J Amer Oil Chem Soc (2007) 84:363–368
DOI 10.1007/s11746-007-1046-4

ORIGINAL PAPER

Effects of Antioxidants on the Oxidative Stability of Oils Containing Arachidonic, Docosapentaenoic and Docosahexaenoic Acids

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Received: 30 May 2006 / Accepted: 8 February 2007 / Published online: 15 March 2007
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Abstract The effects of ascorbyl palmitate at 0, 300, 600, 900, or 1,200 ppm, tocopherol at 0, 200, 400, 600, or 800 ppm, and β-carotene at 0, 3, 6, 9, or 12 ppm on the oxidative stabilities of Oil 1, Oil 2, and Oil 3 containing 0, 0.55, and 0.67% of combined arachidonic acid (AA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), respectively, were studied by a central composite experimental design. The oxidative stability of oil was evaluated by determining the induction time using the Oxidative Stability Index. Ascorbyl palmitate and tocopherol had a significant effect on the stability of all three oils at $\alpha = 0.05$. β-Carotene did not have any effect on the stability of oils at $\alpha = 0.05$. The interaction effect of ascorbyl palmitate and tocopherol was significant for the three oils at $\alpha = 0.05$. The induction time of oils decreased as the total amounts of AA, DPA and DHA increased from 0 to 0.55% or 0.55 to 0.67%. The addition of 1,200 ppm ascorbyl palmitate, 800 ppm tocopherol and 12 ppm β-carotene to Oils 1, 2, and 3 increased the induction time from 13.5 to 29.9 h, from 11.8 to 27.0 h, and from 10.5 to 20.0 h, respectively. The coefficient of determination (r^2) for the linear regression between the experimentally determined and statistically predicted induction time of the three oils was greater than 0.95. The use of an optimum combination of ascorbyl palmitate and tocopherols from the response surface analysis could improve the oxidative stabilities of oils containing AA, DPA and DHA.

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Keywords Antioxidant · Oxidative stability · Arachidonic acid · Docosapentaenoic acid · Docosahexaenoic acid

Introduction

Lipid oxidation can adversely affect the nutritional value, sensory quality, and shelf-life of foods [1–3]. Oils should have a good oxidative stability to maintain the flavor quality of foods [4]. As the concentration of highly unsaturated double bonds increases, the oil becomes more unstable and consequently becomes more susceptible to oxidation [5]. The relative oxidation rates for stearic, oleic, linoleic and linolenic acids are 1, 100, 1,200, and 2,500, respectively [6]. The estimated relative oxidation rates for arachidonic acid (AA, C20:4ω-6), docosapentaenoic acid (DPA, C22:5ω-3), and docosahexaenoic acid (DHA, C22:6ω-3) are about 3,600, 4,800, and 6,000 based on the number of methylene groups separated by double bonds. Fish oil containing AA, DPA and DHA is more easily oxidized than vegetable oil [7]. The off-flavors develop from the oxidation of oils containing AA, DPA and DHA make food products less acceptable to consumers.

Lipid oxidative stability can be determined by measuring induction periods, peroxide value, conjugated dienes, carbonyls and volatiles [8, 9]. Analytical methods can detect oxidation prior to the start of off-flavor and evaluate the oxidative stability of oil. The initial phase of lipid oxidation is called the induction period. Oxidation proceeds slowly and at a uniform rate during the induction period. After the induction period, the rapid oxidation of lipids leads to a deterioration of oils [4]. Antioxidants have been added to oil to extend the induction period and to decrease the oxidation rate [10]. The induction period can

be used as a measurement to determine the oxidative stability of oil, as well as an index of antioxidant effectiveness [8, 10].

The Oxidative Stability Index (OSI) is an automated accelerated method for determining the oxidation rate [11, 12]. The OSI method was adopted and recommended by the American Oil Chemists' Society for oxidation rate determination [13]. The OSI is capable of running up to 24 samples at the same time compared to the Rancimat method which can analyze only a few samples. The OSI has good reproducibility and precisely evaluates the effects of antioxidants on induction time [14].

The beneficial health effects of AA, DPA and DHA have been reported [15–17]. AA, DPA and DHA can reduce the risk of cardiovascular diseases such as arteriosclerosis and myocardial infarction [18] and are essential to the optimal development of the brain and eyes in children [12]. However, they are very susceptible to oxidation due to the polyunsaturation and are very expensive compared to linoleic and linolenic acids. The minimization of AA, DPA and DHA oxidation during the processing and storage of foods has been extremely important and challenging [19, 20]. The objective of this research was to study the effects of ascorbyl palmitate, tocopherol and β-carotene to improve the oxidative stability of oils containing different levels of AA, DPA and DHA.

Materials and Methods

Materials

Soybean oil from Archer Daniel Midland (Decatur, IL), coconut oil and high oleic safflower oil from California Oils (Richmond, VA), fungal oil from Nippon Suisan Kaisha Ltd. (Tokyo, Japan), tuna oil from Suntory, Ltd. (Tokyo, Japan) and egg oil from Eastman Kodak (Kingsport, TN) were used. Ascorbyl palmitate, tocopherol, and β-carotene were purchased from Roche Chemicals (Paramus, NJ), from Eastman Chemical Products, Inc. (Kingsport, TN) and from BASF (Florham Park, NJ), respectively.

Preparation of Oil Samples

Soybean, coconut, and high oleic safflower oils were mixed to obtain the stock oil, which was stored in a 10,000 gallon stainless steel tank at 20 °C for 2 days under nitrogen. Fungal oil, tuna oil or egg oil which provided AA, DPA and DHA were stored in ten gallon stainless steel containers under nitrogen at 0 °C prior to use. Fungal (1.74% w/w), tuna (0.57% w/w), or egg oil (8% w/w) were added to the stock oil to obtain the fatty acid composition of Oil 2 and Oil 3 as shown in Table 1. Oil 1 did not contain fungal, tuna, or egg oil. Oils were agitated for 10 min to have good uniform homogeneity. Oils 1, 2, and 3 were transferred into separate stainless steel containers, purged with nitrogen and tightly sealed.

Three liters of Oil 1, 2, or 3 was transferred into a 5 L glass beaker and heated on a hot plate at 38 °C. Ascorbyl palmitate at 0, 300, 600, 900, or 1,200 ppm, tocopherol at 0, 200, 400, 600, or 800 ppm, and β-carotene at 0, 3, 6, 9, or 12 ppm on the weight basis were added to Oil 1, 2, or 3. Oil 1, 2, and 3 were stirred for 5 min at 38 °C, allowed to cool to room temperature, and then stirred for another 5 min at room temperature to disperse the antioxidants uniformly in the oil. Oils 1, 2, and 3 containing different levels of antioxidants were transferred to plastic amber bottles without any headspace in the bottles. Lower levels of β-carotene were used, as higher concentrations could impart unacceptable color to the oils. Sample bottles were tightly sealed with plastic screw caps and stored in a refrigerator for OSI determination.

Preliminary Study for the Recovery of Antioxidants

To study the solubility and uniformity of ascorbyl palmitate, tocopherol, and β-carotene in oils, different levels of antioxidants were added to Oils 1, 2, or 3 at room temperature according to Table 2. The antioxidant contents in Oils 1, 2, and 3 were analyzed by HPLC using AOCS official method Ce 8–89 [13]. The recovery of antioxidants was expressed according to the following equation: Recovered antioxidant (%) = Concentration of antioxidant after mixing antioxidant in oil/Concentration of antioxidant added in oil $\times$ 100.

Experimental Design

There were 125 possible combinations for the factorial design of five levels of ascorbyl palmitate, tocopherol, and β-carotene. A central composite design was used to select 23 samples out of 125 possible combinations. This design

Table 1 Fatty acid composition of Oils 1, 2, and 3

Fatty acid	Oil 1 (%)	Oil 2 (%)	Oil 3 (%)
Saturated fatty acid	34.83	33.65	33.08
Monoene fatty acid	41.41	41.75	41.53
Linoleic C18:2ω-6	21.19	21.81	22.20
Linolenic C18:3ω-3	2.57	2.24	2.52
Arachidonic C20:4ω-6 (AA)	–	0.41	0.43
Docosapentaenoic C22:5ω-3 (DPA)	–	0.01	0.08
Docosahexaenoic C22:6ω-3 (DHA)	–	0.13	0.16
Total of AA, DPA, and DHA	0.00	0.55	0.67

Table 2 Added and recovered concentration of ascorbyl palmitate, tocopherol and β-carotene in Oils 1, 2, and 3

Ascorbyl palmitate				Tocopherol				β-Carotene			
Added (ppm)	Recovered concentration (%)			Added (ppm)	Recovered concentration (%)			Added (ppm)	Recovered concentration (%)		
	Oil 1	Oil 2	Oil 3		Oil 1	Oil 2	Oil 3		Oil 1	Oil 2	Oil 3
300	80	77	86	200	94	100	92	3	100	100	100
600	95	80	100	400	99	95	100	6	100	100	99
900	100	89	80	600	100	92	95	9	98	100	99
1,200	100	98	90	800	100	100	94	12	100	98	100

was used for fitting a second-order response surface model [21]. The design consisted of the corner points or vertices of two cubes, one inside the other. The outer cube vertices were defined by the ''low'' and ''high'' levels of each antioxidant while the inner cube vertices were defined by the ''mid-low'' and ''mid-high'' levels of each antioxidant. The face points of the outer cube were also included in this study. These points are defined by two of the antioxidants being at mid-level while the third antioxidant is at a ''low'' or a ''high'' level.

Oxidative Stability Index Measurement

The AOCS Standard Method Cd 12b-92 [13] for OSI was used to measure the induction period in hours for 23 samples of Oils 1, 2, or 3 shown in Table 3. The induction time of oils was determined using the Oxidative Stability Instrument (Omnion Inc., Rockland, MA). The samples shown in Table 3 were removed from a refrigerator and allowed to equilibrate to room temperature for 30 min. Bottles were inverted several times

Table 3 Determined and predicted induction time for Oils 1, 2, and 3

Ascorbyl palmitate (ppm)	Tocopherol (ppm)	β-Carotene (ppm)	Determined (D) and predicted (P) induction time (h)					
			Oil 1 (D)	Oil 1 (P)	Oil 2 (D)	Oil 2 (P)	Oil 3 (D)	Oil 3 (P)
0	0	0	13.5	13.8	11.8	12.5	10.5	10.4
0	0	12	18.0	17.9	11.8	12.8	10.9	10.8
0	400	6	20.2	19.5	13.1	13.2	10.9	11.9
0	800	0	18.7	19.1	12.9	12.7	13.5	13.0
0	800	12	19.3	18.6	14.3	14.5	11.5	12.1
300	200	3	28.9	30.0	20.6	18.9	15.5	14.6
300	200	9	23.6	22.8	21.1	19.1	14.6	14.6
300	600	3	17.7	18.4	19.5	19.5	15.8	15.9
300	600	9	20.2	19.3	20.5	20.1	16.6	15.6
600	0	6	25.0	24.5	21.8	21.7	15.5	15.6
600	400	0	26.1	26.0	23.2	23.6	17.0	17.7
600	400	6	26.2	26.8	23.1	23.4	18.4	17.5
600	400	12	21.2	20.9	24.5	24.0	17.9	17.4
600	800	6	27.3	27.9	24.0	24.1	18.2	18.4
900	200	3	26.1	27.1	24.4	24.3	17.8	17.6
900	200	9	26.9	27.3	23.2	24.2	17.6	17.6
900	600	3	29.2	29.3	24.5	15.7	19.2	19.5
900	600	9	25.7	25.0	24.5	25.9	18.3	19.2
1200	0	0	26.4	26.5	23.1	23.0	16.5	16.1
1200	0	12	24.6	24.4	22.3	22.1	16.0	16.4
1200	400	6	28.0	27.9	24.8	24.4	18.8	18.5
1200	800	0	23.0	22.5	26.9	26.1	20.6	20.6
1200	800	12	29.9	29.6	27.1	26.5	20.0	19.6

prior to sampling to have a homogeneous mixture of antioxidants. Then 5 g of oil was transferred into a disposable borosilicate glass reaction tube (Omnion Inc., Rockland, MA). A rubber stopper fitted with a disposable pipette and aeration tube was placed in the reaction tube. A polycarbonate conductivity tube (Omnion Inc., Rockland, MA) was filled with 50 mL of deionized water. A rubber stopper fitted with a disposable pipette and conductivity probe was placed in the conductivity tube. A rubber tube connected with the pipette in the reaction tube to the airflow nozzle and the aeration tube to the pipette in the conductivity tube. The conductivity probe was connected to the OSI instrument. The airflow was set at 2.5 mL/s and temperature was at 110 °C for each heating block. The OSI in hours at 110 °C was determined by the online computer which measured the change in conductivity of the water versus time. The induction time was plotted by a microprocessor-computed slope/change algorithm in accordance with AOCS Method Cd 12b-92 [13]. Each sample was analyzed in duplicate.

Statistical Analysis

Multiple linear regressions using SAS software (SAS Institute, Cary, NC) were used to evaluate the effect of antioxidants on the oxidative stability of oils containing AA, DPA and DHA. The method of least squares was used to estimate the regression coefficients in the model. Regression analysis of variance (ANOVA) was used to study the effects of the antioxidants on the induction time of the oil at $\alpha = 0.05$.

Results and Discussion

Fatty Acid Composition of Oils

Fatty acid composition for Oils 1, 2, and 3 are shown in Table 1. The three oils contain about 34% saturated fatty acid, 41% monoene fatty acid, 22% linoleic acid, and 2.5% linolenic acid. Oil 1 had no AA, DPA and DHA. Oil 2 and Oil 3 contained 0.55% and 0.67% of total AA, DPA and DHA. The World Health Organization recommends the daily consumption of 0.3–0.5 g of AA, DPA and DHA [22]. The concentration of AA, DPA and DHA should be about 0.4–0.7% in the oil when the daily fat intake is 65 g to meet the daily requirement [23]. Oil 2 and Oil 3, which have 0.55 and 0.67% of AA, DPA and DHA can provide the WHO recommended daily intake of 0.3–0.5 g of AA, DPA and DHA when the daily fat intake of 65 g is met.

Preliminary Study for the Solubility and Uniformity of Antioxidants

The added and actual concentrations of ascorbyl palmitate, tocopherol and β-carotene in Oils 1, 2, and 3 at room temperature are shown in Table 2. The recovered concentration percentage of ascorbyl palmitate, tocopherol and β-carotene in the sample ranged from 77 to 100%, 92 to 100%, and 98 to 100 %, respectively. The antioxidant concentrations were not uniform. The difference in the antioxidant concentration in oils may be due to the inhomogeneity of antioxidants and the different solubility of antioxidants in the oils. It was possible that the antioxidants were not completely solubilized throughout the oil to have a uniform concentration at room temperature. However, when the samples were carefully mixed to improve solubility and uniformity at 38 °C for 5 min, the recovered ascorbyl palmitate, tocopherol and β-carotene were very close to the added concentration. The recovered concentration increased as the hydrophobicity of the antioxidant increased. β-Carotene has a higher hydrophobicity than those of tocopherol and ascorbyl palmitate and its recovery was almost 100%. After the preliminary study on the recovery of antioxidants at room temperature, antioxidants were added and homogeneously mixed at 38 °C for 5 min as it was stated in the sample preparation in the materials and methods section. The recovery of ascorbyl palmitate in the oil varied from 77 to 100%. The mixing of samples at 38 °C for 5 min during sample preparation could enhance the solubility and facilitate the uniformity of the antioxidants in the oils.

Induction Time of Oils

The effects of ascorbyl palmitate, tocopherol, and β-carotene on the induction times for Oils 1, 2, and 3 are shown in Table 3. The induction times for Oil 1, 2, or 3 with 0, 0.55, or 0.67% of combined AA, DPA and DHA were 13.5, 11.8 or 10.5 h, respectively. As the amount of combined AA, DPA, and DHA increased from 0 for Oil 1 to 0.67% for Oil 3, the induction time decreased from 13.5 to 10.5 h which is equal to the 25% of the induction time. The induction times for Oils 1, 2, and 3 with 1,200 ppm ascorbyl palmitate, 800 ppm tocopherol and 12 ppm β-carotene were 29.9, 27.1, and 20.0 h, respectively. The induction times of the oils containing antioxidants decreased as the total content of AA, DPA, and DHA in the oils increased. AA, DPA and DHA decreased the oxidative stability of oils as expected.

Table 3 shows that ascorbyl palmitate had the greatest effect to increase the oxidative stabilities of Oils 1, 2, and 3. The induction time was increased from 13.5 to 26.4 h as the ascorbyl palmitate increased from 0 to 1,200 ppm.The 1,200 ppm ascorbyl palmitate had a better effect than the 800 ppm tocopherol on the oxidative stabilities of Oils 1, 2,

Table 4 Analysis of variance of antioxidants effect on the induction time for Oils 1, 2 and 3

Parameter	p value[a]		
	Oil 1	Oil 2	Oil 3
Ascorbyl palmitate	0.01	0.01	0.01
Tocopherol	0.03	0.03	0.01
β-Carotene	0.17	0.49	0.96
Ascorbyl palmitate × tocopherol	0.01	0.03	0.02
Ascorbyl palmitate × β-carotene	0.75	0.31	0.93
Tocopherol × β-carotene	0.85	0.28	0.11

[a] $p < 0.05$ is significantly different at $\alpha = 0.05$

and 3. β-Carotene did not have any effect on the induction time at any of the levels evaluated for Oils 1, 2, and 3. β-Carotene has not been reported as an antioxidant for triplet oxygen free radical lipid oxidation but it is well known as an excellent antioxidant for singlet oxygen under light [3, 24, 25].

Regression Model for the Predicted Induction Time of Oils

The regression model equation used to evaluate antioxidants effect on the induction time was a second degree polynomial form:

$$\begin{aligned} \mathrm{IT} = & b_0 + b_1 a + b_2 t + b_3 c + b_4 at + b_5 ac + b_6 tc \\ & + b_7 a^2 + b_8 t^2 + b_9 c^2 \end{aligned}$$

where

- IT induction time
- a ascorbyl palmitate, ppm
- t tocopherol, ppm
- c β-carotene, ppm
- b_0 intercept
- b_1 linear coefficient of ascorbyl palmitate
- b_2 linear coefficient of tocopherol
- b_3 linear coefficient of β-carotene
- b_4 interaction coefficient of ascorbyl palmitate and tocopherol
- b_5 interaction coefficient of ascorbyl palminate and β-carotene
- b_6 interaction coefficient of tocopherol and β-carotene
- b_7 curvature coefficient of ascorbyl palmitate
- b_8 curvature coefficient of tocopherol
- b_9 curvature coefficient of β-carotene

The analysis of variance (ANOVA) of antioxidants effect on the induction time for Oils 1, 2, and 3 are shown in Table 4. The coefficients of determination (r^2) between determined induction time and predicted induction time for Oils 1, 2 and 3 containing different levels of antioxidants were 0.98, 0.96 and 0.95, respectively. Table 3 shows the determined and predicted induction time for Oils 1, 2, and 3. The determined and predicted induction time for all samples of Oils 1, 2, and 3 were close to each other as shown in Table 3. There was an excellent agreement between regression models of predicted induction time and measured induction time. The regression model could successfully predict the induction time of oils based on the contents and types of antioxidants.

Ascorbyl palmitate and tocopherol had a significant effect on the induction times for Oils 1, 2, and 3 at $\alpha = 0.05$ (Table 4). The addition of β-carotene had no linear, interaction or curvature effect on the induction time for Oils 1, 2, and 3 at $\alpha = 0.05$. The interaction effect of ascorbyl palmitate and tocopherol was highly significant at $\alpha = 0.05$ (Table 4). The synergist effects of ascorbyl palmitate and tocopherol have been previously reported [26] and the results from this study confirmed those findings. The interaction of β-carotene and ascorbyl palmitate or tocopherol did not have a significant effect on the induction time at $\alpha = 0.05$.

The response surfaces plot for the effect of ascorbyl palmitate and tocopherol on Oil 1 are shown in Fig. 1.

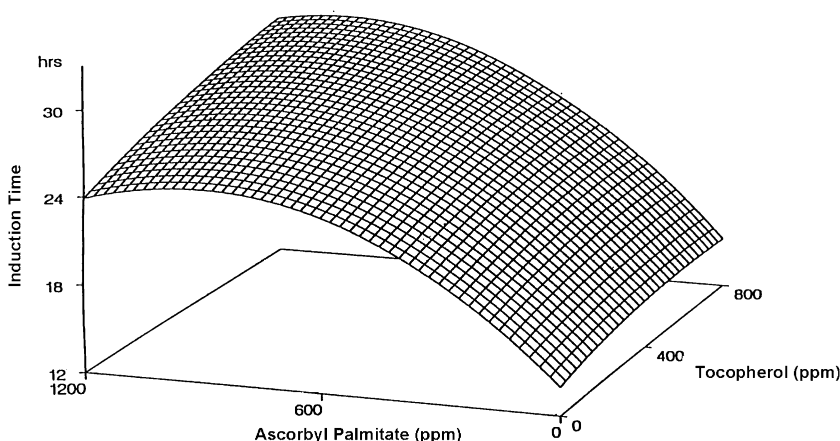

Fig. 1 Response surface curve of Oil 1 for the induction time as a function of ascorbyl palmitate and tocopherol at 6 ppm β-carotene

Response surface analysis for Oil 1 showed that as the ascobyl palmitate increased from 0 to 300, 600, 900 and 1,200 ppm, the induction period of Oil 1 increased as shown in Fig. 1. As the tocopherol content increased from 0 to 200, 400, 600 and 800 ppm, the induction period of Oil 1 increased. The similar results were shown in Oil 2 and Oil 3 (response surface plots not shown). Since β-carotene showed no significant effect on theinduction time at any of the levels studied, it was held at 6 ppm for the response surface curves of Oils 1, 2, and 3. We were able to select the combination of different levels of ascorbyl palmitate and tocopherol to give the same induction time. The use of an optimum combination of ascorbyl palmitate and tocopherol from the response surface analysis could improve the oxidative stability of oils containing AA, DPA and DHA which are easily oxidized.

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