Effects of Tocopherols on Oxidative Stability of Margarine

[1]MARYAM AZIZKHANI, [2]ABOLFAZL KAMKAR AND [3]AMIR SASAN MOZAFFARI NEJAD*
[1]*Department of Food Science and Technology*, *Khazar University*, *Babolsar*, *Iran.*
[2]*Department of Food Hygiene*, *Faculty of Veterinary Medicine*, *University of Tehran*, *Tehran*, *Iran.*
[3]*Department of Food Science and Technology*, *Member of Young Researchers Club*, *Islamic Azad University*, *Damghan Branch*, *Damghan*, *Iran.*

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Summary: Tocopherols are the most important natural antioxidants which are now added to edible oils. Each individual tocopherol (α, γ and δ) was added to the oil phase of margarine in 0 (control), 100, 250, and 500 ppm. Antioxidative activity was evaluated through oven test (at 60 ± 1 °C in 5 days and measuring peroxide value and anisidine value) and Ransimat test (110 ± 1 °C). Antioxidative activity increased progressively as concentration of δ-tocopherol increased. As the concentration of γ-tocopherol increased from 0 to 100, and 250 ppm, oxidation of oil phase decreased. At 500 ppm, α-tocopherol acted as a prooxidant and γ-tocopherol did not have any noticable effect, but δ-tocopherol acted as an antioxidant.The order of antioxidative activity of tocopherols was: α- < γ- < δ-.

Introduction

Margarine is a water-in-oil emulsion containing a minimum of 80% fat, a maximum of 16% water and about 4% additives [1, 2]. Margarine is susceptible to oxidation and its oxidative stability is affected by several factors such as oxygen, light, heat, metal ions, and enzymes, thus leading to oxidative rancidity. The addition of antioxidants to the margarine helps to prevent or decrease oil phase oxidation. Traditionally, synthetic compounds such as *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as antioxidants in oil products. However, some research papers have shown that these compounds are toxic to experimental animals [3]. Therefore, the use of natural antioxidants is now a new trend in both research and industrial applications. Several compounds, including ascorbyl palmitate [4, 5], tocopherols [6-8], catechins [9], rosemary extract [4, 7, 10-12], phospholipids [4, 5] and sage extract [13, 14] have been investigated and some have been more effective than BHA and BHT in decreasing oil oxidation.

There are 4 different tocopherols (vitamin E), α-, β-, γ-, and δ-tocopherols, with minor structural differences. Tocopherols are the most important lypophilic natural antioxidants in vegetable oils. they act as antioxidant by donating a hydrogen atom to peroxyl radicals of unsaturated lipid molecules, forming a hydroperoxide and a tocopheroxyl radical, which reacts with other peroxyl or tocopheroxyl radicals forming more stable adducts [15]. Tocopherols show a synergistic effect with phospholipids for enhancing oxidative stability of oils [6, 8, 16]. Therefore, optimum concentrations of tocopherols may be affected by quantities of phospholipids and other minor compounds present [17]. Some studies have reported pro-oxidant activity for α-tocopherol, *e.g.* in one study, when exposed to Cu^{2+}, α-tocopherol, in detergent dispersion, is rapidly oxidised. Moreover, if phospholipids and traces of their hydroperoxide derivatives are included in these dispersions, Cu^{2+} initiates lipid peroxidation, the rate of which is dramatically stimulated by α-tocopherol. The observation that the rate of α-tocopherol consumption is identical in the absence and in the presence of lipids undergoing peroxidation, apparently rules out any antioxidant effect. These results are consistent with a prooxidant effect of vitamin E, mediated by its capability to reduce Cu^{2+} to Cu^{+} which, in turn, produces, from lipid hydroperoxides, the highly reactive alkoxyl radicals [18]. Margarine contains about 1000 ppm lecithin as an emulsifier [19]. Although tocopherols are most important natural antioxidants which now are added to edible oils, the detailed effect of different tocopherols and different concentrations on oxidative stability of vegetable oils such as margarine is important. The objective of this work was to determine quantitive effects of tocopherols on oxidative stability of margarine.

Results and Discussion

Peroxide value (PV) and anisidine values (AV) of sample containing 100 ppm α-tocopherol stored at 60 ± 1 °C were lower than the control. PV and AV increased as the α-tocopherol increased from 100 to 250 and 500 ppm (Tables-1 and 2).

*To whom all correspondence should be addressed.

Table-1: The effect of tocopherols on PV (meq/kg) in oil phase of margarine during dark storage at 60 °C.

Days	1	2	3	4	5
α-toc (ppm)					
0	10.2 ± 0.05	23.7 ± 0.09	35.5 ± 0.05	52.1 ± 0.11	61.4 ± 0.06
100	8.0 ± 0.02	21.2 ± 0.07	32.3 ± 0.09	46.9 ± 0.09	57.2 ± 0.05
250	9.3 ± 0.01	24.8 ± 0.09	38.2 ± 0.10	54.0 ± 0.11	62.9 ± 0.06
500	11.4 ± 0.11	29.5 ± 0.05	43.2 ± 0.07	60.1 ± 0.05	72.1 ± 0.02
Γ -toc(ppm)					
0	10.5 ± 0.05	25.0 ± 0.06	36.9 ± 0.05	51.4 ± 0.08	59.0 ± 0.07
100	9.0 ± 0.04	23.3 ± 0.04	37.7 ± 0.05	49.0 ± 0.06	58.6 ± 0.05
250	8.8 ± 0.05	23.0 ± 0.05	33.1 ± 0.06	47.9 ± 0.05	54.0 ± 0.03
500	8.5 ± 0.03	23.4 ± 0.04	36.0 ± 0.03	51.8 ± 0.08	63.5 ± 0.09
δ -toc(ppm)					
0	10.1 ± 0.03	24.5 ± 0.03	36.3 ± 0.05	51.1 ± 0.06	60.5 ± 0.01
100	9.6 ± 0.01	22.5 ± 0.05	35.8 ± 0.04	49.9 ± 0.02	59.7 ± 0.08
250	7.4 ± 0.08	21.3 ± 0.12	31.2 ± 0.02	46.5 ± 0.10	55.7 ± 0.05
500	7.2 ± 0.08	21.2 ± 0.07	31.5 ± 0.05	46.8 ± 0. 12	56.2 ± 0.01

[a-d] Values that are significantly different in each column ($p<0.05$).

Table-2: The effect of tocopherols on AV in oil phase of margarine during dark storage at 60 °C.

Days	1	2	3	4	5
α-toc (ppm)					
0	2.6 ± 0.07	6.1 ± 0.05	9.2 ± 0.13	13.5 ± 0.11	15.9 ± 0.05
100	2.0 ± 0.09	5.5 ± 0.10	8.3 ± 0.05	12.1 ± 0.10	14.8 ± 0. 10
250	2.41 ± 0.15	6.4 ± 0.09	9.9 ± 0.09	14.0 ± 0.12	16.3 ± 0.13
500	2.9 ± 0.11	7.6 ± 0.06	11.2 ± 0.10	15.6 ± 0.05	18.7 ± 0.15
γ -toc(ppm)					
0	2.7 ± 0.05	6.4 ± 0.03	9.58 ± 0.06	12.1 ± 0.09	15.9 ± 0.04
100	2.3 ± 0.07	6.0 ± 0.11	9.2 ± 0.09	12.7 ± 0.05	15.2 ± 0.06
250	2.3 ± 0.07	5.97 ± 0.01	8.5 ± 0.03	12.4 ± 0.08	15.0 ± 0.06
500	2.2 ± 0.03	6.0 ± 0.10	9.3 ± 0.08	13.4 ± 0.05	16.1 ± 0.07
δ -toc(ppm)					
0	2.6 ± 0.04	6.3 ± 0.06	9.4 ± 0.01	13.2 ± 0.11	15.7 ± 0.05
100	2.3 ± 0.03	5.8 ± 0.08	9.2 ± 0.04	12.9 ± 0.10	15.3 ± 0.10
250	1.9 ± 0.05	5.5 ± 0.05	8.1 ± 0.07	12.0 ± 0.10	14.4 ± 0.05
500	1.8 ± 0.05	5.5 ± 0.05	8.1 ± 0.07	12.1 ± 0.09	14.5 ± 0.05

[a-d] Values that are significantly different in each column ($p<0.05$).

Peroxide value and anisidine values of sample containing 100 ppm *α*-tocopherol was significantly higher than the control ($p < 0.05$). This result is in agreement with the results of Braunrath *et al.*, [20] by reported that *α*-tocopherol had low antioxidant activity and even prooxidant effects on rapeseed oil triacylglycerols exposed to radiation and induced formation of peroxides. Also, in another study, the PV levels of samples (mackerel oil) with 250 and 500 ppm *α*-tocopherol was higher than samples with 50 and 100 ppm *α*-tocopherol at the same temperature. So, at high concentrations (250 and 500 ppm) *α*-tocopherol was less effective as an antioxidant versus the relatively lower concentrations of 50 and 100 ppm with even higher levels than control samples [21]. Previous studies by Huang *et al.*, [22] with corn oil and by Kulas and Ackman [23] with fish oil also showed 100 ppm *α*-tocopherol as the concentration for maximal antioxidant activity in those oils. It is mentioned here that the reduced antioxidant effects of higher levels of *α*-tocopherol is not unusual, as high *α*-tocopherol levels have been shown to even exhibit prooxidant effects in other studies [17, 22, 24] it has been suggested that tocopherols and/or their radicals are capable of undergoing side reactions besides chelating free radicals, this side reaction may result in the prooxidant effect. The tocopheroxy radical has been implicated in the prooxidant effect on the assumption that the presence of higher concentrations of tocopheroxy radicals facilitates the occurrence of side reactions [25]. Also, this result in our study is in agreement with results of Ohm *et al.*, [26] and Romero *et al.*, [15].

As the concentration of *γ*-tocopherol increased from 0 to 100 and 250 ppm, peroxide value and anisidine values decreased and were significantly lower than the control ($p<0.05$). These results are in agreement with Braunrath *et al.*, [19] and Huang *et al.*, [21] who reported that in general optimum concentrations for tocopherols were found to be for *α*-tocopherol ~100 ppm, for *γ* -tocopherol~500 ppm and for *δ*-tocopherols an inhibition of hydroperoxide formation was observed to high levels up to 2000 ppm. In their studies and also Lampi *et al.*, [27] in the range of 5-500 ppm *α*- and γ –tocopherols, it was found that at concentrations above 100 ppm *γ*–tocopherol was more effective than *α*-tocopherol at the same concentration.

PV and AV decreased progressively as the concentration of *δ*-tocopherol increased from 0 to 500ppm.These values indicated that individual tocopherols acted as antioxidants and prooxidants depending on concentrations. In our study, applying *α*-, *γ*- and *δ*-tocopherols between 100-500 ppm, the results of PV and AV showed that at 500 ppm, *α*-tocopherol can act as prooxidant and *γ*-tocopherol did not have noticable effects, but *δ*-tocopherol acted as an effective antioxidant.

Quantitative effects of *α*-, *γ*- and *δ*-tocopherols on induction period at 110 ± 1 °C (Rancimat test) are shown in Table-3. The induction period (IP) of samples containing 100 ppm *α*-tocopherol was significantly higher than the control containing no tocopherol ($p < 0.05$). However the induction period of samples containing 500 ppm *α*-tocopherol was significantly lower than the others ($p<0.05$). The IP increased as the concentration of *γ*-tocopherol increased from 0 to 100 and 250 ppm. Also, IP increased as the concentration of *δ*-tocopherol increased from 0 to 100, 250 and 500 ppm.

Experimental

Neutralized, bleached and deodorized sunflower oil with no added antioxidant was purchased from Kesht-o-Sana'at-e-Shomal, Iran.

Neutralized, bleached, deodorized and antioxidant-free palm stearine was donated by PORIM (Palm Oil Research Institute of Malaysia). Tocopherols (α, γ and δ) and emulsifier (mono and diacylglycerols) were purchased from Danisco (Copenhagen, Denmark), soy lecithin from ADM (Archer Daniels Midland, Koog aan de Zaan, Netherlands), citric acid, potassium sorbate and other analytical grade chemicals and solvents were purchased from Merck Co. (Darmstadt**,** Germany), *β*-Carotene (dissolved in edible oils) was purchased from Roche (Basel,Switzerland), vitamin A and D_3 were purchased from BASF (Karlsruhe, Germany), Sodium casienate was purchased from Iran Caseinate, low fat milk powder was obtained from Moghan Co. (Tehran, Iran) and diacetyl was obtained from Robert (Tehran, Iran).

Table-3: Effect of tocopherols on induction period in oil phase of margarine at 110 °C.

	Induction period (hour)		Induction period (hour)		Induction period (hour)
α-toc (ppm)		*γ* –toc (ppm)		*δ* –toc (ppm)	
0	1.70 ± 0.03	0	1.70 ± 0.03	0	1.70 ± 0.06
100	5.18 ± 0.08	100	4.65 ± 0.05	100	4.71 ± 0.03
250	1.41 ± 0.04	250	5.11 ± 0.05	250	5.40 ± 0.05
500	1.15 ± 0.01	500	1.53 ± 0.02	500	5.60 ± 0.05

Sample Preparation

Samples of margarine were produced in the laboratory scale (3 kg) containing approximately 16% water and 80% oil. The oil phase was consisted of sunflower oil and palm stearine (80:20), emulsifier (0.5%), *β*-carotene solution (0.003%), diacetyl (0.02%), vitamins (A and D_3, 0.01%), soy lecithin (1000ppm) and tocopherols. Each individual tocopherol was added to the oil phase in 0 (control), 100, 250 and 500 ppm.

The aqueous phase consisted of water, NaCl (0.5%), milk powder (1%), potassium sorbate (0.02%), citric acid (0.06%), and sodium caseinate (1%). The oil and aqueous phases were mixed at 40-45 °C, and then the emulsion was cooled and mixed to obtain proper texture. Samples were packed in 250 g polyethylene cups and stored in the freezer (-18 °C) for 48 hours in order to complete crystallization [4].

Oven Test

The samples were transferred in beakers to an oven maintained at 60 ± 1 °C. Oxidative stability was determined by measuring peroxide and anisidine values every 2 days over a 6-day period according to official methods of AOCS [28]. Primary oxidation products were determined by peroxide measurements. Formation of secondary oxidation products was measured using the *p*-anisidine value [28].

Rancimat Test

The induction period of the oil phase of the samples was determined by ranciamt at 110 ± 1 °C by Metrohm679 [4].

Statistical Analysis

All measurements were carried out three times. The results obtained for peroxide, anisidine values and induction period were statistically analyzed by Tukey's Range test (spss13) at p = 0.05.

Conclusion

The heating induced formation of peroxides/hydroperoxides in oil phase of margarine was significantly reduced by *γ*- and *δ*-tocopherols, whereas of *α*-tocopherol had adverse effect on the PV, AV and IP. These findings demonstrate the different reaction pathways of the *α*-tocopherol having two CH_3-groups vicinal to OH-/O-, compared to *γ*- and *δ*-tocopherols, having H-/CH_3- and H-/H-, respectively. Summarising it can be stated that the optimum concentration of the tocopherols for oxidative stability of margarine seems related to the oxidative stability of each individual tocopherol. The order of antioxidative activity of tocopherols was: *α*- < *γ*- < *δ*-.

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