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β-carotene in sunflower oil oxidation

By **N. V. Yanishlieva, V. G. Raneva** and **E. M. Marinova**

Institute of Organic Chemistry, Bulgarian Academy of Sciences,
Kv. Geo Milev, Acad. G. Bontchev Str., Bl. 9, 1113 Sofia, Bulgaria
E-mail: nelly@orgchm.bas.bg

RESUMEN

β-caroteno en la oxidación de aceite de girasol.

Se estudió la cinética de oxidación de aceite de girasol (SO), así como la de triacilgliceroles puros de aceite de girasol (TGSO) en presencia de diferentes concentraciones (0.001-0.02 %) de β-caroteno. El proceso se llevó a cabo a altas (régimen cinético) y bajas (régimen de difusión) concentraciones de oxígeno a temperatura ambiente con luz natural y en la oscuridad, y se presentan también los resultados de la oxidación de SO y TGSO a 100°C en presencia de β-caroteno. Los resultados indicaron que en los sistemas lipídicos libres de antioxidantes, el β-caroteno no dió protección antioxidante. Funcionó como prooxidante durante la oxidación a temperatura ambiente y a concentraciones de oxigeno suficientemente elevadas, siendo el efecto más pronunciado en la oscuridad que con luz natural. El β-caroteno aumentó la estabilidad del SO conteniendo tocoferol durante su oxidación a temperatura ambiente y con luz natural. Este efecto es expresado más intensamente en un régimen cinético de oxidación. El sinergismo del β-caroteno con los tocoferoles se caracterizó por el factor de estabilidad F y la actividad A. En el régimen cinético de oxidación, F y A variaron en el intervalo F= 2.0-6.3, y A= 2.7-21.0. En el régimen de difusión F = 1.3-1.5, y A= 1.5-2.8.

PALABRAS-CLAVE: Aceite de girasol - β-caroteno - Oxidación.

SUMMARY

β-carotene in sunflower oil oxidation.

The oxidation kinetics of sunflower oil (SO), as well as of pure triacylglycerols of sunflower oil (TGSO) in the presence of different concentrations (0.001-0.02 %) β-carotene was studied. The process was performed at high (kinetic regime) and low (diffusion regime) oxygen concentrations at room temperature in the dark and under daylight. The results from the oxidation of SO and TGSO at 100°C in the presence of β-carotene were also presented. It was established that in the antioxidant-free lipid system, the β-carotene did not give any antioxidative protection. It worked as a prooxidant during the oxidation at room temperature and at sufficiently high oxygen concentration, the effect being more pronounced in the dark than under daylight. β-carotene increased the stability of tocopherol-containing SO during its oxidation at room temperature and under daylight. This effect is more strongly expressed in a kinetic regime of oxidation. The synergism of β-carotene with the tocopherols was characterized by the stabilization factor F and the activity A. In the kinetic regime of oxidation F and A varied in the interval F=2.0-6.3, and A =2.7-21.0. In the diffusion regime F=1.3-1.5, and A=1.5-2.8.

KEY-WORDS: β-Carotene - Oxidation - Sunflower oil.

1. INTRODUCTION

Carotenoids are present in a wide variety of food ingredients. They are mainly used as natural colorants in foods and in dietary fats (Bhagavan and Nair, 1992), and they have also antioxidative properties (Rajalakshi and Narasimhan, 1996).

Carotene research has progressed rapidly in the past few years. β-Carotene has the highest provitamin A activity of all carotenoids (Kotareddy and Devi, 1997). Burri (1997) recently discussed the important new developments in β-carotene research, and provided an interpretation of how these results reflect the impact of β-carotene on human health. β-Carotene reduces the risk of certain cancer (Peto *et al.*, 1981; Appel and Woutersen, 1996; Ziegler *et al.*, 1996; Hughes, 1998) and is high effective in preventing coronary cardiac disease (Seelert, 1991; Kohlmeier and Hastings, 1995).

The role of β-carotene in lipid oxidation was discussed many years ago. It was established that β-carotene increased the rate of oxygen absorption by fats (Olcott, 1934; Henk, 1941; Werner, 1942; Thompson and Steenbock, 1944; Holman, 1949). Wodsak (1951) reported that in the light β-carotene accelerated the fat oxidation, whereas in the dark it had an antioxidative effect. Herriset (1948) also found that β-carotene and vitamin A were inhibitors of spontaneous fat and oil oxidation.

Recently, however, contradictory results on the role of carotenoids, and in particular, of β-carotene, in lipid oxidation are also reported - antioxidative (Kiritsakis and Dugan, 1985; Fakourelis *et al.*, 1987; Lee and Min, 1990; Jung and Min, 1991), as well as prooxidative (Terao *et al.*, 1980; Faria and Mukai, 1983; Warner and Frankel, 1987; Haila and Heinonen, 1994) effects are observed.

The numerous investigations demonstrate that the behaviour of β-carotene in the oxidation process depends strongly on its concentration (Krinsky, 1968), on the environment (Pryor *et al.*, 1993), as well as on whether the process proceeds in the dark or in the light (Yanishlieva *et al.*, 1998). The anti-or prooxidative activity of β-carotene is also related closely to both oxygen concentration and to the

presence of other antioxidants (Krinsky, 1993; Palozza and Krinsky, 1993). Numerous reviews discuss the ability of β-carotene to act as a chain-breaking antioxidant and as a singlet oxygen quencher (Burton, 1989; Krinsky, 1989; Palozza and Krinsky, 1992a; Handelman, 1996).

There are no data in the literature concerning the role of β-carotene in sunflower oil oxidation at storage conditions. As known, this oil is one of the most commonly used valuable oils containing 60-70 % of the essential 9-cis,12-cis-octadecadienoic (linoleic) acid.

The objective of this study is to throw light on the influence of β-carotene on the kinetics of sunflower oil oxidation at room temperature.

The experiments were performed with commercially available sunflower oil (SO), as well as with pure triacylglycerols of sunflower oil (TGSO) at different storage conditions - in the dark and under daylight, at high (kinetic regime) and low (diffusion regime) oxygen concentrations. Different β-carotene concentrations (0.001-0.02 %) were investigated. Kinetic results from the autoxidation of SO and TGSO at 100°C (the most frequently used temperature for accelerated lipid stability determination) in the presence of β-carotene were also presented.

2. EXPERIMENTAL

2.1. Materials

β-Carotene was from Merck (Darmstadt, Germany).

A commercially available sample of sunflower oil (SO) was used.

Pure triacylglycerols of sunflower oil (TGSO) were obtained by cleaning the sunflower oil from pro-and antioxidants and trace metals by adsorption chromatography (Popov *et al.*, 1968): 50 g lipid substrate in 500 ml distilled hexane were passed through a column (i.d. 2 cm) filled with 35g alumina (type 507C, neutral, Fluka AG, Buchs, Switzerland) activated for 4 h at 180°C, and collected in nitrogen in the dark. The solvent was removed in a rotary evaporator at 30°C in the dark. The product obtained was stored in an inert atmosphere at -20°C no more than 10 days.

Lipid samples containing 0.001-0.02 % (1.86×10^{-5} - 3.70×10^{-4} M) β-carotene were prepared by adding aliquots of a solution of β-carotene in purified acetone to a weighted lipid sample followed by the removal of the solvent in nitrogen.

2.2. Methods

The fatty acid composition of SO was determined by gas chromatography of its methyl esters using a Pye Unicam instrument, model 304, equipped with a dual flame-ionization detector and a gass capillary column (30 m x 0.2 mm i.d.) coated with SILAR 10C (Supelco Inc., Bellefonte, PA). The carrier gas was nitrogen at a flow rate of 14 ml min^{-1}. The temperature was mantained at 165°C for 5 min, then increased to 220°C at 2°C/min.

The tocopherol content of the sunflower oil was determined by normal-phase HPLC (Ivanov and Aitzetmüller, 1995) using a Merck-Hitachi apparatus equiped with a pump L-6000 and a fluorescence detector Merck - Hitachi F-1050. A column with Nucleosil SI 50-5, 250x4 mm (Macherey - Nagel), and an elution system hexane: dioxane = 96:4 with a rate of 1 ml/min were used.

Oxidation at room temperature, 22°C (±2°C) in the dark and under daylight (southern window) was carried out at oxygen sufficient conditions (kinetic regime), as well as at oxygen - insufficient conditions (diffusion regime). The kinetic regime was realized by storage of the samples in a 1 mm layers in Petri dishes having a diameter of 52 mm. The diffusion controlled oxidation was performed by storage of 10 g of the samples in glass jars with a diameter of 32 mm. Oil samples were thoroughly stirred before sampling.

Oxidation at 100°C (±0.2°C) was carried out by blowing air through the samples (2 g) in the dark at a rate of 100 ml min^{-1}.

The process was followed by withdrawing samples at measured time intervals and subjecting them to iodometric determination of the primary product (peroxide) concentration, i.e. the peroxide value (PV) (Yanishlieva *et al.*, 1978). As established, one of the most reliable methods for investigating the role of the carotenoids in lipid oxidation is the study of the peroxide accumulation (Terao *et al.*, 1992; Haila and Heinonen, 1994; Kasaikina, 1995; Tsuchihashi *et al.*, 1995; Servili et al., 1996; Haila *et al.*, 1996). All kinetic curves are the mean result of two independent experiments.

The influence of β-carotene on lipid oxidation was estimated on the basis of the induction periods (IP) determined by the method of the tangents to the two parts of the kinetic curves (Yanishlieva and Popov, 1971; Le Tutour and Guedon, 1992). The oxidation rate of the control sample (W_O) and of the samples with β-carotene ($W_{\beta\text{-Car}}$) were found from the tangents to the initial phase of the kinetic curves of peroxide accumulation and expressed as M s^{-1}. Recalculation of the rate from meq kg^{-1} h^{-1} into M s^{-1} was performed according to the following formula (Marinova and Yanishlieva, 1992):

$$1 \text{ meq kg}^{-1} \text{ h}^{-1} = 1.4 \times 10^{-7} \text{ M s}^{-1}$$

3. RESULTS AND DISCUSSION

The fatty acid composition of TGSO was as follows: palmitate 6%, stearate 5%, oleate 25%,

linoleate 64%. The oil contained 0.062% tocopherols (Toc): α-Toc 88.4%, β-Toc 3.9%, γ-Toc 5.1%, δ-Toc 1.6%, and α-tocotrienol (α-Toc-3) 1.0%. The initial PV of the commercial sunflower oil was 13.0 meq kg^{-1}.

3.1. Triacylglycerols of sunflower oil (TGSO)

Figures 1 and 2 represent the kinetic curves of peroxide accumulation during oxidation of pure triacylglycerols of sunflower oil (TGSO) at room temperature at a sufficiently high oxygen concentration in the dark (Figure 1) and under daylight (Figure 2). Figures 1 and 2 show that β-carotene works as a prooxidant .

These results are in agreement with the data reported by other authors. Haila and Heinonen (1994) established that β-carotene concentrations between 0.005 and 0.2% accelerated hydroperoxide formation during autoxidation of purified rapeseed oil under light at 25°C. At the same concentrations Heinonen et al. (1996) found a prooxidative effect of β-carotene in a 10% purified rapeseed oil-in-water emulsion. The acceleration of autoxidation of different lipid systems by β-carotene at high oxygen pressure was observed also by Burton and Ingold (1984); Pryor *et al.* (1988), Kennedy and Liebler (1992), Jorgensen and Skibsted (1993), Tsuchihashi et al. (1995), Palozza *et al.*, (1995).

The prooxidative effect of β-carotene may be due to the oxidation of β-carotene alone leading to the formation of chain propagating radicals, and to the formation of products which act as initiators of the process (Kennedy and Liebler, 1992).

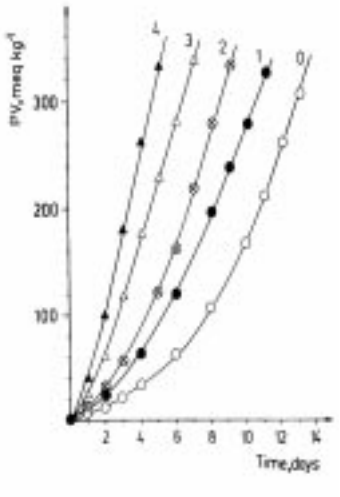

Figure 1
Kinetic curves of peroxide accumulation during oxidation of TGSO at room temperature in the dark at a sufficiently high oxygen concentration (kinetic regime) in the presence of different β-carotene concentrations: 0 - 0%; 1 - 0.001% ($1.86x10^{-5}$M); 2 - 0.005% ($9.30x10^{-5}$M); 3 - 0.01% ($1.86x10^{-4}$M); 4-0.02% ($3.72x10^{-4}$M)

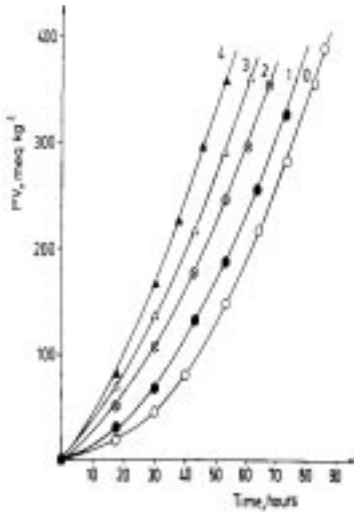

Figure 2
Kinetic curves of peroxide accumulation during oxidation of TGSO at room temperature under daylight at a sufficiently high oxygen concentration (kinetic regime) in the presence of different β-carotene concentrations: 0 - 0%; 1 - 0.001% ($1.86x10^{-5}$M); 2 - 0.005% ($9.30x10^{-5}$M); 3 - 0.01% ($1.86x10^{-4}$M); 4-0.02% ($3.72x10^{-4}$M)

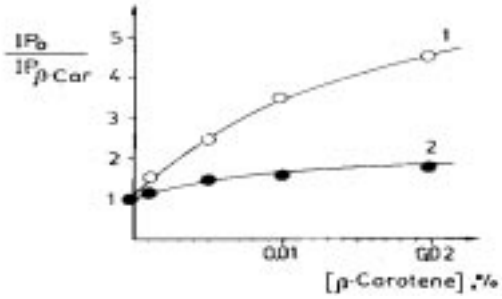

Figure 3
Dependence of the decrease of the induction period in the presence of β-carotene ($IP_o/IP_{β-Car}$) on β-carotene concentration during oxidation of TGSO in a kinetic regime at room temperature in the dark (1) and under daylight (2).

After treatment of the curves in Figures 1 and 2 the dependence of the decrease of the induction period (IP) in the presence of β-carotene ($IP_o/IP_{β-Car}$) on its concentration was obtained (Figure 3).

The induction period of TGSO without additive IPo in the kinetic regime was 38 h under daylight , and 7.7 days in the dark. It could be seen from Figure 3 that the prooxidative effect of β-carotene is much more pronounced in the dark than under daylight.

The results obtained during oxidation of TGSO at unsufficiently oxygen concentration (diffusion regime) showed that at both conditions, in the dark (IP_o = 18 days), as well as under daylight (IP_o = 4.7 days), β-carotene did not change the kinetics of the process (data not shown). The same was true when TGSO were oxidized at 100°C in the dark (IP_o = 0.5 h).

The data presented above show that β-carotene does not give any antioxidative protection during the oxidation of pure triacylglycerols of sunflower oil, e.g. of the antioxidant free lipid system.

3.3. Sunflower oil (SO)

Figures 4 and 5 illustrate the kinetic curves of peroxide accumulation during oxidation of SO at room temperature under daylight in a kinetic (Figure 4) and in a diffusion regime (Figure 5), respectively. One can see that at these conditions β-carotene retards the oxidation of SO, increasing the induction period IP and decreasing the rate of the process W in the initial phase.

For quantification of the retardation effect of β-carotene on SO oxidation we determined the following kinetic parameters characterizing the lipid oxidation during its initial stage: stabilization factor F, oxidation rate ratio ORR and activity A (Yanishlieva and Marinova, 1992). F is a measure of the effectiveness:

$$F = IP_{\beta\text{-Car}} / IP_o$$

where $IP_{\beta\text{-Car}}$ is the induction period in the presence of β-carotene, and IP_o is the induction period of SO without additive.

ORR is an inverse measure of the strength:

$$ORR = W_{\beta\text{-Car}}/W_o$$

where $W_{\beta\text{-Car}}$ is the oxidation rate in the presence of β-carotene, and Wo is the initial oxidation rate of the control.

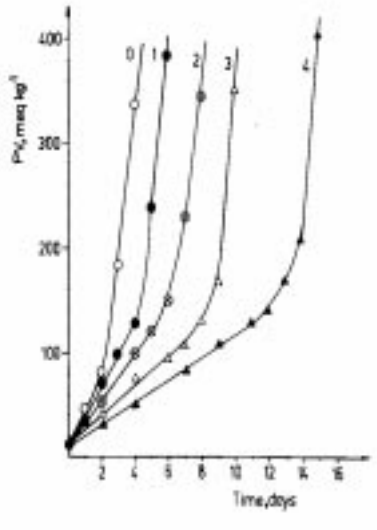

Figure 4
Kinetic curves of peroxide accumulation during oxidation of SO at room temperature under daylight at a sufficiently high oxygen concentration (kinetic regime) in the presence of different β-carotene concentrations: 0 - 0%; 1 - 0.001% ($1.86x10^{-5}$M); 2 - 0.005% ($9.30x10^{-5}$M); 3 - 0.01% ($1.86x10^{-4}$M); 4-0.02% ($3.72x10^{-4}$M)

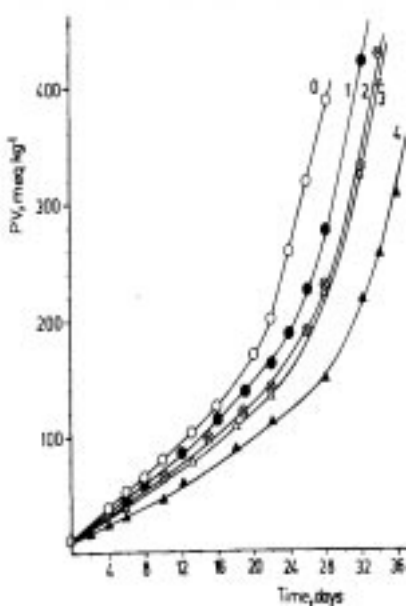

Figure 5
Kinetic curves of peroxide accumulation during oxidation of SO at room temperature under daylight at oxygen insufficient conditions (diffusion regime) in the presence of different β-carotene concentrations: 0 - 0%; 1 - 0.001% ($1.86x10^{-5}$M); 2 - 0.005% ($9.30x10^{-5}$M); 3 - 0.01% ($1.86x10^{-4}$M); 4 - 0.02% ($3.72x10^{-4}$M)

The general parameter activity A unifies the effectiveness on the one hand, and the strength of the additive in the oxidation process, on the other:

$$A = F/ORR$$

After processing the kinetic curves in Figures 4 and 5 the kinetic parameters F, ORR and A characterizing the oxidation of SO at room temperature under daylight in the presence of β-carotene were obtained (Table I). The data in Table I illustrate that β-carotene retards the oxidation of SO under light in a much higher degree when the process proceeds at a sufficiently high oxygen concentration (kinetic regime).

The experiments at room temperature in the dark showed that β-carotene did not influence the oxidation stability of sunflower oil in a kinetic (IP_o = 48 days), as well as in a diffusion regime (IP_o = 140 days). The same was true when SO was oxidized at 100°C in the dark (IP_o = 7.3 h). The results obtained with the thermal oxidation of safflower seed oil at 75°C in the presence of different concentrations of β-carotene (Henry *et al.*, 1998) were similar.

The presented data demonstrated that β-carotene increased the oxidation stability of tocopherol containing sunflower oil during its oxidation at room temperature and under daylight. It should be pointed out that the natural sensitisers in sunflower oil, e.g. the chlorophylls, may generate singlet oxygen during

Table I
Kinetic parameters characterising the oxidation of sunflower oil at room temperature under daylight in the presence of β-carotene (β-Car), PV_o = 13 meq kg^{-1}

Conditions	β-Carotene concentration		F	ORR	A
	[β-Car] (wt%)	[β-Car] x 10^5 (M)			
Sufficiently high oxygen concentration (Kinetic regime) IP_o = 2.2 days $W_o = 1.93x10^{-7}Ms^{-1}$	0.001	1.86	2.0	0.75	2.7
	0.005	9.30	3.0	0.60	5.0
	0.010	18.60	4.1	0.43	9.5
	0.020	37.20	6.3	0.30	21.0
Oxygen insufficient conditions (Diffusion regime) IP_o = 21.0 days $W_o = 3.62x10^{-8}Ms^{-1}$	0.001	1.86	1.3	0.87	1.5
	0.005	9.30	1.4	0.70	2.0
	0.010	18.60	1.4	0.67	2.1
	0.020	37.20	1.5	0.53	2.8

oil oxidation under light (Foote and Denny, 1968; Korycka-Dahl and Richardson, 1978). The stabilizing effect of β-carotene observed may be due to its ability to quench the singlet oxygen (Raws and Van Santen, 1970; Foote, 1979; Kiritsakis and Dugan, 1985; Lee and Min, 1990), which explains its cooperative (synergistic) action with tocopherols.

The presented data are in accordance with the findings of other authors concerning the synergistic action of β-carotene with α-tocopherol (Palozza and Krinsky, 1992b; Haila and Heinonen, 1994; Li *et al.,* 1995; Perrin *et al*., 1996; Yin and Cheng, 1997), γ-tocopherol (Heinonen *et al*., 1996), phospholipids (Bratkowska and Zwierzykowski, 1988), quercitrin (Maoka and Ito, 1994), tert-butylated hydroquinone (Tsai *et al*., 1989), and 3,4-dihydroxyphenyl ethanol (Servili *et a*l., 1996).

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