J Am Oil Chem Soc (2009) 86:895–902
DOI 10.1007/s11746-009-1411-6

ORIGINAL PAPER

Effects of Temperature and UV Light on Degradation of α-Tocopherol in Free and Dissolved Form

Cristina M. Sabliov · C. Fronczek · C. E. Astete · M. Khachaturyan · L. Khachatryan · C. Leonardi

Received: 22 December 2008 / Revised: 21 May 2009 / Accepted: 26 May 2009 / Published online: 21 June 2009

Abstract The effects of heat and UV exposure on the degradation of free α-tocopherol (oil form), α-tocopherol dissolved in methanol, and α-tocopherol dissolved in hexane were measured. Results showed that degradation of free α-tocopherol due to heat followed first order kinetics, with the samples held at 180 °C showing the greatest degradation rate. Free α-tocopherol degraded faster at high temperatures than dissolved α-tocopherol. In contrast, free α-tocopherol did not degrade when exposed to UV light for as long as 6 h, but the hexane and methanol samples degraded significantly as a matter of time. The α-tocopherol dissolved in hexane and methanol degraded by 20 and 70%, respectively over this time span. A mechanism for degradation of α-tocopherol was proposed to explain the higher degradation rate of α-tocopherol in methanol, as compared to hexane for times longer than 180 min. Knowledge of degradation kinetics of pure α-tocopherol as a result of temperature or exposure to UVA light whether in free or dissolved form is critically needed to understand how different processing parameters affect the amount of α-tocopherol during extraction, stabilization, storage or encapsulation processes.

Keywords α-Tocopherol · Antioxidant · Photooxidation · Hexane · Methanol · Heat degradation

C. M. Sabliov (✉) · C. Fronczek · C. E. Astete · M. Khachaturyan
Biological and Agricultural Engineering Department, Louisiana State University Agricultural Center, 141 E. B. Doran Bldg, Baton Rouge, LA 70803, USA
e-mail: csabliov@lsu.edu

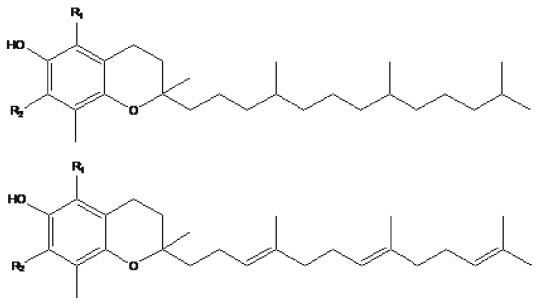
L. Khachatryan
Department of Chemistry, Louisiana State University, Baton Rouge, USA

C. Leonardi
Department of Experimental Statistics, Louisiana State University, Baton Rouge, USA

Introduction

Vitamin E, a fat-soluble vitamin exists in eight different forms, including four tocotrienols (α, β, γ, δ) and four tocopherols (α, β, γ, δ) [1]. The components of vitamin E possess an amphyphilic structure containing a hydrophobic isoprenoid side chain, and a hydrophilic chromanol ring (Fig. 1) [2]. The most biologically active component of vitamin E is α-tocopherol [1]; α-tocopherol is a pale, yellow, viscous oil that is insoluble in water but soluble in oils, fats, and certain organic solvents, such as acetone, ether, alcohol, and chloroform [3]. α-Tocopherol is capable of capturing free radicals and breaking lipid peroxidation chain reactions, thereby preventing the destruction of lipids [1]. Because of its antioxidant activity, α-tocopherol is claimed to possibly curb the effects of oxidative stress, preventing cardiovascular diseases, cancer, the aging process, and atherosclerosis. Its activity is believed to be mainly through scavenging reactive oxygen species (ROS), especially peroxyl radicals, and forming resonance-stabilized tocopheroxyl radicals (α-T•) [4].

Generally, the recommended intake of α-tocopherol through vitamin E supplements is approximately 22.7 mg/day for adults and possibly as high as 600–800 mg/day to address therapeutic concerns [5]. Good sources of α-tocopherol are plants such as wheat germ (~1,920 mg/L), sunflower seed (~590 mg/L), extra virgin olive oil (188 mg/L), peanut oil (174 mg/L), corn oil (225 mg/L) and



Tocopherol & Tocotrienol	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
Δ	H	H

Fig. 1 Vitamin E family formed of α, β, γ, δ tocopherols and tocotrienols. Adapted from reference [2]

soybean oils (89 mg/L) [6, 7]. While several methods of extracting α-tocopherol from such sources have been presented, it has been noted that at high temperatures, some quantity of α-tocopherol degrades over time [1, 2]. Alpha-tocopherol is stable at high temperatures if no oxygen is present [8, 9], but in normal, atmospheric conditions, the rate of oxidation of α-tocopherol increases, leading to increased α-tocopherol degradation [8].

Shin et al. [8] demonstrated that the amount of α-tocopherol lost through stabilization by extrusion of rice bran at higher temperatures was higher than the amount stabilized at lower temperatures. When stabilized at a temperature of 110 °C, 86.1% α-tocopherol was retained after a 6-min holding time whereas at 140 °C, only 76.2% α-tocopherol was still available after the same holding time. Hakansson and Jagerstad [10] showed that white flour lost up to 70–80% of its vitamin E at temperatures over about 150 °C when heated for 15 min. Studies on α-tocopherol degradation in typical oil models such as olive oil and triolein were also reported [9, 11, 12]. The losses of α-tocopherol were 5.7% in triolein exposed to 180 °C for 60 min at 40 mbar [9]. In comparison, when the samples were under nitrogen, the α-tocopherol losses were not significant (0.3%). The highest loss of α-tocopherol (13.7%) was observed under 260 °C for 80 min at 40 mbar. Nissiotis and Tasioula-Margari [12] found that samples of olive oil heated to 100 °C at atmospheric pressure lost significantly more α-tocopherol than did samples heated at 60 °C, which was reflected in the different time scales required to degrade 100% of the α-tocopherol. A complete degradation of α-tocopherol occurred in samples stored at 60 °C for 30 days. The samples at 100 °C only required 100 h of heating time before a complete degradation of α-tocopherol occurred.

In addition to extended exposure to certain temperatures, several other factors such as oxygen, alkali, light, minerals, and hydroperoxides degrade α-tocopherol [13]. The photooxidation of α-tocopherol has been studied by different groups [14–19]. Pirisi et al. [14] showed that a solution of olive oil in hexane irradiated with artificial light ($\lambda = 290$ nm), or sunlight degraded at a rate constant (k) of 1.86×10^{-4} s^{-1} and 1.03×10^{-4} s^{-1}, respectively with a halftime $t_{1/2}$ values of 112 and 62 min, respectively. After olive oil was exposed for 2,500 min to artificial light, the majority of α-tocopherol was degraded (only 17% of the initial amount remaining). Psomiadou and Tsimidou [15] reported a pseudo-first order reaction for α-tocopherol degradation in olive oil by fluorescent light, with a rate constant estimated at 0.0461×10^{-4} s^{-1} [15]. After 100 h, less than 10% of α-tocopherol was detected.

While these studies present relevant data on α-tocopherol degradation as a function of temperature and UV exposure, their focus is on the degradation of the vitamin as a component of a lipid matrix or a constituent of a cereal. The purpose of our study was to quantify degradation of pure α-tocopherol as a function of temperature and UV radiation when exposed to these factors in free and dissolved forms. This information is critically needed to understand the effects of temperature and UV light on degradation of α-tocopherol during extraction, storage, and encapsulation processes.

Materials and methods

Materials

The 97% α-tocopherol (5,7,8-trimethyl-tocol) used in this study was obtained from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO). Isopropyl alcohol was purchased from EMD (EMD Chemicals Inc., Gibbstown, NJ) and methanol, anhydrous, was obtained from Mallinckrodt Chemicals (Mallinckrodt Chemicals Inc., St. Louis, MO). Hexane (95%) was purchased from JT Baker (JT Baker Chemical Co., Phillipsburg, NJ).

Methodology

Temperature Degradation of Free α-Tocopherol

Degradation of free α-tocopherol was measured at temperatures of 40, 60, 120, and 180 °C under atmospheric pressure. A 2–4 mg sample of α-tocopherol was weighed in 5 ml amber vials, and the vials were placed into a

preheated Isotemp Model 285A Vacuum Oven (Fisher Scientific, Hampton, NH). The various initial amounts were a result of the high viscosity of the oil, which prohibited weighing the same amounts of α-tocopherol each time. The appropriate samples were removed from the oven after each hour for a maximum time of 6 h. Four ml of hexane was added to the degraded sample, of which 1 ml was removed, diluted with 1 ml of mobile phase and analyzed by HPLC (see method below). Degradation was calculated via the ratio of the measured amount to the initial amount.

Degradation of Dissolved α-Tocopherol in Methanol and Hexane Solution at 40 °C

Five mg of α-tocopherol was dissolved in 25 ml of hexane to produce a stock solution with 0.2 mg/ml α-tocopherol. One ml aliquots of this solution were transferred to amber chromatographic vials. The vials were placed in a preheated Isotemp Model 285A Vacuum Oven (Fisher Scientific, Hampton, NH) and were maintained at 40 °C. Samples were removed every hour for a maximum of 6 h and were analyzed by HPLC. Degradation was calculated via the ratio of the measured amount to the initial amount. The degradation of α-tocopherol at 40 °C when dissolved in methanol was assessed in a similar manner.

Degradation of α-Tocopherol Under UV Light

Degradation of α-tocopherol under UV light was studied for free α-tocopherol, and for dissolved α-tocopherol in methanol and hexane. For the free α-tocopherol degradation study, 2–4 mg of free α-tocopherol was added to clear, 4-ml glass vials. The capped vials were placed at a distance of 15 cm from the light source under a Blak Ray 1B 100P MDSK long wave ultraviolet lamp (ThermoSpectronic, Madison, WI) with a wavelength of 365 nm operating at 100 W. Samples were removed after 15, 30 and 60 min, after which samples were removed at 1-h intervals for a maximum of 6 h. Four ml of hexane was added to the degraded sample, 1 ml was removed, placed in an HPLC vial and following addition of 1 ml of hexane, the sample was injected into the HPLC. The ratio of the measured amount by HPLC at each time to the initial α-tocopherol amount was calculated and used as an indicator of α-tocopherol degradation. The degradation of α-tocopherol in dissolved form by UV light was evaluated in a manner similar to that described previously by first making a stock solution of the sample dissolved in the solvent of choice (methanol or hexane) at a concentration of 0.22 mg/ml followed by the exposure process described for the free form.

HPLC Measurement of α-Tocopherol

The α-tocopherol concentration was measured by normal-phase HPLC. An Agilent Series 1200 HPLC (Agilent, San Jose, CA) equipped with an auto-sampler and an Eclipse XDB-CN, 4.6 × 150, 5-μm column was used. The mobile phase was a mixture of 98% hexane and 2% isopropanol, flowing at 0.3 ml/min (with an elution time of 8 min). Alpha-tocopherol was detected with a multi-wavelength Fluorescence 1200 detector set at an excitation wavelength of 295 nm, and an emission wavelength of 330 nm. The column temperature was set to 25 °C, and the injection volume of the samples was 0.5 μl. Chromatograms were recorded and processed using 03.01 Agilent Chemstation software. Areas were converted to concentrations using a standard curve of α-tocopherol in hexane ($R^2 = 0.9978$) in the range of concentrations relevant for the sample concentrations.

All experiments were performed under atmospheric conditions, in triplicate (i.e. three different samples were exposed to the same conditions and degradation measured once per sample).

Statistical Analysis

Data was analyzed using the MIXED procedure of SAS (SAS system, SAS Institute Inc., Cary, NC). Residuals produced within each analysis were randomly distributed around 0 unless otherwise specified indicating that each model was a good fit. When multiple comparisons between treatment means or degradation rates were performed the Bonferroni post hoc adjustment was applied. Statistical significance was declared at $P \leq 0.05$. Specifics for each analysis are reported below.

Temperature Degradation of Free α-Tocopherol The fixed categorical effect of temperature, time and their interaction on free α-tocopherol degradation ratio (c/c_0, where c-concentration of α-tocopherol at a specific time point and c_0- initial α-tocopherol concentration) was tested. The response variable was analyzed on a natural log transformed base. The slice option in SAS was used to determine if there was an overall temperature effect within each time point. When a significant temperature effect within a time point was observed, estimated temperature means were compared. First order degradation rate for free α-tocopherol were estimated and compared across temperatures by transforming the degradation ratio (c/c_0) using a natural log. Time treated as a continuous variable was regressed against the $\ln(c/c_0)$. Regression lines for each temperature were forced trough the origin (0, 0) by fitting a no intercept model.

Degradation of α-Tocopherol Either Under UV Light or at 40 °C in Free and Dissolved Form The fixed categorical

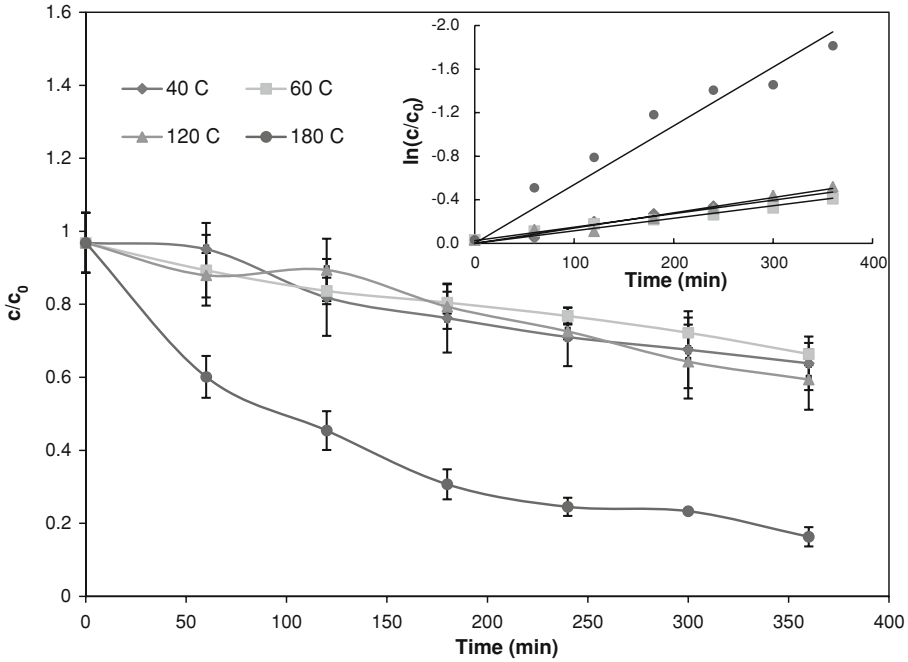


Fig. 2 Free α-tocopherol thermal degradation as a function of time showing change of concentration relative to the initial α-tocopherol concentration, c/c_o. *Insert* shows first order degradation kinetics with the following correlation coefficients 0.962, 0.947, 0.961, and 0.932 for 40, 60, 120, and 180 °C, respectively. The c/c_0 ratio at all time points except 0 and degradation rates significantly differed between 180 °C and remaining temperatures ($P \leq 0.05$). No significant differences were observed in c/c_0 ratio among 40 °C, 60 °C and 120 °C at any time point. Degradation rates for 40 °C, 60 °C and 120 °C were not significantly different either

effect of solvent type (hexane, methanol or no solvent), time and their interaction on α-tocopherol degradation ratio (c/c_0) was tested. The slice option in SAS was used to determine if there was an overall solvent effect within each time point. When a significant solvent effect within a time point was observed, estimated solvent type means were compared. The 95% confidence intervals (95% CI) were calculated for each mean within each time point to verify if estimated mean was significantly different from 1 which indicated no degradation. First order degradation rate for free α-tocopherol was estimated and compared between free and hexane dissolved α-tocopherol exposed to UV light as described in the previous section. The degradation rate of α-tocopherol dissolved in methanol was calculated using the first 180 min, because after 180 min the degradation was no longer following a first order kinetics mechanism.

Results and Discussions

Thermal Degradation of Free α-Tocopherol

Degradation of free α-tocopherol as a function of temperature showed that the higher the temperature, the greater the degradation (Fig. 2). In particular, the c/c_0 ratio was significantly lower at 180 °C compared to the remaining temperatures at all time points. The c/c_0 ratio did not significantly differ across 40 °C, 60 °C and 120 °C at any time point. The samples heated at 40, 60, and 120 °C degraded at a non significantly different rate, with $t_{1/2}$ values of 8.2, 10.1, and 8.2 h, respectively (Table 1).

Table 1 Free α-tocopherol first order degradation kinetics at 40, 60, 120, and 180 °C

	Temperature				Temperature effect	
	40 °C	60 °C	120 °C	180 °C	SEM	*P* value
k (h^{-1})	0.085^b	0.069^b	0.085^b	0.325^a	0.007	<0.0001
$t_{1/2}$ (h)	8.2	10.1	8.2	2.1		

Means within a row marked with different letter are significantly different ($P \leq 0.05$)

SEM standard error of the mean

Samples heated to 180 °C degraded at a significantly higher rate compared to the remaining temperatures with a $t_{1/2}$ value of 2.1 h and a degradation constant "k" of 0.325 h^{-1} (0.903×10^{-4} s^{-1}). More than half (55%) of the α-tocopherol had degraded after 2 h at 180 °C, and nearly 80% of the free α-tocopherol had degraded following 5 h of exposure at this temperature. By contrast, at 60 °C, only about 30% of the free α-tocopherol had degraded after 5 h. Less than 20% of the α-tocopherol degraded if heating was applied for less than 2 h, and temperature did not exceed 120 °C (Fig. 2). At 180 °C, degradation of α-tocopherol was much more rapid and should be avoided during extraction of α-tocopherol and stabilization of α-tocopherol containing crops, when maintaining the activity of this component is desirable.

As a comparison, Verleyen et al. [11] showed that the residual amount of α-tocopherol in triolein exposed to 150 and 200 °C for a period of 2 h was 23.5 and 5.3%, respectively. The different degradation kinetics reported by Verleyen et al. [11] as compared to those found in our

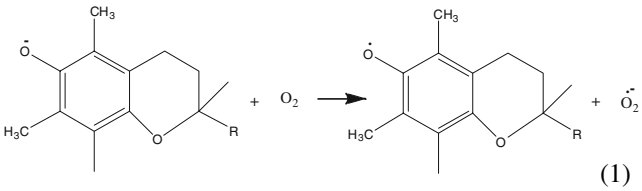
study were attributed to differences in the form of α-tocopherol exposed to high temperatures, free (for our study) or as a component of an oil (Verleyen's study), as well as different temperature tested in the two studies.

Degradation of α-Tocopherol Dissolved in Methanol and Hexane at 40 °C

The c/c_0 ratio significantly changed across solvent types (methanol, hexane or no solvent) depending on the exposure time. The 95% CI for α-tocopherol dissolved in methanol solution and exposed to a temperature of 40 °C included the value of 1 across all time points revealing that no degradation of α-tocopherol occurred in 6 hours (Fig. 3). By contrast, free α-tocopherol heated at the same temperature significantly degraded by 40% after 6 h. At any time point past 2 h of exposure, the c/c_0 ratio was significantly lower for free α-tocopherol than α-tocopherol dissolved in either methanol or hexane. There was no difference in c/c_0 ratio between methanol and hexane at all time points except after 6 h of exposure. This shows that organic solvents such as methanol and hexane protected vitamin E from heat degradation. The higher rate of degradation of α-tocopherol in free form can be explained considering that a volume of 1 ml of α-tocopherol in dissolved form was 475 bigger than the volume occupied by 2 mg of free α-tocopherol. Hence, more oxygen was available and in contact with the free α-tocopherol as compared to the dissolved α-tocopherol, which explains the higher rate of degradation of α-tocopherol in free form. The results were compared against literature findings, which also showed that 40 °C was not high enough to cause major degradation of α-tocopherol in the solubilized form, regardless of the solvents used (i.e. methanol or hexane) [13].

After 4 h of exposure at 40 °C, α-tocopherol dissolved in hexane showed some degradation and after 6 h, degradation of α-tocopherol dissolved in hexane was significantly higher than that in methanol. The amount of dissolved oxygen in hexane or methanol is one of the factors that made a difference in degradation of α-tocopherol dissolved in these solvents, as described below.

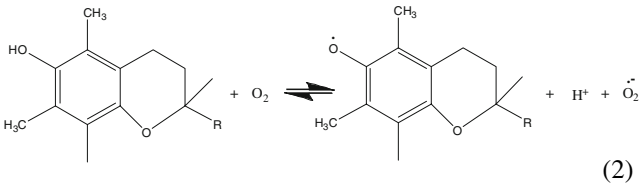
α-Tocopherol dissociates to some extent in organic solvents (to a higher extent in methanol than in hexane) to form α-tocopherol anion which can undergo one electron oxidation in the presence of dissolved oxygen resulting in formation of tocopheroxy radical and superoxide radical, reaction 1 [20, 21],



(1)

where the R group represents the side hydrocarbon chain.

More generally, the degradation of α-tocopherol by slow oxidation can be presented as reaction 2:



(2)

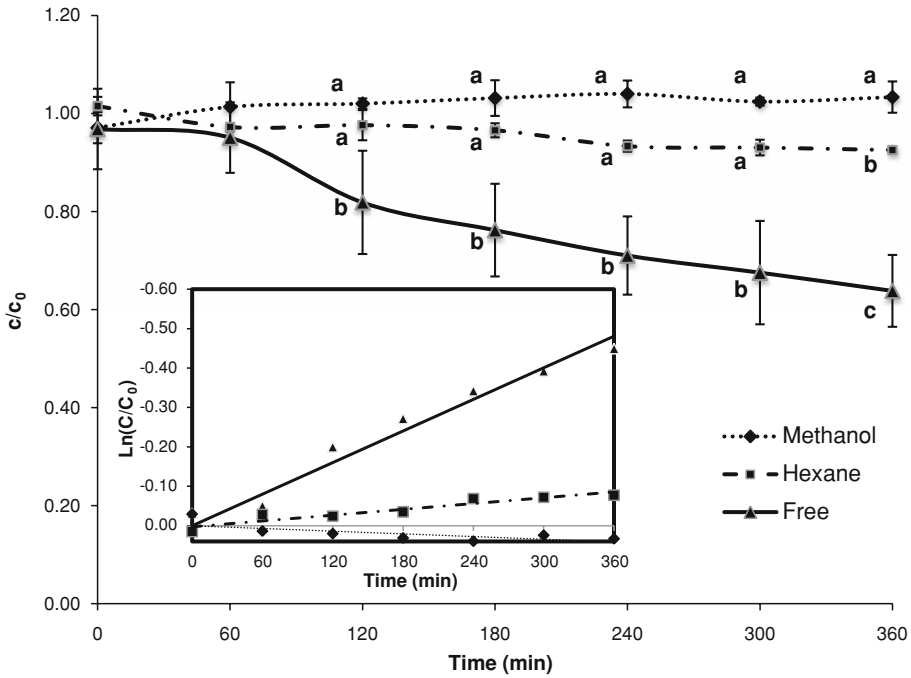


Fig. 3 Degradation of α-tocopherol in free form, and dissolved form in either methanol or hexane at 40 °C. The presence of letters within each time point indicates a significant solvent type effect ($P \leq 0.05$) and different letters within each time point indicate significant differences between solvent types ($P \leq 0.05$). Correlation coefficient for free α-tocopherol was 0.962, and α-tocopherol in hexane was 0.896

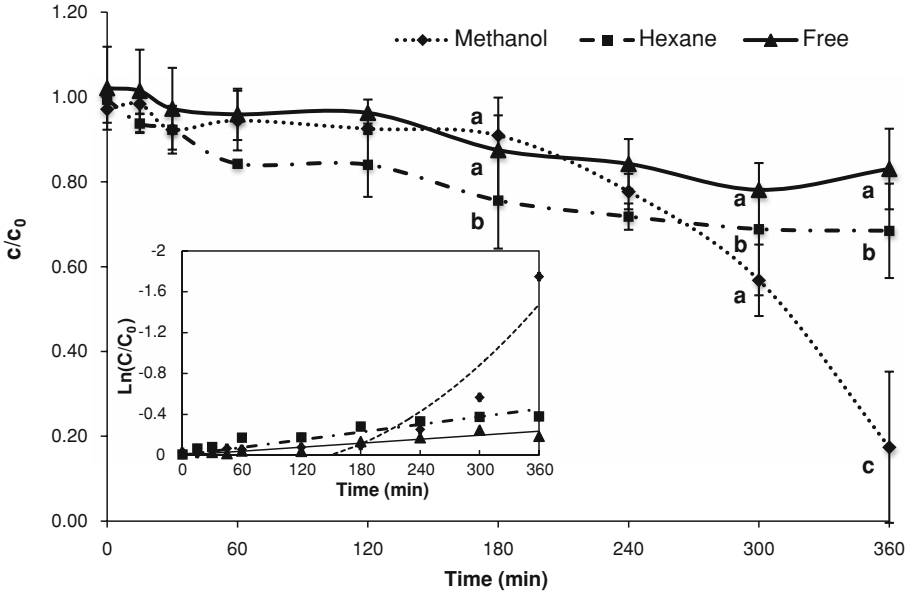
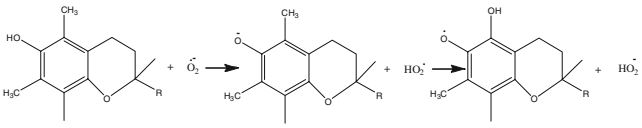


Fig. 4 Degradation of α-tocopherol in free form, dissolved in methanol, and dissolved in hexane when exposed to UV light at room temperature. The presence of letters within each time point indicates a significant solvent type effect ($P \leq 0.05$) and different letters within each time point indicate significant differences between solvent types ($P \leq 0.05$). The correlation coefficients were 0.855 for hexane, and 0.894 for free α-tocopherol for first order reactions. Methanol presented a value of 0.883 for a second order reaction

Further interaction of the superoxide radical with the initial α-tocopherol can cause the chain reaction 3:



(3)

The $HO_2^{\cdot}$ radicals are somewhat more reactive than superoxide radicals [20] and they can additionally interact with α-tocopherol to degrade it further. It is evident from reactions 1–3 that the more oxygen is dissolved in the solvents the more degradation of α-tocopherol will be experimentally seen. In this respect, thermal degradation of α-tocopherol will be more significant in hexane than in methanol as observed in Fig. 3 after 360 min exposure at 40 °C, due to higher oxygen concentrations in hexane (1.93×10^5 mol fraction) in comparison with methanol (0.41×10^3 mol fraction) [22].

Degradation of α-Tocopherol Under UV Light in Free and Dissolved Form

After 3 h of UV light exposure, the c/c_0 ratio was significantly different across solvent types (hexane, methanol and no solvent, Fig. 4). Furthermore, the estimated 95% CI did not include the value of 1 for each solvent after 3 h of exposure indicating that neither of the solvents inhibited α-tocopherol degradation. The dissolved form of α-tocopherol degraded significantly more than the free form when exposed to UV degradation for more than 3 h. Alpha-tocopherol degraded following first order kinetics when in free form, and when dissolved in hexane. Alpha-tocopherol in methanol solution degraded according to second order kinetics.

Table 2 Kinetics of degradation of α-tocopherol exposed to UV light in free form and dissolved in methanol and hexane

	Solvent type			Solvent effect
	Free	Hexane	SEM[a]	*P* value
k (h^{-1})	0.040[b]	0.077[a]	0.005[&]	<0.0001
$t_{1/2}$ (h)	17.5	9.0		

Means within a row marked with different letter are significantly different ($P \leq 0.05$)

[a] SEM = standard error of the mean. [&]SEM for free alpha-tocopherol and hexane solution

The degradation constant (k) of free α-tocopherol was 0.040 h^{-1} (0.1×10^{-4} s^{-1}), and the half-time ($t_{1/2}$) was 17.5 h (Table 2). While this degradation occurred at a low rate, as indicated by a small degradation constant and a large $t_{1/2}$ value, it did appear to occur according to first order kinetics. A possible explanation for this fairly low rate of degradation of free α-tocopherol is the fact that the solubility of oxygen through pale, free oils is low [23]. The data compiled on olive oil, as an example, show a Bunsen coefficient of 0.1162, with a mole fraction of 5.07×10^3 [23]. As demonstrated by this low solubility of oxygen in pale oils, UVA induced oxidation of α-tocopherol occurs at a very low rate, causing a very low degree of degradation of the free α-tocopherol. In comparison, dissolved α-tocopherol showed higher degradation rates (Table 2). Other studies also showed that α-tocopherol oxidation occurred under UVB light in the absence of lipid peroxyl

radicals, when α-tocopherol was dissolved in acetonitrile/water solution [18].

Alpha-tocopherol dissolved in hexane and exposed to UV light degraded according to first order kinetics (Fig. 4), with a $t_{1/2}$ value of 9.0 h and a constant value of "k" at 0.077 h^{-1} (0.214×10^{-4} s^{-1}). While degradation in hexane solution appeared to be less severe over 6 h than degradation in methanol solution, it was significant. This degradation occurred via UV-induced oxidation of the dissolved α-tocopherol as described above (reactions 1–3) as a result of the fact that oxygen is quite soluble in hexane, allowing for photooxidation of α-tocopherol to occur (the Bunsen coefficient of oxygen solubility in hexane is 0.329, with a mole fraction of 19.3×10^4 [24], process accelerated by the formation of excited molecules of α-tocopherol under UV light irradiation. Alpha-tocopherol in hexane solution degraded to about 68% of its original concentration after 6 hours, whereas free α-tocopherol degraded to 83% of its concentration after the same time of exposure to UV light.

Samples of α-tocopherol dissolved in methanol, following exposure to UV light showed that there was significant degradation within a time frame of 6 h, which could be described by second order kinetics (Fig. 4). The lower solubility of oxygen in methanol with a Bunsen coefficient of 0.2268 and a mole fraction of 0.4122×10^3 [22] when compared to that in hexane is the responsible factor for the initial lower rate of α-tocopherol degradation in methanol, as compared to hexane.

The significant increase in the degradation rate of α-tocopherol dissolved in methanol after 180 min and the higher rate of degradation observed in methanol as compared to hexane can be explained by several mechanisms.

1. In organic solvents, the superoxide radical-anions generated by reactions 1–3 have the ability to convert the available protons to very active hydroxyperoxyl radical [25] by reaction 4 (more prevalent in methanol than hexane):

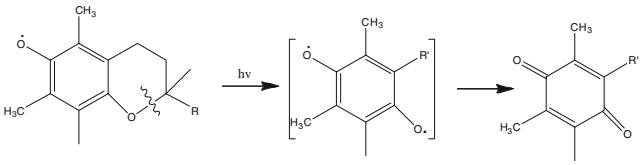
$$H^+ + O_2^{\cdot -} \rightarrow HO_2^{\cdot} \quad (4)$$

The hydroxyperoxyl radicals can be further converted to hydrogen peroxide by hydrogen abstraction or disproportionation reactions; the hydrogen peroxide in turn initiates the degradation process of α-tocopherol. Hydrogen peroxide is also very sensitive to the UV light [26, 27] and can generate OH radicals under 365 nm UV irradiation by reaction 5:

$$H_2O_2 + hv \rightarrow 2OH \quad (5)$$

The OH radicals are commonly known as a very effective oxidative species and can also contribute to the fast degradation of α-tocopherol. As a result, after 180 min UV exposure when a critical amount of hydrogen peroxide was accumulated, α-tocopherol degraded drastically in methanol solution, much faster than α-tocopherol dissolved in hexane (Fig. 4).

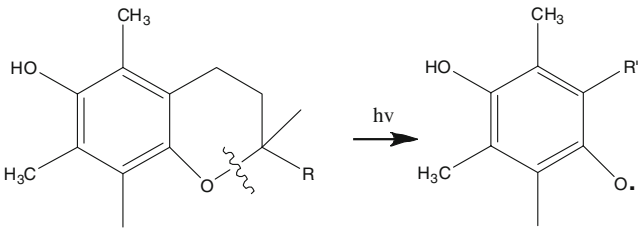
2. While hexane is transparent to UV light, methanol can absorb UV light and generate methoxy radicals [26]. The methoxy radicals are very active and enhance the chain process generating additional tocopherol oxy radicals. These radicals undergo further photo excitation [28], converting finally to quinons by reactions sequence:



(6)

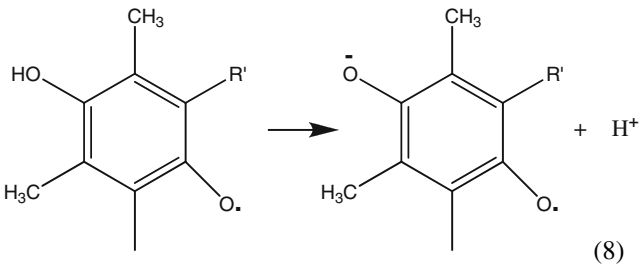
where R' is a modified hydrocarbon group formed after cleavage of the ether bond.

3. UV light initiates formation of not only α-tocopherol oxy radical (breaking O–H bond [27]), but it can also break the weak ether linkage at the marked place in following reaction 7.



(7)

The oxy radical formed can be dissociated in methanol to some extent easier than in hexane, making a semiquinone anion-radical:



(8)

The new semiquinone anion-radical interacts with oxygen [21] to convert into a di-radical, which is further converted to quinones in analogy to reaction 6.

Therefore it can be concluded from this analysis and the experimental data that dissolving α-tocopherol in an organic

solvent does not prevent UV degradation of the compound; rather it appears that degradation due to UV exposure increases under these circumstances, especially in methanol.

Conclusions

Heat degradation of free α-tocopherol followed first order kinetics, with the samples held at 180 °C showing the greatest degradation rate. While free α-tocopherol degraded at temperature as low as 40 °C, organic solvents such as hexane and methanol protected α-tocopherol from degradation for 4 h at 40 °C. The rate of degradation of α-tocopherol dissolved in hexane was significantly higher than that in methanol due to the higher solubility of oxygen in hexane. In contrast to the heat experiment, α-tocopherol dissolved in methanol and hexane solutions clearly degraded under UV light. While degradation of α-tocopherol in hexane followed first order kinetics, α-tocopherol degradation in methanol was described by a combination of first order and second order kinetics. The lower rate of degradation of α-tocopherol dissolved in methanol at times shorter than 4 h was associated with lower solubility of oxygen in methanol, as compared to hexane. The drastic increase in the α-tocopherol degradation rate at longer times in methanol (not observed in hexane) was attributed mostly to formation of methoxy radicals, hydrogen peroxide formation and conversion of α-tocopherol to oxy radicals that enhanced oxidation of α-tocopherol. The kinetics and mechanisms of α-tocopherol degradation unveiled in this study are significant for selecting the optimum conditions for processes such as α-tocopherol extraction, stability, encapsulation, and storage studies such as temperature and UV light, parameters which have been shown to affect degradation of the vitamin.

References

1. Traber MG (1999) Utilization of Vitamin E. Biofactors 10:115–120
2. Ricciarelli R, Zingg JM, Azzi A (2002) The 80th Anniversary of Vitamin E: beyond its antioxidant properties. Biol Chem 383:457–465
3. Gyorgy P, Tomarelli RM (1945) The stabilization of carotene in linoleic acid by tocopherol and rice bran extract. Fed Proc 4:155 155
4. Sabliov CM, Astete CE (2008) Encapsulation and controlled release of antioxidants and vitamins. In: Garti N (ed) Delivery and controlled release of bioactives in foods and nutraceuticals. CRC Press, New York, pp 297–330
5. Berman K, Brodaty H (2004) Tocopherol (Vitamin E) in Alzheimer's disease and other neurodegenerative disorders. CNS Drugs 18:807–825
6. Cabrini L, Barzanti V, Cipollone M, Fiorentini D, Grossi G, Tolomelli B, Zambonin L, Landi L (2001) Antioxidants and total peroxyl radical-trapping ability of olive and seed oils. J Agric Food Chem 49:6026–6032
7. Schwartz H, Ilainen V, Pfironen V, Lampi MA (2008) Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. J Food Compos Anal 21:152–161
8. Shin TS, Godber JS, Martin DE, Wells JH (1997) Hydrolytic stability and changes in E vitamers and oryzanol of extruded rice bran during storage. J Food Sci 62:704–708
9. Verleyen T, Verhe R, Huyghebaert A, Dewettinck K, De Greyt W (2001) Identification of alpha-tocopherol oxidation products in triolein at elevated temperatures. J Agric Food Chem 49:1508–1511
10. Hakansson B, Jagerstad M (1990) The effect of thermal inactivation of lipoxygenase on the stability of Vitamin-E in wheat. J Cereal Sci 12:177–185
11. Verleyen T, Kamal-Eldin A, Dobarganes C, Verhe R, Dewettinck K, Huyghebaert A (2001) Modeling of alpha-tocopherol loss and oxidation products formed during thermoxidation in triolein and tripalmitin mixtures. Lipids 36:719–726
12. Nissiotis M, Tasioula-Margari M (2002) Changes in antioxidant concentration of virgin olive oil during thermal oxidation. Food Chem 77:371–376
13. Zigoneanu IG, Williams L, Xu Z, Sabliov CM (2008) Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. Bioresour Technol 99:4910–4918
14. Pirisi FM, Angioni A, Bandino G, Cabras P, Guillou C, Maccioni E, Reniero F (1998) Photolysis of alpha-tocopherol in olive oils and model systems. J Agric Food Chem 46:4529–4533
15. Psomiadou E, Tsimidou M (2002) Stability of virgin olive oil. 2. Photo-oxidation studies. J Agric Food Chem 50:722–727
16. Psomiadou E, Tsimidou M (2002) Stability of virgin olive oil. 1. Autoxidation studies. J Agric Food Chem 50:716–721
17. Grams GW, Inglett GE (1972) Sensitized photooxidation of alpha-tocopherol and of 2, 2, 5, 7, 8-pentamethyl-6-chromanol in ethyl acetate. Lipids 7:442–444
18. Kramer KA, Liebler DC (1997) UVb induced photooxidation of vitamin E. Chem Res Toxicol 10:219–224
19. Miyazawa T, Yamashita T, Fujimoto K (1992) Chemiluminescence detection of 8a-hydroperoxy-tocopherone in photooxidized alpha-tocopherol. Lipids 27:289–294
20. Halliwell B, Gutteridge JMC (1990) Role of free-radicals and catalytic metal-ions in human-disease—an overview. Methods Enzymol 186:1–85
21. Zang LY, Stone K, Pryor WA (1995) Detection of free-radicals in aqueous extracts of cigarette tar by electron-spin-resonance. Free Radical Biol Med 19:161–167
22. Kretschmer CB, Nowakowska J, Wiebe R (1946) Solubility of oxygen and nitrogen in organic solvents from -25-Degrees-C to 50-Degrees-C. Ind Eng Chem 38:506–509
23. Battino R, Evans FD, Danforth WF (1968) Solubilities of 7 gases in olive oil with reference to theories of transport through cell membrane. J Am Oil Chem Soc 45:830–833
24. Van Slyke DD (1939) Determination of solubilities of gases in liquids with use of the Van Slyke–Neill manometric apparatus for both saturation and analysis. J Biol Chem 130:545–554
25. Kohl DH, Wright JR, Weissman M (1969) Electron spin resonance studies of free radicals derived from plastoquinone, alpha- and gamma-tocopherol and their relation to free radicals observed in photosynthetic materials. Biochim Biophys Acta 180:536–544
26. Schinke R (1993) Photodissociation dynamics; spectroscopy and fragmentation of small polyatomic molecules. Cambridge University Press, Cambridge
27. Calvert JG, Pitts JN Jr (1966) Photochemistry. Wiley, New York
28. Hales BJ, Case EE (1981) Immobilized radicals. 4. Biological semi-quinone anions and neutral semiquinones. Biochim Biophys Acta 637:291–302