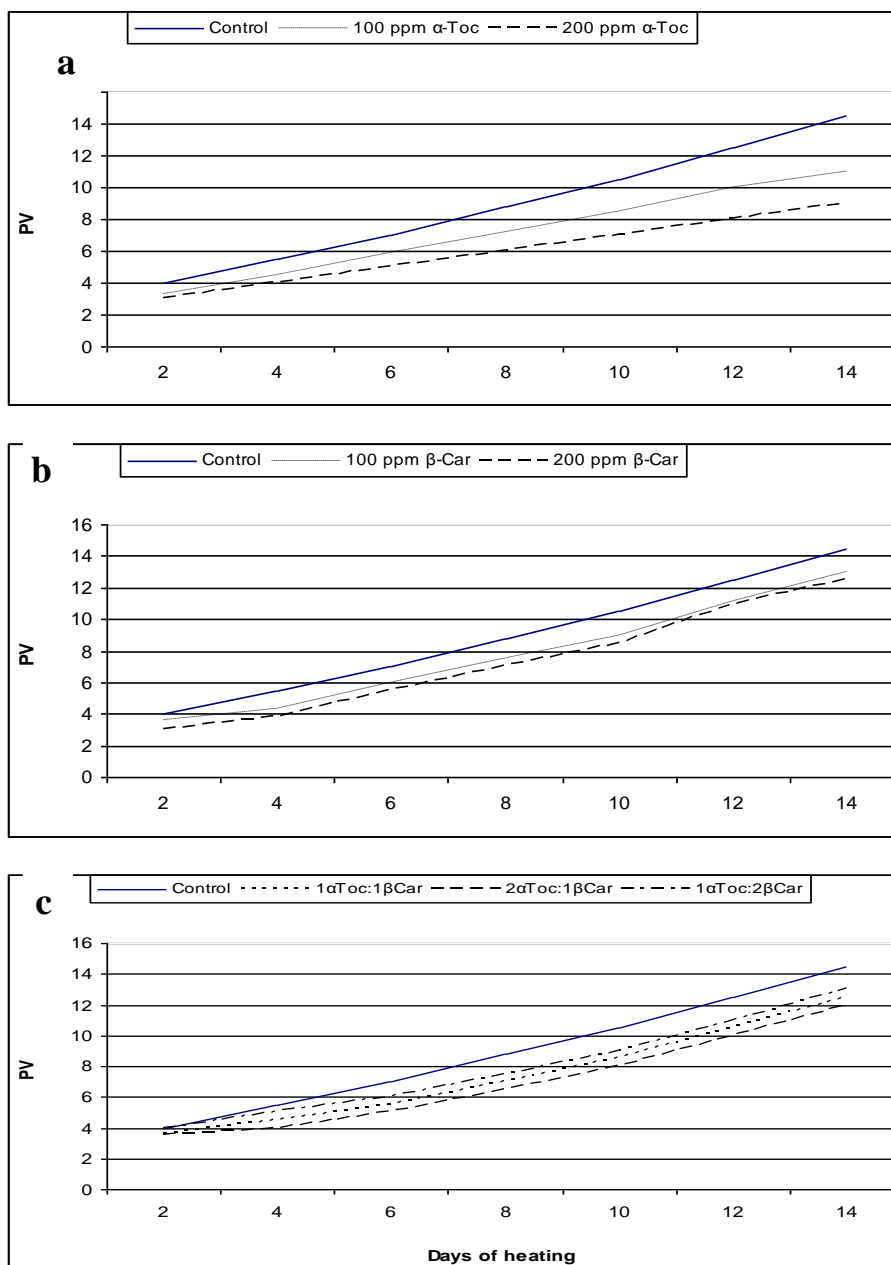


Figure (1): Effect of different concentrations of α -toc and β -car and their mixtures on stripped sunflower oil on hydroperoxides formation at 40 °C.

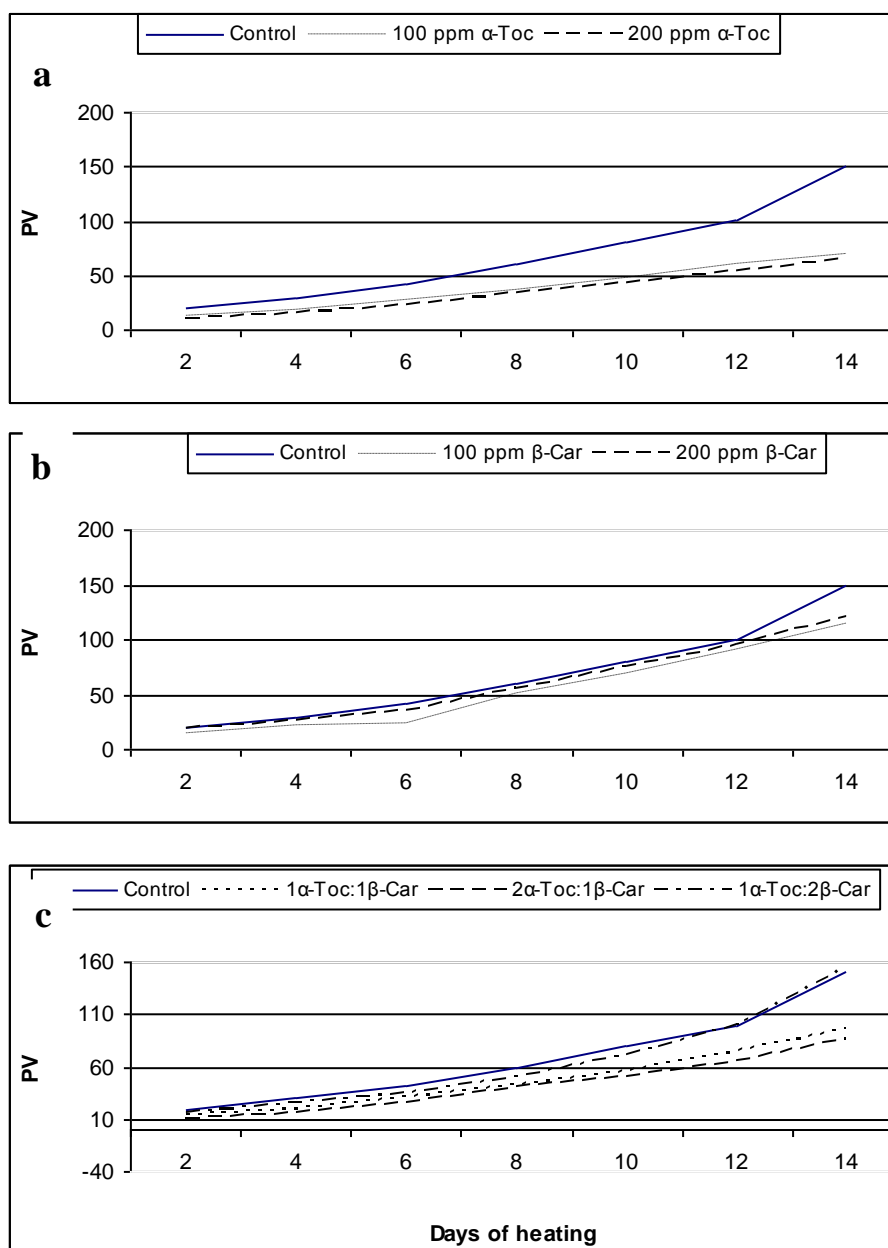


Figure (2): Effect of different concentrations of α -toc and β -car and their mixtures on stripped sunflower oil on hydroperoxides formation 60 °C.

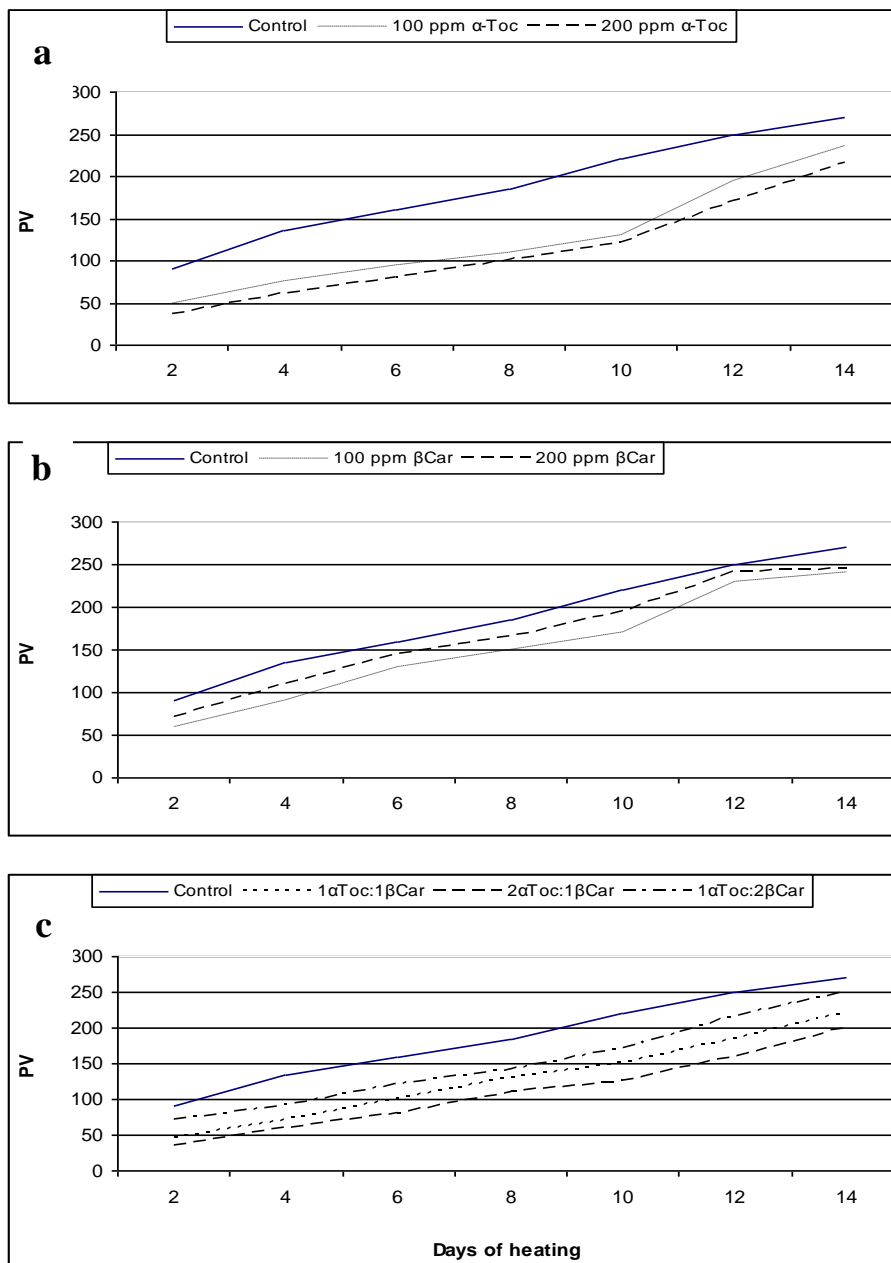


Figure (3): Effect of different concentrations of α -toc and β -car and their mixtures on stripped sunflower oil hydroperoxides formation at 100 °C.

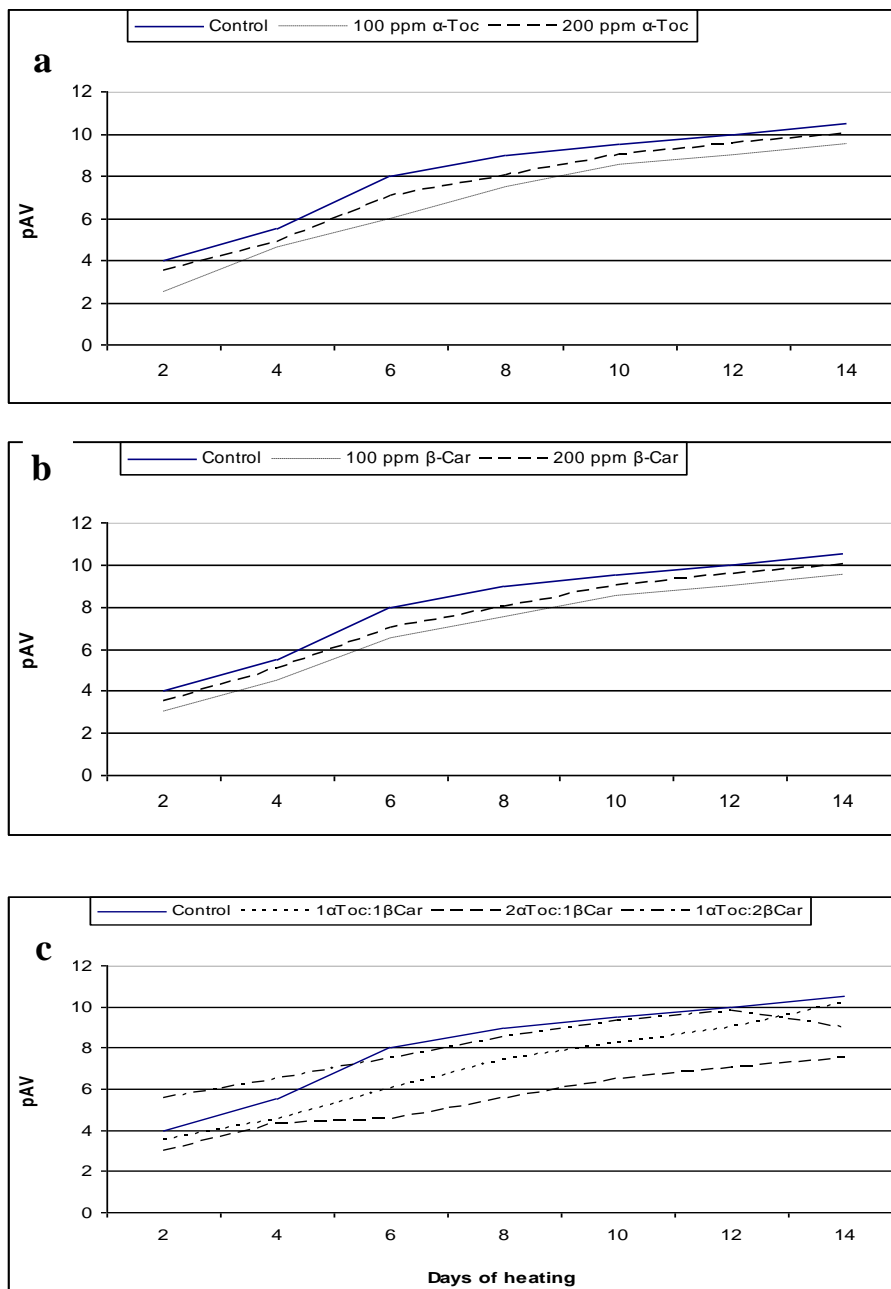


Figure (4): Effect of different concentrations of α -toc and β -car and their mixtures on stripped sunflower oil on secondary compounds formation at 40 °C.

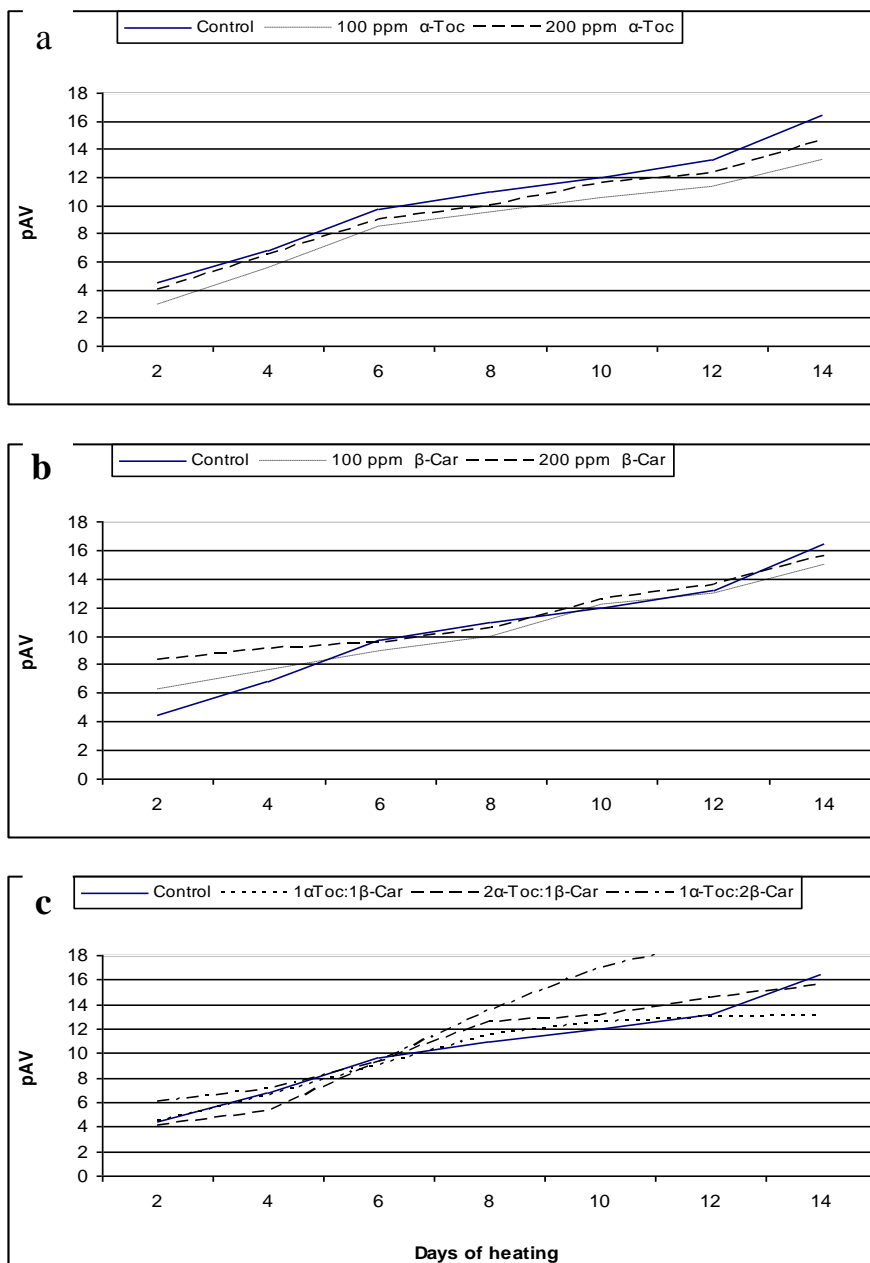


Figure (5) Effect of different concentrations of α -toc and β -car and their mixtures on stripped sunflower oil on secondary compounds formation at 60 °C.

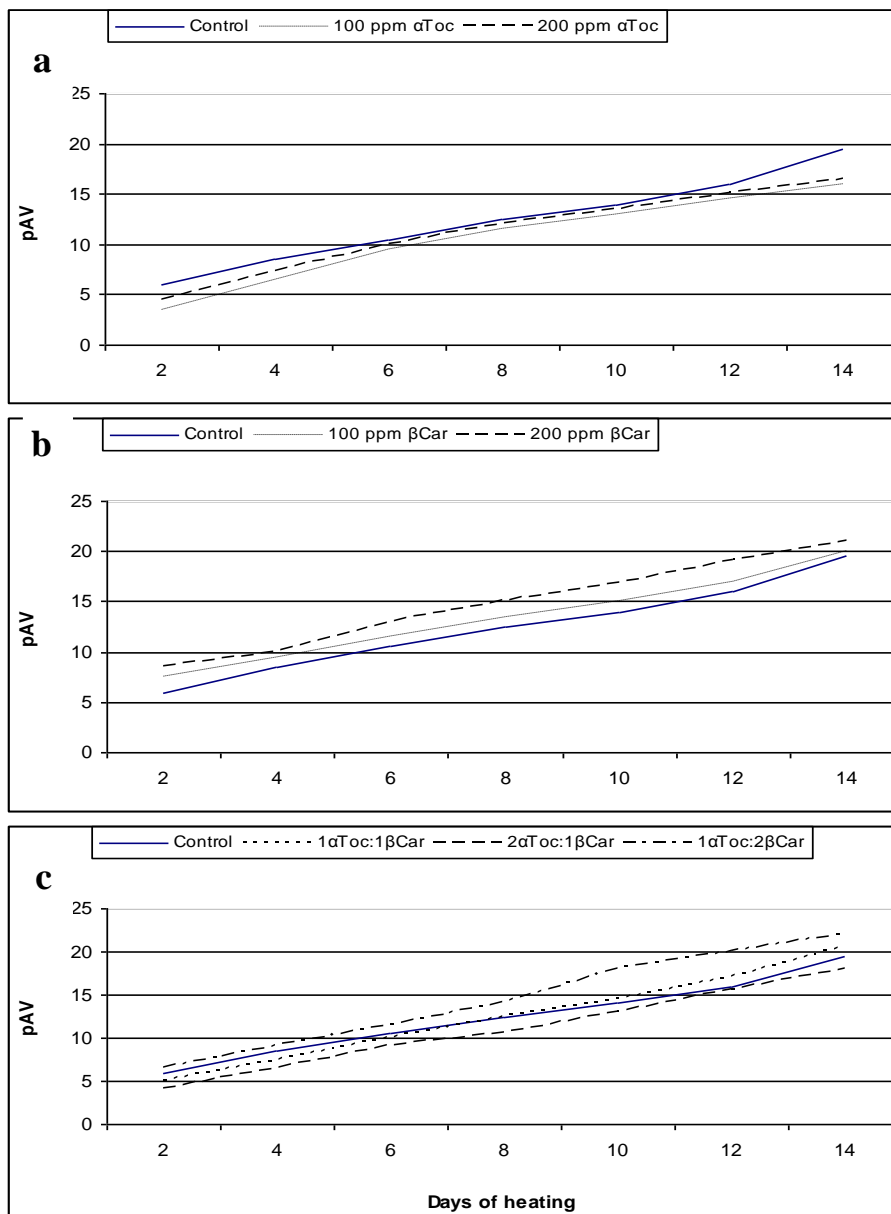


Figure (6): Effect of different concentrations of α-toc and β-car and their mixtures on stripped sunflower oil on secondary compounds formation at 100 °C.

4 Totox value

Totox value of the oil samples after addition of 100 and 200 ppm α-toc, β-car and their binary mixtures was periodically calculated at 40, 60 and 100 °C. Comparing total oxidation at 40, 60 and 100 °C, the total oxidation

values were greatly higher at 60 and 100 °C in comparison than those calculated at 40 °C. Whereas, 100, 200 ppm of α -toc and 2 α -toc: 1 β -car were still satisfactory in suppressing the oxidation processes particularly at 40 and 60 °C (Table 2, a, b, c).

Table (2): Effect of different concentrations of α -toc, β -car and their mixtures on stripped sunflower oil on total oxidation (calculated totox value)

Sample	(a) Days of heating at 40 °C						
	2	4	6	8	10	12	14
Control	12.0	16.5	22.0	26.6	30.5	35.0	39.5
100 ppm α -toc	9.1	13.6	17.8	21.9	25.5	29.0	31.5
200 ppm α -toc	9.5	12.8	17.0	20.0	23.0	25.5	28.0
100 ppm β -car	10.2	13.1	18.5	22.5	26.5	31.4	35.5
200 ppm β -car	9.5	12.6	18.0	22.0	26.0	31.3	35.0
1 α -toc : 1 β car	10.7	13.5	17.0	21.4	25.2	30.0	35.2
2 α -toc : 1 β car	10.0	12.3	14.5	18.5	22.5	27.0	31.5
1 α -toc : 2 β car	13.5	16.5	19.5	23.5	27.3	31.8	35.0

Sample	(b) Days of heating at 60 °C						
	2	4	6	8	10	12	14
Control	44.5	66.8	94.7	131.0	172.0	213.0	316.4
100 ppm α -toc	28.0	40.5	63.5	84.5	106.5	131.3	153.2
200 ppm α -toc	24.0	36.5	53.9	75.0	96.5	118.2	144.6
100 ppm β -car	36.3	52.6	54.9	109.9	152.2	193.0	245.0
200 ppm β -car	43.3	59.1	79.5	120.5	162.5	203.5	255.5
1 α - toc : 1 β -car	32.5	46.5	69.0	96.5	122.5	162.9	203.0
2 α - toc : 1 β -car	24.0	35.2	59.3	92.5	113.0	144.5	185.5
1 α - toc : 2 β -car	38.0	57.0	79.3	113.5	157.0	219.0	329.5

Sample	(c) Days of heating at 100 °C						
	2	4	6	8	10	12	14
Control	186	278.5	330.5	382.5	454	516	559.5
100 ppm α -toc	103.5	156.5	199.5	231.5	273	404.5	486
200 ppm α -toc	74.5	127.2	170	212	253.5	355	446.5
100 ppm β -car	127.5	189.5	271.5	313.5	355	477	500
200 ppm β -car	148.5	230	303	345	406.8	499.0	511.0
1 α -toc : 1 β -car	95	147.5	210	272.5	314.5	387	460.5
2 α -toc : 1 β -car	74	126.5	169	230.5	263	335.5	418
1 α -toc : 2 β car	146.5	189	251.5	294	358	450.0	522.0

CONCLUSION

During the periodical accelerated oxidation of sunflower oil, it was found that α -toc alone at higher concentration (200 ppm) inhibits effectively the formation of hydroperoxides little bit more than its lower concentration (100 ppm). In addition it was reported that the antioxidant activity of the tocopherols diminished at higher levels. Above the optimal concentrations,

the individual and tocopherol mixture behaved as prooxidant which was more pronounced with increasing temperature from 40 to 60 °C (Evans *et al.*, 2002). The results reported by (Huang *et al.*, 1994a, 1994b and Henry *et al.*, 1997) showed that the optimum concentration to inhibit hydroperoxide formation, was 200 ppm for α -toc. Moreover, α -toc showed an initial prooxidant effect at 250 ppm or higher levels. Thus it was concluded that low tocopherol concentration may be due to the insufficient scavenging capacity of free radicals produced during the oxidation process. In general, it seems clearly that individual α -toc (200ppm) and its combination with β -car (100 ppm) exhibit better antioxidation potency and accordingly lower primary oxidation reactions occurs. The effective antioxidant potency was observed with individual α -toc at 200 ppm and when mixed with 100 ppm β -car it delays the accelerated oxidation in general. This may be explained on the possibility that the antioxidant of linoleic acid may be favoured by the combination of α - toc and β -car in the new variety of sunflower oil (i.e. higher linoleic acid content).

As carotenoids are highly unsaturated molecules, comprising many conjugated double bonds, they are very susceptible to oxidation (Henry *et al.*, 1998). In the process of protecting compounds from harmful oxidative reaction via trapping free radicals (Kennedy, and Liebler, 1991) or quenching singlet oxygen (Foote, and Denny, 1968; Warner, and Frankel, 1987 and Kiritsaki, and Dugan, 1985), carotenoids may become the primary oxidizable substrate. However, beta-carotenoids acted as prooxidant when used at a concentration equal to that of the tocopherols, because β -car may react with oxygen to form β -car peroxy radicals (Tsuchihashi, *et al.*, 1995), these peroxy radicals may favor the propagation stage of the oxidation reaction because they supply the system with more oxidizable substrates. Therefore, the antioxidant capacity or 'sparing efficiency' of tocopherols decreases (Henry *et al.*, 1997). Carotenoids alone are the most common singlet oxygen quenchers, and vitamin E is the most widely used chain-breaking antioxidant (Loliger *et al.*, 1996) In general; these individual antioxidants and their combinations prevent or delay lipid oxidation by decreasing the oxygen active compounds in the medium. Some of them decrease the concentration of oxygen (triplet oxygen, $^3\text{O}_2$) through a direct reaction. A much more active oxygen species is the singlet oxygen ($^1\text{O}_2$). It is known that singlet oxygen react with unsaturated lipids more than 1000 times faster than triplet oxygen (Dawson, and Gartner, 1983). Some antioxidants such as carotenoids convert singlet oxygen into the more stable triplet oxygen and therefore called singlet oxygen quenchers.

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تأثير الالفاتوكوفيرول والبيتا-كاروتين ومخاليطهما كمضادات أكسدة طبيعية على ثبات زيت زهرة الشمس المنزوع منه مضادات الاكسدة
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تم دراسة تأثير تركيزات مختلفة من الالفاتوكوفيرول والبيتا-كاروتين ومخاليطهما كمضادات اكسدة طبيعية عند درجات حرارة 40، 60، 100 °س و لفترات زمنية مختلفه: 2، 4، 6، 8، 10، 12، 14 يوم وذلك بقياس قيمة البيروكسيد (نواتج الاكسدة الاوليه) و البارا أنيزيدين (نواتج الاكسدة الثانويه) وكذلك حساب الاكسدة الكلية (2بيروكسيد+1 بارا أنيزيدين) اضافة الى تتبع تكون الروابط المزدوجة المتبادلة. وقد وجد ان التركيز العالى من الالفاتوكوفيرول (200 جزء فى المليون) هو اكثر فاعلية فى تقليل تاكسد زيت زهرة الشمس عن التركيز الاقل (100 جزء فى المليون).

وجد ايضا ان مخلوط من الالفاتوكوفيرول والبيتا-كاروتين بنسبة 2 : 1 اثبت فاعلية فى تقليل اكسدة الزيت عند كل درجات الحرارة المستخدمة افضل من المخاليط الاخرى. و فى المقابل فقد وجد أن البيتاكاروتين عند استخدامه منفردا بتركيز 200 جزء فى المليون يعمل كعامل مؤكسد (على العكس من الالفاتوكوفيرول منفردا او مخلوطا بالبيتاكاروتين)

وفى النهاية يمكن الاستنتاج من ان النشاط المضاد للاكسدة للالفاتوكوفيرول يقل بصورة كبيرة عندما يزيد مستوى الالفاتوكوفيرول عن التركيز الامثل. كذلك يجب التذكير بان البيتا كاروتين تعمل كمحفز للاكسدة عند استخدامة بتركيز مساو لتركيز التوكوفيرول وذلك لان البيتاكاروتين من المحتمل انة يتفاعل مع الاوكسجين ليكون الشق البيروكسيدى للبيتاكاروتين وهذا الشق البيروكسيدى من المحتمل انة يسرع من مرحلة انتشار تفاعل الاكسدة ونتيجة لذلك يقل النشاط المضاد للاكسدة للالفاتوكوفيرول فى وجود البيتاكاروتين.

وقد انتهت هذه الدراسة الى ان التركيز العالى (200 جزء فى المليون) من الالفاتوكوفيرول افضل من التركيز الاقل (100 جزء فى المليون) وكذلك المخلوط من الالفاتوكوفيرول والبيتا-كاروتين بنسبة 2: 1 افضل من المخاليط الاخرى.

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