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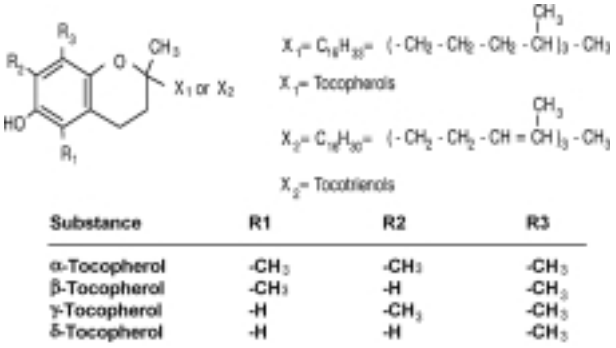
Antioxidative potential of tocotrienols and tocopherols in coconut fat at different oxidation temperatures

The objective of this study was to compare the stabilizing effects of tocotrienols and their corresponding tocopherols at 60 °C and 160 °C. Stability was determined in coconut fat by observing the Oil Stability Index (OSI), the peroxide value (POV) and conjugated dienes (CD). α- and β-tocotrienols as well as α-tocopherol induced susceptibility of the systems against oxidative deterioration and reduced the life time of coconut fat. δ- and γ-tocotrienols increased the fat's shelf-life at ambient temperature (60 °C). At frying conditions the antioxidative potential increased in the following order: α<γ<δ (Tocopherols) α<β<γ<δ (Tocotrienols). Under these conditions γ- and δ-tocotrienols were significantly more active than their corresponding tocopherols. Irrespective of the temperature employed, the protective effects of tocochromanols were dose dependent at 160 °C (mg/kg): 1000>500>100 (tocotrienols) and 5000 > 2000 > 1000 > 100 (tocopherols). Among the tested antioxidants δ-tocopherol and δ-tocotrienol were found to be most efficient against lipid oxidation both at 60 °C and at 160 °C. This study showed, that γ- and δ-tocotrienols, similar to the much better investigated tocopherols, are good food antioxidants to enhance shelf-life of coconut fat both at frying and at low temperature.

Keywords: Tocotrienols, tocopherols, coconut fat, antioxidative potential, oxidation temperature.

1 Introduction

The chemical reaction cascade during lipid oxidation is the most limiting factor in maintaining food quality. Especially the increasing emphasis on the use of polyunsaturated plant or fish oils in human nutrition and the different cooking and storage conditions in households increase the evidence for the improvement of stabilization. To enhance stability and lifetime several antioxidants are approved to be used as additives in respect to their effectiveness. Tocopherols are the major group of primary antioxidants occurring in plant oils and fats and therefore numerous investigations were focused on their antioxidant activity [1–5]. However, the optimum tocopherol concentration to slow down the hydroperoxid formation was established only for specific and well-defined systems. The reason for this selectivity is the complex pathway during the formation of oxidation products, the influence of the fatty acid pattern, oil source, initial content of antioxidants and the conditions of use. Beside tocopherols there is a high practical interest in other free radical scavengers and in finding synergistic substances. Tocotrienols are similar in structure but differ from their corresponding tocopherols by their three unsaturated units in the isoprenoid side chain (Fig. 1). With regards to the formation of tocopheroxy radicals α-tocotrienol was shown to be a more potent antioxidant than α-tocopherol [6] due to its higher recycling efficiency from chromanoxyl radicals which correlates with inhibition of lipid peroxidation. In the first reported oxidation tests, using concentrations of 500 mg/kg *Lehmann* and *Slover* [7] showed higher antioxidative potentials for γ- and δ- tocotrienols than for α-tocotrienol, which was similar to the potential of α-tocopherol. Test media were methylmyristate and methyllinoleate. Under heating conditions in stripped oils γ-tocotrienol was the most effective antioxidant, followed by γ-tocopherol, with the α-forms being much less effec-



Substance	R1	R2	R3
α-Tocopherol	-CH_3	-CH_3	-CH_3
β-Tocopherol	-CH_3	-H	-CH_3
γ-Tocopherol	-H	-CH_3	-CH_3
δ-Tocopherol	-H	-H	-CH_3

Fig. 1. Structure of tocopherols and tocotrienols.

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tive [8]. In simulated frying tests γ-tocotrienol was the least stable tocol, but no conclusions about their antioxidant potentials could be made [9].

Tocotrienols are considered to be primary antioxidants in humans [10] and cell lines [11], however, in foods less data is available. For a better understanding of the tocochromanols impact on lipid oxidation related to oxidation temperature this study was designed to evaluate their free radical scavenging properties at frying conditions and at the lower temperature of 60 °C in coconut fat, with special focus on the tocotrienols potential.

2 Materials and methods

Coconut fat was purchased at retail outlets in Vienna, Austria. Tocopherols and tocotrienols were obtained from *Merck* (Vienna, Austria), only γ-tocopherol was kindly donated by *Roche* (Basel, Switzerland).

2.1 Determination of tocopherols

As described previously [12] fat initial tocopherol and tocotrienol contents were analyzed in duplicate with reversed phase HPLC. The system consisted of a L-7100 pump, L-7400 UV-detector, D-7000 Interface module and a 250 x 4, 5 μm LiChrospher RP-18 column, all by *Merck*. Methanol/dichlormethane (85:15, v/v) was used as mobile phase, the flow rate was 0.8 ml/min. Detection wavelength was set to 295 nm. Fat samples were dissolved in hexane, diluted and evaporated until dryness under vacuum, resolved in mobile phase and injected.

2.2 Fat enrichment with tocopherols/tocotrienols

α-, β-, γ- and δ-tocotrienols as well as α-, γ- and δ-tocopherols (dissolved in hexane), were evaporated at 35 °C. Simultaneously coconut fat was liquefied at 40 °C and an appropriate amount was added to the evaporated tocotrienols/tocopherols to receive final concentrations of 0.01–0.1% for tocotrienols and 0.01–0.5% for tocopherols. The final concentrations were monitored with HPLC.

2.3 Fatty acid composition

The fatty acids of the coconut fat were converted into methyl esters and analyzed with gas chromatography (GC) by using an Auto-System Gas Chromatograph, *Perkin Elmer* (Vienna, Austria), equipped with a split/splitless capillar injector as described previously [13]. FAME were separated by a 30 m x 0.25 mm ID fused silica column (RTx-2330) and detected with flame ionisation detector (FID). The FID temperature was set to 250 °C. The fatty acid pattern was analyzed in duplicate.

2.4 Frying experiments

The oxidation experiments at frying temperatures were simulated using the Rancimat 679, *Metrohm* (Herisau, Switzerland). The current American Oil Chemists' Society (AOCS) Official Method Cd 12b-92 [14] is a direct determination of the induction period expressed as the Oil Stability Index (OSI). Tests were performed at 160 °C with an air flow as low as possible (3 l/h) as described previously [13]. Results were expressed as oil stability index (OSI) in hours.

2.5 Low temperature experiments

The finally enriched fat (0.01–0.1 %) was filled into screw-capped flasks and oxidized at 60 °C in the dark. Oxidative changes were observed by analyzing the peroxide value (POV) and the formation of conjugated dienes (CDs).

POV were determined by the AOCS Cd 8-53 Acetic Acid-Chloroform procedure [15]. A single analysis was performed on each sample, the coefficient of variation (CV) of this method was 2.5%.

CDs were observed according the official method Ti 1a-64 of the AOCS [16]. Again in consideration of the low sample amount single analysis was performed.

2.6 Statistics

Frying tests were assessed in duplicate. The obtained data was analyzed by one way ANOVA. Statistical analyses were conducted using SPSS for Windows 9.0. Differences were considered to be significant at $p<0.05$.

3 Results and discussion

Effects of tocopherols and tocotrienols were tested in commercially available coconut fat which was purchased in shops and used without any technical interventions. Many studies investigated the potential of tocochromanols on stripped oils or triacylglycerols purified from different fats/oils [1, 4, 5, 17], however we decided to use a fat which is popular in Austria for frying in households and likewise low in initial antioxidants. The coconut fat used for the tests was poor in polyunsaturates (p/s ratio: 0.02) and therefore less susceptible against oxidation. The used fat had a very low initial amount of tocopherols and tocotrienols with a total vitamin E activity of <3 mg/100 g fat.

3.1 Frying experiments

3.1.1 Tocotrienols

OSI value of control coconut fat amounted to 2.04 h. The influence of tocotrienols on the fat stability at 160 °C is presented in Tab. 1.

Tab. 1. Effects of tocotrienols on coconut fat compared to the control fat at frying conditions of 160 °C.

	OSI [h]			
Addition of T_3* [mg/kg]	α-T_3	β-T_3	γ-T_3	δ-T_3
0 (CO**)	2.04[a]	2.04[a]	2.04[a]	2.04[a]
100	2.14[a]	2.32[b]	3.87[b]	3.77[b]
500	2.40[b]	3.23[c]	5.54[c]	6.34[c]
1000	2.55[b]	3.02[c]	6.60[d]	7.85[d]

* T_3 = tocotrienols, ** CO = control oil.
[a–d] Values within each column (OSI) followed by different letters are significantly different ($p < 0.05$).

α-tocotrienol proved to be the least effective tocotrienol even though the concentration of 500 and 1000 mg/kg could prolong the OSI significantly. Only the lowest concentration added (100 mg/kg) showed no difference to the control sample. β-tocorienol was able to increase OSI of the control as a matter of concentration. Similar to α-tocotienol the enrichment with 1000 mg/kg β-tocotrienol was as effective as 500 mg/kg. Although the effects of α- and β-tocotrienols were significant there was only a slight increase in stability. Significantly higher effects were observed for γ-tocotrienol which increased the OSI of control fat as a matter of concentration with highest protection at 1000 mg/kg (+224%). 100 mg/kg of γ-tocotrienol was significantly more effective than both α- and β-tocotrienol in each other concentration added. Most efficient in scavenging free radicals was found to be δ-tocotrienol. In respect to the speed of lipid oxidation each increase of γ- and δ- tocotrienol prolonged the fat stability significantly (Tab. 1) with 1000 mg/kg of δ-tocotrienol as the most effective (+285% to control). In each added equi-molecular concentration δ-tocotrienol protected more significantly than γ-tocotrienol.

3.1.2 Tocopherols

The experimental results clearly showed that in the presence of the additional amounts of δ-tocopherol (100–5000 mg/kg), the fat stability increased depending on the concentrations added. The significantly highest protection was seen by 5000 mg/kg (+726%; OSI of 14.82 h) (Tab. 2). Similar results but less pronounced were found for the same concentrations of γ-tocopherol. The same observations about the activity of γ-tocopherol (in different oil samples at similar but also lower temperatures of 60 °C and 40 °C) were reported previously [1, 13, 17]. Although the addition of γ-tocopherol was significantly less effective than that of δ-tocopherol in the same concentrations, the OSI increased significantly in comparison to the control fat. In contrast to these significantly positive findings, α-tocopherol was not as efficient in increasing the lifetime as the other two tocopherols. 100 mg/kg was ineffective, 1000–5000 mg/kg of α-tocopherol increased the fats lifetime, but significantly less effective than γ- and δ-tocopherol (Tab. 2).

Tab. 2. Effects of tocopherols on coconut fat compared to the control fat at frying conditions of 160 °C.

	OSI [h]		
Addition of T* [mg/kg]	α-T	γ-T	δ-T
0 (CO**)	2.11[a]	2.11[a]	2.11[a]
100	2.08[a]	3.20[b]	3.29[b]
1000	2.73[b]	5.58[c]	6.78[c]
2000	3.10[c]	7.16[d]	8.84[d]
5000	4.00[d]	10.70[e]	14.82[e]

* T= tocopherols, ** CO = control oil.
[a–e] Values within each column (OSI) followed by different letters are significantly different ($p < 0.05$).

The comparison of the equi-molar amounts of tocopherols/tocotrienols of 100 and 1000 mg/kg is shown in Fig. 2. The α–forms were similar in their impacts without differences. The γ- and δ-forms of tocotrienols, either with 100 or 1000 mg/kg, were significantly more potent than their corresponding tocopherols.

As far as known these results show for the first time the antioxidative implications of tocotrienols at frying temperatures in oils. The antioxidative properties of the individual tocotrienols (T3) considering also the tocopherols (T) at frying conditions after addition of 1000 mg/kg each, in increasing order were: α-T= α-T_3<β-T_3< γ-T<γ-T_3< δ-T<δ-T_3.

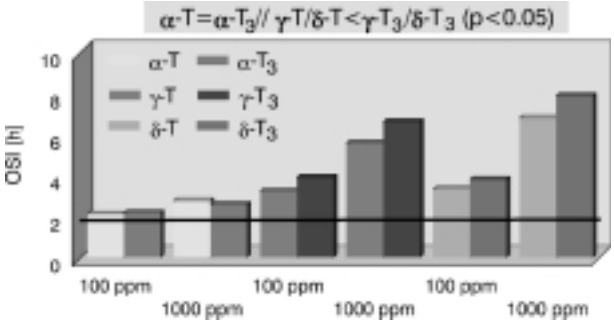

Fig. 2. Oil stability (OSI) of coconut fat after addition of tocopherols (T) or tocotrienols (T_3) at 160 °C.

Since it is known that the effects of tocopherols as antioxidants depend highly on the characteristic of the oxidizing material [2, 3, 18, 19] comparison of results obtained by using different methods is needed. Although the OSI test is an official method of the AOCS to determine the oil stability, its use is discussed contradictionary due to the high availability of oxygen [20]. Therefore we tried to adapt the method by using as less air as possible, which was 3 l/h. Even the different conditions from actual frying conditions with the absence of fried substance the rank order for tocopherols principally agree with other studies, which performed other tests to determine the antioxidative potential [3]. The higher activity of γ- and δ-tocopherol was also shown for higher unsaturated fats and oils [5, 13]. Even if the relative stability of the α-forms were highest under frying conditions [9] their antioxidative potential was the weakest. In fact tocotrienols were found to be very effective in extending shelf-life of coconut oil at those high temperatures and therefore they should be considered as food antioxidants.

3.2 Low temperature experiments

3.2.1 Tocotrienols

To observe the development of rancidity peroxide value (POV) and conjugated dienes (CDs) were determined. Both methods correlated fairly well ($r = 0.79$, $p < 0.01$) therefore only POV results are presented.

It took the control coconut fat about 12 weeks to reach the limit which was set to the POV of 5 mV/kg sample. This limit is normally used in experiments with highly saturated fats.

Both α- and β-tocotrienols reduced the stability of the control fat showing pro-oxidative effects which increased with higher concentrations added. 1000 mg/kg of both α- and β-tocotrienols reached a POV of 5 after 11 days, 100 mg/kg after about 25 days. At this time the POV of the control sample was below 1.5 mV/kg (Fig. 3A). γ-tocotrienol had a positive impact on shelf-life and was able to increase the fat stability from 12 to about 16 weeks (Fig. 3B). The increase was not dependent on concentrations added.

The highest enrichment of 1000 mg/kg of δ-tocotrienol proved to be most effective in scavenging free radicals. Therefore the stability of the control sample was lengthened up to 18 weeks (a 50% increase) before the limit of POV = 5 was exceeded. The lowest δ-tocotrienol concentration of 100 mg/kg seemed to have no effect.

In fact it was reported that between high and low temperature tests occur differences due to a change of oxidation mechanisms and antioxidants reactions [21]. The rank order for the antioxidative potential of the tocotrienols was similar for both test conditions.

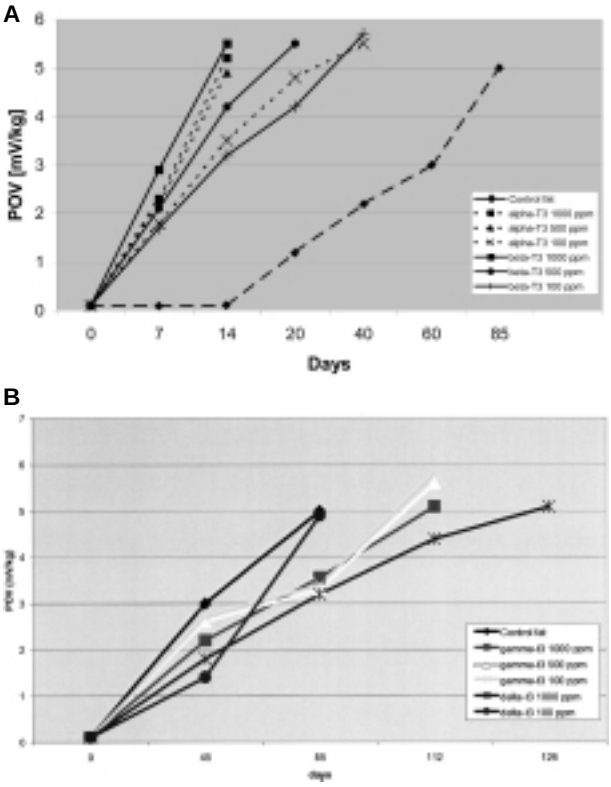

Fig. 3. Development of the peroxide value (POV) of coconut fat. **A.** after enrichment with alpha- and beta-tocotrienol (mg/kg) at 60 °C, **B.** after enrichment with gamma- and delta-tocotrienol (mg/kg) at 60 °C.

Even though tocotrienols are similar to tocopherols in structure, their antioxidative behavior is less studied in foods. Some information is available on their biological effects. Tocotrienols are thought to act antioxidatively in rats and in human lipoproteins [22] and are proven to lower plasma concentrations of atherogenic LDL cholesterol in various animal species [23, 24]. *Serbinova* et al. [6] evaluated a higher antioxidative potential of α-tocotrienol as compared to its corresponding tocopherol based on its higher recycling efficiency from the chromanoxyl radical, the more uniform distribution in the membrane bilayer and its stronger disordering of membrane lipids.

In vitro studies with microsome membranes or cancer cell lines identified tocotrienols as efficient protectors of lipids and proteins against oxidation [25]. However, less studies on the antioxidative potential of tocotrienols in foods are reported. Comparable results with the study performed were shown by *Feng* [8] who tested purified isolates of vitamin E homologues (tocopherols and tocotrienols) from vegetable oils at heating temperatures. He also observed that the γ-forms had a higher antioxidative strength than

the corresponding α-forms with the highest protection by γ-tocotrienol.

At lower temperatures the different tocotrienol forms behave like their corresponding tocopherols in similar studies [1, 13, 17].

We could observe, that α- and β-tocotrienols as well as α-tocopherol were the least effective food antioxidants both at room and at frying temperature. At room temperature the pro-oxidative activity increased as a matter of concentration. This is due to the known ability of tocochromanols to become pro-oxidants at higher concentrations, thus forming peroxyl radicals which propagate autoxidation [13, 26]. Both γ-tocopherol and γ-tocotrienol proved to be antioxidative both at room temperature similar conditions of 60 °C and under frying conditions. Under all tested conditions the potential increased significantly with concentrations added. Similar to observations of other working groups [27, 28] and our own results [13] the δ-forms reached their highest antioxidants potential at high temperatures. 1000 mg/kg of δ-tocotrienol followed by the same concentration of γ-tocopherol were the most effective in reducing the lipid oxidation at frying temperatures. At ambient temperature both γ- and δ-tocotrienols prolonged the fats shelf-life, δ-tocotrienol concentration dependent, whereas γ-tocotrienol did not depend on the amount given and δ-tocotrienol depended on the concentration.

This study could show that tocotrienols are good antioxidants to prolong shelf-life of coconut fat both at frying and at room temperature. With regard to their ability of reducing serum cholesterol levels in humans and the now presented antioxidative effects in foods at different temperatures, tocotrienols should increasingly be considered in the human daily diet and food technology.

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