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Food Chemistry 66 (1999) 189–195

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidant activity of 1,4-dihydropyridine derivatives in β-carotene-methyl linoleate, sunflower oil and emulsions

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Received 27 October 1998; received in revised form 2 December 1998; accepted 16 December 1998

Abstract

The antioxidant activity of six synthetic 1,4-dihydropyridine (DHP) derivatives was tested in an azobis-amidinopropane dihydrochloride initiated β-carotene-methyl linoleate peroxidation model system. Radical scavenging activity of the derivatives was estimated by measuring their reactivity with stable *N*,*N*-diphenyl-*N*′-picrylhydrazyl radicals. The antioxidant activity of these different derivatives was also evaluated in traditional sunflower oil and its 20% oil-in-water emulsion in the dark at 60°C, as well as during storage of sunflower oil in the dark and with light exposure at ambient temperatures. The primary (conjugated diene hydroperoxides) and secondary (hexanal, pentanal) oxidation products were monitored during different periods of storage. Butylated hydroxytoluene was used as a standard antioxidant and different plant extracts were used for comparison of antioxidant activities throughout this study. All synthetic compounds showed antioxidant activity, while plant extracts acted as pro-oxidants upon light exposure. Three derivatives of DHP, without substitution at position 4, showed highest antioxidative activity in model system and sunflower oil and its emulsion in the dark at 60°C as well as during storage of sunflower oil in the dark and with light exposure at ambient temperature. The hydrophilic derivative of DHP with a phenyl group at position 4 showed high radical scavenging activity, and high antioxidant activity in sunflower oil-in-water emulsion, but was less active in model system and sunflower oil. The hydrophobic derivatives of DHP with a phenyl group at position 4 showed high antioxidant activity in model system but were the lowest active derivatives in sunflower oil and its emulsion.

1. Introduction

Numerous synthetic compounds have been evaluated as antioxidants in fats, oils, and fatty foods. However, only a few can be used in products for human consumption due to their non-toxicity and effectiveness at low concentration. Also, absence of undesirable effects on colour, odour, taste and other characteristics of the food are important together with compatibility with the food and ease of application, like stability under the conditions of processing and/or storage of food (Pratt, 1996). The phenolic compounds, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroxyquinone (TBHQ) were authorized as antioxidants for use in food. The effectiveness and the biological impact of these phenolic antioxidants have been evaluated (Sherwin, 1985) as well as the effect of their interaction with food components during food processing (Hamama & Nawar, 1991). There are some serious problems concerning the toxicity of these phenolic compounds (Linderschmidt, Trylka, Goad, & Witschi, 1986). The main concern about the safety of synthetic compounds is related to their metabolism and possible absorption and accumulation in body organs and tissues (Tappel, 1995). Besides, BHA and BHT are quite volatile and decompose easily at high temperatures (Warner, Daniel, Lin, Joe, & Fazio, 1986).

1,4-Dihydropyridine (DHP) derivatives, which were unsubstituted at position 4 (Scheme 1a–c), posess pronounced free radical quenching and antioxidant properties (Tirzitis & Duburs, 1972). One of these derivatives is 'diludine' (Scheme 1a) which has been found to be active in edible oil stabilization and proven to be a good synergist of α-tocopherol (Tirzitis & Duburs, 1977; Tirzitis, Kirule, & Duburs, 1988). Diludine showed longer induction periods than BHA and BHT during oxidation of rapeseed, sunflower and olive oils (Kourimska, Pokorny, & Tirzitis, 1993). Some work was done to determine the toxicity of these compounds (Tirzitis et

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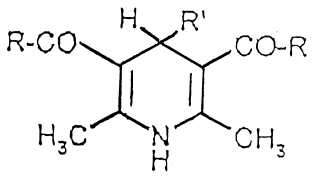
0308-8146/99/$ - see front matter
PII: S0308-8146(98)00265-9



a R = OC_2H_5 R' = H (diludine) **b** R = $OC_4H_{9\text{-}n}$ R' = H

c R = $OC_{10}H_{19}$ (menthyl) R' = H **d** R = OCH_2COONa R' = C_6H_5

e R = NHC_6H_5 R' = C_6H_5 **f** R = $NHC_6H_4OCH_3$ R' = C_6H_5

Scheme 1. 1,4-Dihydropyridine (DHP) derivatives.

al., 1988). However, more studies should be carried out to get permission for human consumption.

The purpose of this work was to investigate the antioxidant activity of six different 1,4-DHP synthesized derivatives in model systems and in traditional sunflower oil and its emulsion. Their activity was also compared with different plant extracts during storage of sunflower oil in the dark and with light exposure at ambient temperatures.

2. Materials and methods

2.1. Materials

Six DHP derivatives denoted in Scheme 1 as a–f were synthesized by Dr J. Uldrikis at the Latvian Institute of Organic Synthesis, Riga, Latvia (Stale, 1994). All reagents used in this study were obtained from Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO, USA).

Traditional sunflower oil containing 570 ppm natural α-tocopherol, and without addition of any synthetic antioxidant, was obtained from Cargill, Amsterdam, Netherlands. Initial quality was checked by determining peroxide value (1.0 meq O_2/kg oil) and conjugated diene hydroperoxides (absorbance at 234 nm was 0.042/50 mg oil dissolved in 5 ml cyclohexane). Fatty acid composition was determined by gas chromatography (Carlo Erba Strument, HRGC) giving 6.6% C16:0, 3.9% C18:0, 21.4% C18:1 and 68.1% C18:2.

2.2. Methods

2.2.1. Antioxidant activity in model system

The modified β-carotene-methyl linoleate system of Miller (1971) was used. Two milligrams of β-carotene was dissolved in 10 ml of chloroform. β-Carotene-chloroform solution (1 ml) was pipetted in a flask containing 20 μl of methyl linoleate and 0.1 g of Tween 40. The chloroform was evaporated at 40°C under reduced pressure and 50 ml of 0.1 M phosphate buffer (pH 7.4) was added under vigorous mixing. For each determination, 20 μl of ethanolic solution of DHP derivatives or standard antioxidant solution (BHT) at appropriate concentrations was added to 5 ml of β-carotene-methyl linoleate dispersion in a Bausch & Lomb 5-ml test tube. Five microlitres of 0.5 M 2,2-azobis(amidinopropane)dihydrochloride (ABAP) in 0.1 M phosphate buffer (pH 7.4) was added and the tubes stoppered. The final concentration of each of the derivatives or BHT was 2 μM. The test tubes were incubated at 37°C and the absorbance was measured at 460 nm (Spectronic 70, USA). Antioxidant activity was calculated as τ/τ_o, in which τ and τ_o are time periods needed to decrease absorbance by 33% (β-carotene concentration) with and without added synthetic compound.

2.2.2. Determination of radical scavenging activity

Ethanolic solution (2.5 ml) of 100 μM *N*,*N*-diphenyl-*N*′-picrylhydrazyl (DPPH) was incubated at 30°C with 50 μl of 50 μM ethanolic solution of DHPa–c, DHPe and DHPf, and of DHPd dissolved in deionized water. Final concentration of compounds was 100 μM in the measuring cell. The decay of DPPH was measured at 517 nm on a Hitachi 557 UV–VIS spectrophotometer. The '*k*' calculations were made from five to six time-points (intervals) till absorbance diminishes by 50%. The scavenging activity was expressed as the reduction rate constant (*k*) of DPPH, which was calculated as:

$$k(M^{-1}s^{-1}) = [DPPH]_0 - [DPPH]_t/t[DPPH]_0[DPPH]_t,$$

where $[DPPH]_0$ is the starting concentration and $[DPPH]_t$ is concentration at the time '*t*'.

2.2.3. Antioxidant activity in sunflower oil and its emulsion at 60°C

Oil samples (25 g) and 20% oil-in-water emulsion samples (50 ml) were transferred into screw-capped 380-ml glass bottles covered externally with aluminium foil and subjected to accelerated oxidation in the dark in an oven (Gallenkamp, Germany) at 60°C. Each synthetic compound (300 ppm) in absolute ethanol was added to the traditional sunflower oil. The samples were incubated for 1 h at 60°C in the dark to evaporate the solvent. Twenty per cent sunflower oil-in-water emulsions were made with 20 g of sunflower oil containing 300 ppm of each derivative and 1 g of Tween 80 as described by Abdalla and Roozen (1999). Two bottles were prepared from each sample. Oxidative stability was monitored by measuring conjugated diene hydroperoxides and volatile compounds in oil samples during 25 days of storage and in emulsion samples during 8 days of storage.

The methods used to measure the conjugated diene hydroperoxides and volatile compounds were described previously by Abdalla and Roozen (1999).

2.2.4. Antioxidant activity in sunflower oil during storage in the dark and with light exposure at ambient temperature

Sunflower oil samples with 300 ppm of each derivative and 600 ppm of each plant extract of catnip, hyssop, lemon balm, oregano, sage and thyme (Abdalla & Roozen, 1999) were stored in the dark at ambient temperature ($20 \pm 2°C$) for 8 months and with light exposure at the same temperature for 8 weeks. The latter samples were surrounded with uniform light intensity (800 lux at the surface of the lids) generated by fluorescent lamps (Lumlux Interna L8w/41, Italy). Oxidation stability was monitored during storage as described before.

2.2.5. Statistical analyses

Student's *t*-test (O'Mahoney, 1986) was used to determine significant differences between average values of oxidation products formed in oils and emulsions with different synthetic compounds and plant extracts ($p < 0.05$).

3. Results and discussion

3.1. β-Carotene-methyl linoleate model system and radical scavenging activity

The influence of DHP substituents (Scheme 1) on the reactivity of stable *N*,*N*-diphenyl-*N*′-picrylhydrazyl (DPPH) radicals is presented in Table 1. DHPd was the most active compound bearing strong electron-donating carboxyl anion groups. The presence of a substituent in position 4 ($R = C_6H_5$) in both DHPe and f causes a strong decrease of activity. The same influence of substituents was detected in reactions of DHP substituents with active oxygen forms (Rubene, Tirzitis, & Duburs, 1982).

Antioxidant activity τ/τ_o was highest for derivatives DHPb, e and f; however, they were less effective than BHT. The higher τ/τ_o of DHPb (primary butyl derivative) in comparision with DHPa (diludin) obviously is due to the larger electron donating activity of a butyl radical. Special attention should be paid to diminished τ/τ_o of DHPc which is less than expected from electron-donating properties of a menthyl group. Similar data were obtained in the methyl oleate model system of Tirzitis et al. (1988). It is possible that diminishing of antioxidant activity and reactivity with DPPH is due to involvement of secondary C-atoms in radical reactions. The relatively high τ/τ_o detected for DHPe and f, inspite of low radical scavenging activity of these compounds, can be explained by the results of Denisov and Klhudyakov (1987). They showed that τ/τ_o depends, to a great extent, on the stability of an antioxidant radical produced in reaction with a lipid peroxi-radical. When a radical is stable, it cannot propagate the autoxidation/peroxidation chain, which increases the antioxidant power. In the case of DHPe and f the antioxidant radical is stabilized by the bulk substituent (phenyl groups) in position 4 (like tert-butyl groups in BHT) and cannot participate in the propagation reaction. The introduction of additional electron-donating methoxy groups in the phenyl rings of the substituents in positions 3 and 5 does not render sufficient influence on their activitiy with DPPH (cf. DHPf with e). The low τ/τ_o of DHPd in spite of its high reactivity with DPPH is probably due to hydrophilicity (dilution in bulk water) and, therefore, lack of penetration in peroxidizing micelles. The study of Frankel (1996) indicated that hydrophilic antioxidants such as Trolox and ascorbic acid were more active in bulk oil than in oil-in-water emulsion, because they would not be as concentrated in the oil-in-water interphase as lipophilic α-tocopherol and ascorbyl palmitate.

Table 1
Radical scavenging activity of 1,4-dihydropyridine (DHP) derivatives and butylated hydroxytoluene (BHT) reactivity with *N*,*N*-diphenyl-*N*′-picrylhydrazyl (*k*) and antioxidant activity in β-carotene-methyl linoleate model system (τ/τ_o)

Compound	k (M^{-1} s^{-1})	τ/τ_o
DHPa	13.96 ± 0.62	4.2
DHPb	7.38 ± 0.53	11.8
DHPc	5.68 ± 0.41	5.7
DHPd	42.97 ± 0.79	1.1
DHPe	0.67 ± 0.06	9.8
DHPf	0.57 ± 0.05	10.4
BHT	0.60 ± 0.05	19.8

Values are means of five determinations.

3.2. Sunflower oil

The effects of individual DHP derivatives on the oxidative stability of traditional sunflower oil were evaluated by measuring the formation of conjugated diene hydroperoxides as primary oxidation products and generation of both hexanal and pentanal as secondary oxidation products. The formation of hydroperoxides increased significantly more in control sunflower oil than in oil with individual derivatives added and stored over 25 days at 60°C (Table 2). All derivatives used in this study showed antioxidant activities in sunflower oil. Derivatives without substitution at position 4 (DHPa–c) decreased the formation of hydroperoxides more than derivatives with substitution at position 4 (DHPd–f) and were as effective as BHT during storage.

The generation of hexanal and pentanal was monitored by static headspace gas chromatography (GC) during incubation of sunflower oil in the presence of DHP derivatives. Hexanal formation was detected earlier in the control oil and oil with DHPd–f compounds than others (Table 3). The formation of hexanal increased significantly more in control sunflower oil

than in oil with different synthetic compounds. Derivatives without substitution at position 4 (DHPa–c) inhibited the formation of hexanal more than derivatives with substitution at position 4. Pentanal formation was detected at low levels in DHPd–f [0.50—0.85 FID peak area (V_s) in static headspace GC] after 15 and 20 days and was not detected in others. The results indicate that derivatives without substitution at position 4 were as active as BHT during primary oxidation step (Kourimska et al., 1993) but BHT was more active than all derivatives during secondary oxidation step.

3.3. Sunflower oil-in-water emulsion

Twenty per cent sunflower oil-in-water emulsions were prepared under room temperature to get droplet sizes ranging between 0.1 and 5 μm (Abdalla & Roozen, 1999). These prepared emulsions were physically stable during 8 days of incubation at 60°C. The formation of conjugated diene hydroperoxides increased more in emulsion samples than in sunflower oil samples (Tables 2 and 4). Derivatives without substitution at position 4 (DHPa,b) showed similar antioxidant activities in 20% emulsion as in sunflower oil and inhibited the formation of hydroperoxides. DHPc with menthyl group followed by hydrophylic DHPd compound, were the most active derivatives and showed higher antioxidant activities in 20% emulsion than in sunflower oil.

The generation of hexanal was detected from the first day of incubation and increased much more in emulsion samples than in sunflower oil samples (Table 5). DHPc followed by DHPd decreased the formation of hexanal more than control and other samples except BHT, which made BHT more effective in overall oxidation delay.

Pentanal was detected at very low levels in DHPe and DHPf [0.45–0.75 FID peak area (V_s) in static headspace GC] during last the two days of storage, and it was not detected in other samples. Note, that these two derivatives score lower for dienes and hexanal than other DHP derivatives in both bulk oil and emulsion.

3.4. Storage of sunflower oil in the dark and with light exposure

Samples of sunflower oil with 300 ppm of each DHP derivative or with 600 ppm of each plant extract were stored in the dark for 8 months and with light exposure for 8 weeks. The primary and secondary oxidation products

Table 2
Effect of 1,4-dihydropyridine (DHP) derivatives (300 ppm) on the formation of conjugated diene hydroperoxides in sunflower oil during storage in the dark at 60°C (mean ± SD; $n = 4$)

Samples	Storage period (days)						
	2	4	7	10	15	20	25
Sunflower oil	0.051 ± 0.004a	0.091 ± 0.003a	0.209 ± 0.005	0.409 ± 0.002	0.644 ± 0.014	0.814 ± 0.006	1.064 ± 0.010
+ BHT	0.043 ± 0.001b	0.057 ± 0.001b	0.083 ± 0.003a	0.115 ± 0.002	0.229 ± 0.002	0.371 ± 0.003	0.442 ± 0.006
+ DHPa	0.045 ± 0.001bc	0.054 ± 0.004bc	0.082 ± 0.001a	0.112 ± 0.002	0.223 ± 0.005	0.361 ± 0.009	0.430 ± 0.003
+ DHPb	0.041 ± 0.001	0.052 ± 0.001c	0.073 ± 0.001	0.095 ± 0.001	0.216 ± 0.005	0.347 ± 0.005	0.416 ± 0.016
+ DHPc	0.053 ± 0.001a	0.066 ± 0.001	0.124 ± 0.018	0.170 ± 0.003	0.339 ± 0.004	0.422 ± 0.002	0.458 ± 0.013
+ DHPd	0.057 ± 0.003	0.088 ± 0.001a	0.189 ± 0.013	0.207 ± 0.003	0.428 ± 0.008	0.626 ± 0.005	0.682 ± 0.015a
+ DHPe	0.047 ± 0.002c	0.085 ± 0.001	0.172 ± 0.001	0.195 ± 0.003	0.417 ± 0.005	0.612 ± 0.008	0.673 ± 0.010a
+ DHPf	0.053 ± 0.004a	0.096 ± 0.001	0.229 ± 0.003	0.310 ± 0.007	0.480 ± 0.011	0.677 ± 0.007	0.703 ± 0.003

Sunflower oil containing 570 ppm natural α-tocopherol. BHT, butylated hydroxytoluene. Conjugated diene values expressed as change in absorbance at 234 nm of 50 mg oil. Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

Table 3
Effect of 1,4-dihydropyridine (DHP) derivatives (300 ppm) on the formation of hexanal in sunflower oil during storage in the dark at 60°C (mean ± SD; $n = 4$)

Samples	Storage period (days)						
	2	4	7	10	15	20	25
Sunflower oil	BD	2.78 ± 0.6	8.24 ± 1.1	22.83 ± 2.1	31.75 ± 4.9	32.60 ± 5.6	36.48 ± 5.3
+ BHT	BD	BD	BD	1.04 ± 0.2	1.79 ± 0.3	1.90 ± 0.2	3.11 ± 0.3
+ DHPa	BD	BD	BD	2.47 ± 0.2	8.66 ± 2.1a	10.60 ± 2.3a	12.25 ± 2.5a
+ DHPb	BD	BD	BD	2.50 ± 1.2	7.83 ± 1.8b	8.60 ± 0.9	11.40 ± 0.8a
+ DHPc	BD	BD	4.50 ± 0.8b	6.70 ± 1.2	9.80 ± 2.1a	10.20 ± 1.5a	17.30 ± 1.5
+ DHPd	BD	0.48 ± 0.2a	6.45 ± 1.2a	9.25 ± 2.2b	13.30 ± 4.1	20.77 ± 4.5b	27.53 ± 5.5
+ DHPe	BD	0.50 ± 0.3a	5.24 ± 0.7b	4.40 ± 1.5	8.40 ± 1.9ba	11.50 ± 2.1a	20.72 ± 2.3
+ DHPf	BD	0.62 ± 0.2	6.60 ± 1.1a	8.70 ± 1.4b	12.40 ± 2.3	19.20 ± 1.6b	26.56 ± 2.1

Hexanal expressed as FID peak area (V_s) in static headspace gas chromatography (GC). BD is below detection level. Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

Table 4
Effect of 1,4-dihydropyridine (DHP) derivatives (300 ppm) on the formation of conjugated diene hydroperoxides in 20% sunflower oil-in-water emulsions during storage in the dark at 60°C (mean ± SD; $n = 4$)

Samples	Storage period (days)							
	1	2	3	4	5	6	7	8
Emulsion (control)	0.182 ± 0.003	0.305 ± 0.003	0.426 ± 0.009	0.576 ± 0.011	0.621 ± 0.005	0.706 ± 0.008	0.855 ± 0.006	1.168 ± 0.014
+ BHT	0.080 ± 0.002a	0.149 ± 0.003a	0.301 ± 0.010	0.383 ± 0.004	0.425 ± 0.006	0.494 ± 0.011	0.614 ± 0.008	0.635 ± 0.004
+ DHPa	0.081 ± 0.004a	0.151 ± 0.004a	0.280 ± 0.004	0.322 ± 0.003	0.342 ± 0.002	0.394 ± 0.004a	0.533 ± 0.004	0.564 ± 0.010
+ DHPb	0.089 ± 0.002b	0.140 ± 0.002b	0.232 ± 0.003	0.306 ± 0.007	0.333 ± 0.003	0.386 ± 0.007a	0.489 ± 0.011	0.547 ± 0.011a
+ DHPc	0.072 ± 0.001	0.136 ± 0.003	0.214 ± 0.004	0.252 ± 0.004	0.271 ± 0.003	0.334 ± 0.003b	0.431 ± 0.007	0.495 ± 0.014
+ DHPd	0.082 ± 0.004a	0.142 ± 0.003b	0.224 ± 0.003	0.261 ± 0.004	0.291 ± 0.003	0.338 ± 0.002b	0.469 ± 0.004	0.546 ± 0.006a
+ DHPe	0.089 ± 0.005b	0.162 ± 0.003	0.333 ± 0.007	0.478 ± 0.058	0.496 ± 0.004	0.673 ± 0.006	0.843 ± 0.007	1.013 ± 0.020
+ DHPf	0.129 ± 0.003	0.331 ± 0.004	0.412 ± 0.007	0.511 ± 0.004	0.610 ± 0.003	0.738 ± 0.010	0.873 ± 0.011	1.078 ± 0.015

Conjugated diene values expressed as change in absorbance at 234 nm of 50 mg extracted oil from emulsion. Twenty per cent sunflower oil-in-water emulsions were prepared from sunflower oil containing 570 ppm natural α-tocopherol. Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

Table 5
Effect of 1,4-dihydropyridine (DHP) derivatives (300 ppm) on the formation of hexanal in 20% sunflower oil-in-water emulsions during storage in the dark at 60°C (mean ± SD; $n = 4$)

Samples	Storage period (days)							
	1	2	3	4	5	6	7	8
Emulsion (control)	0.44 ± 0.2a	2.57 ± 0.7a	5.16 ± 0.8a	10.53 ± 1.2a	13.12 ± 1.3a	23.40 ± 2.2a	32.15 ± 3.1	50.43 ± 7.5
+ BHT	0.40 ± 0.1b	1.78 ± 0.4b	2.30 ± 0.2b	4.16 ± 0.3	5.81 ± 0.6	8.52 ± 0.9	8.92 ± 0.8	9.67 ± 1.1
+ DHPa	0.37 ± 0.1	2.10 ± 0.5c	6.80 ± 1.3c	10.88 ± 1.3a	13.34 ± 1.1a	19.50 ± 2.0b	23.35 ± 2.2a	26.10 ± 2.8a
+ DHPb	0.43 ± 0.2a	1.81 ± 0.4bc	6.95 ± 0.9c	11.80 ± 1.4b	13.60 ± 1.5a	18.44 ± 2.3b	21.38 ± 2.6a	24.50 ± 2.9a
+ DHPc	0.40 ± 0.2b	1.51 ± 0.3	2.65 ± 0.4b	6.38 ± 0.8	8.65 ± 1.4	11.23 ± 1.4	14.62 ± 1.6	18.38 ± 2.0
+ DHPd	0.25 ± 0.1	1.72 ± 0.2b	4.65 ± 0.8a	9.69 ± 1.3a	11.89 ± 1.4	17.10 ± 1.7	20.49 ± 1.9a	21.22 ± 2.1
+ DHPe	1.14 ± 0.4	2.92 ± 0.5ad	7.75 ± 0.8	12.90 ± 1.4b	16.95 ± 1.7b	21.60 ± 2.2a	26.10 ± 2.5b	29.60 ± 3.1ab
+ DHPf	0.82 ± 0.2	3.11 ± 0.6d	9.10 ± 0.9	14.50 ± 1.5	17.50 ± 1.5b	22.90 ± 2.4a	26.96 ± 2.4b	31.54 ± 3.3b

Hexanal expressed as FID peak area (V_s) in static headspace gas chromatography (GC). Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

Table 6
Effect of 1,4-dihydropyridine (DHP) derivatives (300 ppm) and plant extracts (600 ppm) on the formation of conjugated diene hydroperoxides in sunflower oil during storage in the dark at ambient temperature (mean ± SD; $n = 4$)

Samples	Storage period (month)						
	1	2	3	4	5	6	8
Sunflower oil	0.048 ± 0.002a	0.063 ± 0.003a	0.120 ± 0.006	0.223 ± 0.007	0.342 ± 0.009	0.410 ± 0.012	0.515 ± 0.014
+ BHT	0.040 ± 0.002b	0.045 ± 0.002b	0.047 ± 0.002a	0.050 ± 0.003a	0.056 ± 0.004a	0.060 ± 0.005a	0.065 ± 0.004a
+ DHPa	0.042 ± 0.002b	0.043 ± 0.002b	0.044 ± 0.002a	0.051 ± 0.002a	0.055 ± 0.003a	0.058 ± 0.004a	0.066 ± 0.004a
+ DHPb	0.043 ± 0.002b	0.044 ± 0.002b	0.045 ± 0.002a	0.048 ± 0.002a	0.049 ± 0.003	0.051 ± 0.003	0.055 ± 0.003
+ DHPc	0.044 ± 0.003b	0.052 ± 0.002c	0.054 ± 0.003	0.058 ± 0.003	0.068 ± 0.003	0.072 ± 0.003	0.088 ± 0.003b
+ DHPd	0.044 ± 0.002b	0.057 ± 0.004d	0.082 ± 0.004b	0.160 ± 0.004b	0.247 ± 0.007b	0.291 ± 0.007b	0.356 ± 0.010c
+ DHPe	0.043 ± 0.002b	0.060 ± 0.004d	0.085 ± 0.004b	0.151 ± 0.005	0.236 ± 0.007c	0.288 ± 0.009b	0.334 ± 0.012d
+ DHPf	0.043 ± 0.003b	0.057 ± 0.003d	0.090 ± 0.004c	0.161 ± 0.005b	0.270 ± 0.008d	0.344 ± 0.012	0.387 ± 0.014
+ Catnip	0.047 ± 0.003a	0.054 ± 0.003d	0.094 ± 0.004c	0.160 ± 0.005b	0.267 ± 0.008d	0.322 ± 0.009	0.364 ± 0.012c
+ Hyssop	0.043 ± 0.002b	0.061 ± 0.003ad	0.080 ± 0.003b	0.164 ± 0.006b	0.233 ± 0.006c	0.282 ± 0.010b	0.334 ± 0.011d
+ Lemon balm	0.044 ± 0.002b	0.062 ± 0.004ad	0.098 ± 0.005c	0.159 ± 0.006b	0.204 ± 0.006	0.260 ± 0.009	0.276 ± 0.012
+ Oregano	0.043 ± 0.002b	0.062 ± 0.003ad	0.098 ± 0.004c	0.162 ± 0.007b	0.243 ± 0.007b	0.274 ± 0.011b	0.298 ± 0.013
+ Sage	0.042 ± 0.002b	0.054 ± 0.003d	0.068 ± 0.003d	0.072 ± 0.003	0.078 ± 0.003	0.081 ± 0.003	0.092 ± 0.004b
+ Thyme	0.042 ± 0.002b	0.053 ± 0.002d	0.066 ± 0.004d	0.101 ± 0.004	0.125 ± 0.004	0.139 ± 0.004	0.158 ± 0.005

Conjugated diene values expressed as absorbance at 234 nm of 50 mg sunflower oil. Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

were monitored during storage periods. In general, all derivatives and plant extracts used in this study showed antioxidant activity in sunflower oil during storage in the dark (Table 6). Derivatives without substitution at position 4 (DHPa–c) showed highest antioxidant activity in the dark at ambient temperature similar to storage in the dark at 60°C. These derivatives and both sage and thyme extracts decreased the formation of hydroperoxide more than other derivatives and plant extracts. Derivatives with a phenyl group at position 4 (DHPd–f) showed lower antioxidant activity than others, and similar to storage in the dark at 60°C. Hexanal was detected only in the control oil [3.22 FID peak area (V_s) in static headspace GC] at the end of storage period of 8 months.

The effect of light exposure on the oxidative stability of sunflower oil samples in the presence of DHP derivatives and plant extracts is shown in Table 7. The formation of conjugated diene hydroperoxides increased much more in samples stored with light than in the dark. The DHP derivatives and BHT decreased the formation of hydroperoxides compared with control oil, and with the pro-oxidant activity of natural extracts. Hexanal was detected in all samples stored with light exposure (Table 8). The formation of hexanal increased significantly more in oil with plant extracts than in control oil. The DHP derivatives decreased the generation of hexanal less than BHT. Plant extracts increased both the formation of hydroperoxides (Table 7) and the generation of both hexanal and pentanal [1.2–2.5 flame ionization detector (FID) peak area (V_s) in static headspace GC], probably due to their content of chlorophylls. These compounds play an important role in the oxidative stability of oils and fatty foods. They are well-known photosensitizers and act as pro-oxidants in the light (Endo, Usuki, &

Table 7
Effect of 1,4-dihydropyridine (DHP) derivatives (300 ppm) and plant extracts (600 ppm) on the formation of conjugated diene hydroperoxides in sunflower oil during storage with light exposure at ambient temperature (mean ± SD; $n = 4$)

Samples	Storage period (weeks)					
	1	2	3	4	6	8
Sunflower oil	0.185 ± 0.006	0.224 ± 0.008	0.404 ± 0.012	0.483 ± 0.010	0.556 ± 0.018	0.607 ± 0.020a
+ BHT	0.110 ± 0.005a	0.162 ± 0.006	0.202 ± 0.006	0.225 ± 0.009	0.262 ± 0.010	0.338 ± 0.020
+ DHPa	0.133 ± 0.005	0.175 ± 0.006a	0.277 ± 0.008a	0.346 ± 0.015	0.464 ± 0.015	0.560 ± 0.025
+ DHPb	0.114 ± 0.004a	0.174 ± 0.006a	0.268 ± 0.007a	0.304 ± 0.012	0.430 ± 0.012	0.516 ± 0.025
+ DHPc	0.145 ± 0.005	0.188 ± 0.007	0.324 ± 0.007	0.388 ± 0.010	0.506 ± 0.011	0.574 ± 0.021
+ DHPd	0.175 ± 0.007	0.198 ± 0.009	0.375 ± 0.011	0.445 ± 0.011	0.521 ± 0.015	0.598 ± 0.018a
+ Catnip	0.256 ± 0.009	0.343 ± 0.010b	0.545 ± 0.015	0.686 ± 0.025a	0.818 ± 0.030	0.990 ± 0.036b
+ Hyssop	0.297 ± 0.010b	0.339 ± 0.009b	0.487 ± 0.015	0.705 ± 0.022a	0.876 ± 0.033a	1.100 ± 0.050
+ Lemon balm	0.441 ± 0.015	0.495 ± 0.015	0.574 ± 0.018	0.704 ± 0.026a	0.860 ± 0.028a	0.980 ± 0.035b
+ Oregano	0.336 ± 0.010	0.408 ± 0.012b	0.662 ± 0.022	0.774 ± 0.028	0.947 ± 0.036	1.240 ± 0.045
+ Sage	0.225 ± 0.008	0.292 ± 0.009	0.469 ± 0.012	0.570 ± 0.012	0.757 ± 0.026	0.920 ± 0.035
+ Thyme	0.295 ± 0.008b	0.395 ± 0.011b	0.605 ± 0.020	0.712 ± 0.025a	0.868 ± 0.032a	0.990 ± 0.040b

Conjugated diene values expressed as absorbance at 234 nm of 50 mg sunflower oil. Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

Table 8
Effect of 1,4-dihydropyridine (DHP) derivatives (300 ppm) and plant extracts (600 ppm) on the formation of hexanal in sunflower oil during storage with light exposure at ambient temperature (mean ± SD; $n = 4$)

Samples	Storage period (weeks)					
	1	2	3	4	6	8
Sunflower oil	BD	2.12 ± 0.50	5.65 ± 0.85a	7.54 ± 1.12a	9.15 ± 1.82a	12.25 ± 2.10a
+ BHT	BD	BD	BD	0.80 ± 0.10	1.10 ± 0.30	3.85 ± 0.65
+ DHPa	BD	BD	1.10 ± 0.25b	2.60 ± 0.45	3.92 ± 0.65	7.12 ± 1.20b
+ DHPb	BD	BD	0.82 ± 0.10	1.25 ± 0.25	2.75 ± 0.40	5.60 ± 0.85
+ DHPc	BD	BD	1.55 ± 0.40bc	3.34 ± 0.55	4.45 ± 0.65b	7.68 ± 1.60b
+ DHPd	BD	0.96 ± 0.55	1.86 ± 0.65c	4.12 ± 1.10	5.35 ± 1.20b	9.28 ± 1.80
+ Catnip	1.85 ± 0.50a	3.55 ± 0.65a	7.22 ± 1.50d	10.16 ± 1.55b	14.28 ± 2.2c	16.88 ± 3.40c
+ Hyssop	1.70 ± 0.35a	3.10 ± 0.50a	6.10 ± 0.80	10.12 ± 1.45b	15.40 ± 3.8c	18.10 ± 4.20c
+ Lemon balm	1.85 ± 0.45a	4.15 ± 0.75a	8.25 ± 1.10d	10.10 ± 1.55b	13.65 ± 2.60c	15.80 ± 3.20c
+ Oregano	2.10 ± 0.30a	3.22 ± 0.85a	8.80 ± 2.15d	11.75 ± 2.60b	14.40 ± 3.15c	18.15 ± 3.20c
+ Sage	0.88 ± 0.10	2.82 ± 0.50b	5.30 ± 0.60a	7.60 ± 0.88a	10.15 ± 1.60a	13.90 ± 2.10a
+ Thyme	1.20 ± 0.20	3.15 ± 0.55b	6.50 ± 0.90a	9.10 ± 1.50b	12.15 ± 2.30a	14.20 ± 3.30a

Hexanal expressed as FID peak area (V_s) in static headspace gas chromatography (GC). BD is below detection level. Values within each column followed by a different letter or without a letter are significantly different ($p < 0.05$).

Kaneda, 1984) and as antioxidants in the dark (Gutierrez-Rosales, Garrido-Fernandez, Gallardo-Guerrero, Gandul-Rojas, & Minguez-Mosquera, 1992). All edible fats and oils deteriorate under the effect of light exposure. In olive oil, it reduces the antioxidant activity of phenolic compounds (Servili, Baldioli, Miniati, & Montedoro, 1996), and it increases the flavonoid photodestruction during olive oil refining (Criado et al., 1996). Light decreases also the oxidative stability of canola oil triacylglycerols during storage (Neff, Mounts, & Rinsch, 1997). The study of Madsen, Sorensen, Skibsted, and Bertelsen (1998) showed that oil-in-water emulsion dressings with summer savoy or rosemary added as dried leaves or as an extract, had a significant antioxidative effect during storage in the dark. Exposure to fluorescent light during storage had a clear pro-oxidative effect on dressings with or without spices. In the present study the DHP derivatives showed less antioxidant activity during storage with light exposure than in the dark, and plant extracts exhibited pro-oxidant activity.

4. Conclusions

The derivatives of DHP without substitution at position 4 showed highest antioxidative activity in model system and sunflower oil and its emulsion in the dark at 60°C, as well as during storage of sunflower oil in the dark and with light exposure at ambient temperature. The hydrophilic derivative of DHP with phenyl group at position 4 showed high activity with DPPH and sunflower oil-in-water emulsion but was hardly active in model system and in sunflower oil. The hydrophobic derivatives of DHP with phenyl group at position 4 showed high antioxidant activity in model system but were the lowest active derivatives in sunflower oil and its emulsion. In most cases the DHP derivatives were less active antioxidants than BHT.

Acknowledgements

This research work was supported by Copernicus-IC project (15 CT 961006). Ahmed Abdalla is grateful for sabbatical leave from the Faculty of Agriculture, Saba Bacha, Alexandria University, Egypt. The authors thank Jan Cozijnsen for excellent technical assistance.

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