J Food Sci Nutr
Vol 9, p 295~299 (2004)

J Food Science and Nutrition

Characterization of Thermal Products of Alpha-Tocopherol

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Abstract

Alpha-tocopherol was thermally oxidized and degraded at high temperatures and the resulting products were chromatographically separated and identified by LC-MS. Alpha-tocopherol dissolved in glycerol was heated at 200°C for 30 min. The thermal products were separated by hexane extraction and analyzed by HPLC using a reversed phase μ-Bondapak C_{18}-column with two kinds of elution solvents: a mixture of acetonitrile and methanol (3:2), and of acetonitrile, methanol, 2-propanol, chloroform and methylene chloride (3:2:5:0.5:0.5) in a gradient mode. The isolated thermal products of alpha-tocopherol were more viscous than alpha-tocopherol, and dark brown in color. Major thermal degradation products of alpha-tocopherol were identified by LC-MS, and the structures of thermal products were proposed. Alpha-tocopherol and its thermal degradation products were degraded into fragments, mainly at the non-aromatic parts. The degradation products of alpha-tocopherol were combined with oxidized product (tocopherylquinone) to make thermal products through dimerization.

Key words: alpha-tocopherol, thermal degradation products, HPLC

INTRODUCTION

Tocopherols naturally occur mainly in a variety of plant materials, especially oil seed crops (vegetable oils), some grains, nuts, and green leafy vegetables (1-5). The amount in plant foods is affected by species, variety, stage of maturity, season, time and manner of harvesting, processing procedures, and storage time (6).

Many studies have evaluated antioxidant effects of tocopherols in foods (7-12) on changes in the content of tocopherols (5,13-16), and on singlet oxygen quenching effects of tocopherols (17-21).

Strauch et al. (22) studied the metabolism of α-tocopherol in rats and mice and the structures of dimers and trimers of α-tocopherol by gas-liquid chromatography and mass spectrometry. The isolation and determination of the structure of a dimeric metabolite of α-tocopherol in mammalian liver has also been reported (23).

Previous studies (24-26) have separated the thermal degradation products of tocopherols by HPLC using reversed phase column to compare the chromatographic profile for each tocopherol and the thermal degradation pattern of tocopherols in a model food system during heating, and to investigate the kinetics of the degradation of tocopherols and how they are affected by heating time and heating temperatures in the presence or absence of oxygen.

Here, the major thermal degradation products of α-tocopherol produced during heating in a model food system are identified by LC-MS (liquid chromatography-mass spectrometry). Possible chemical structures of thermal degradation products of α-tocopherol are proposed. Identification of the structure of the thermal degradation products of tocopherol requires an understanding of the mechanism of thermal degradation of tocopherol.

MATERIALS AND METHODS

Materials

α-Tocopherol was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Glycerol was purchased from Junsei Chemical Co. (Tokyo, Japan) and membrane filter was from Acrodisc Gelman (Gelman LC13, 0.45 μm, Ann Arbor, MI, USA). All solvents (hexane, acetonitrile, methanol, 2-propanol, chloroform, methylene chloride) were of HPLC grade.

Extraction of thermal degradation products

A mixture of 50 mg α-tocopherol and 10 mL glycerol was placed in a crucible in furnace (Thermolyne 6000, Dubuque, IA, USA) and heated at 200°C for 30 min. A furnace was used for controlling the temperature. The stability of temperature was maintained within ±2%. After heating, the crucible containing α-tocopherol, glyc-

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erol and the nonvolatile thermal degradation product was removed from the furnace. After cooling the mixture for 30 min at room temperature, the thermal degradation products of α-tocopherol were extracted from glycerol with hexane. The hexane layer containing the thermal degradation products of α-tocopherol was filtered through a membrane filter (Gelman LC13, 0.45 μm, Ann Arbor, MI, USA) to analyze the components.

Separation of thermal degradation products

α-Tocopherol and its thermal degradation products, which were extracted with hexane, were simultaneously separated by HPLC under the same analytical conditions as described in the previous studies (24-26). The HPLC system consisted of a Waters 501 and 510 HPLC pumps, and a Waters 745B Data Module integrator (Millipore, Milford, MA, USA). The peaks of α-tocopherol and its thermal degradation products were observed at 295 nm using a Waters 484 UV detector. A reversed phase HPLC chromatography was performed on a μ-Bondapak C_{18}-column, using elution solvents with A, acetonitrile:methanol (3:2); B, acetonitrile:methanol:2-propanol:chloroform:methylene chloride (3:2:5:0.5:0.5) in a linear gradient mode according to the previous studies (24-26). The injection volume was 3 μL.

Collection of thermal degradation products

The major thermal degradation products (the b peak in Fig. 1; 24) of α-tocopherol was collected 30 times. Mass-separation of the fraction of thermal degradation products was performed by using the micro HPLC (Jasco, Tokyo, Japan, flow rate 10 μL/min).

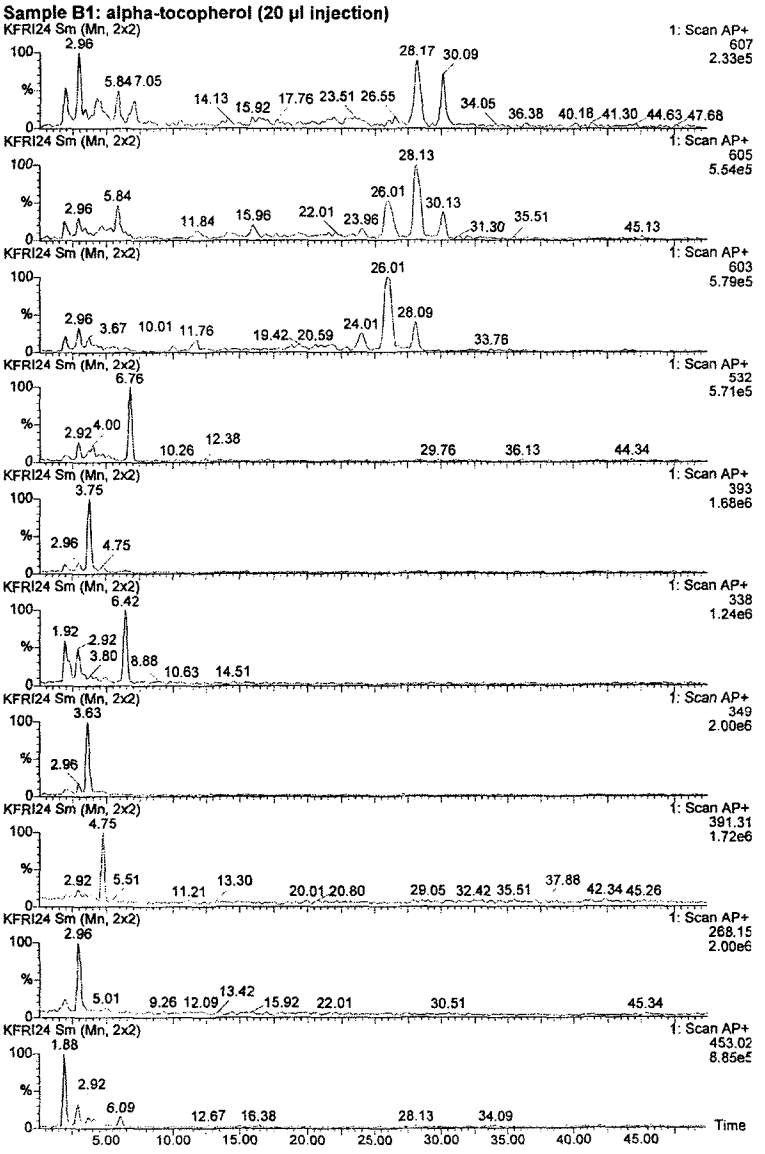

Fig. 1. LC-MS chromatograms of the thermal degradation products of α-tocopherol.

LC-MS analysis

Molecular mass (mass/charge: *m/z*) was analyzed by Platform II Mass Spectrometry (Micromass, Manchester, U.K.). Electrospray$^+$ ionization (positive ion electrospray) and APcI$^+$ (Atmospheric Pressure chemical Ionization$^+$) MS were used for analyzing the tocopherol and the thermal products of tocopherol, respectively. Mass range was 100 ~800 a.m.u (dalton/e). Other operating conditions followed the conventional conditions.

RESULTS AND DISCUSSION

LC-MS analysis results

The electrospray mass spectrum of α-tocopherol (figure not shown) shows that the molecular mass of α-tocopherol is 429.33 (calculated data is 430). Also, α-tocopherol can be fragmented to 164.70 (dalton/e) by electrospray mass spectrometry (cone voltage is 30 eV). This mass spectrum is the same as the spectrum reported in McLafferty and Stauffer (27). The LC-MS chromatograms of the thermal degradation products of α-tocopherol are shown in Fig. 1. The APcI$^+$ mass spectra of the thermal degradation products of α-tocopherol from each fraction of chromatogram (Fig. 1) are shown in great detail in a previous study (28).

The *m/z* 135 (Fig. 2) is a fragment of α-tocopherol, and the remaining side of the fragment is around *m/z* 297 that is detected as a small peak by mass spectrometry. The *m/z* 268 (Fig. 2) is another fragment of α-tocopherol which is also found with natural tocopherol, and *m/z* 162 that is detected as a small peak by mass spectrometry is the remaining side of the fragment of α-tocopherol (*m/z* : 430-162=268).

Tocopherol is oxidized to α-tocopherylquinone and α-tocopheroxide (17,20,21,29). The α-tocopherylquinone, a divalent oxidation product, is formed by rearrangement of a 9-substituted intermediate arising from the reaction between a cationic mesomer and a nucleophile (23).

Grams et al. (21) suggested that α-tocopherylquinone, a product from α-tocopherol oxidation, derived from several endoperoxide intermediates, which results from cycloaddition across the aromatic ring and aromatic addition of oxygen, has precedence in oxidation reactions.

The *m/z* 150 (Fig. 2) represents the fragment of α-tocopherylquinone, and another fragment must be *m/z* 297. The *m/z* 443 (Fig. 2) (this should be 446, but 443 was obtained due to the calibration error) is the α-tocopherylquinone. This result is consistent with the Grams et al. (21) conclusion that tocopherol is oxidized to α-tocopherylquinone.

Structure of thermal degradation products of α-tocopherol

The fragmentation patterns of α-tocopherol and α-tocopherylquinone are proposed by analyzing all the spectra as shown in Fig. 3 and Fig. 4. The aromatic ring is more stable and has stronger bond than aliphatic ring because of the resonance of electrons (30). Therefore, the non-aromatic parts of α-tocopherol and α-tocopherylquinone are affected by heating or oxidation, and decomposed into two fragments as shown in Fig. 3 and Fig. 4.

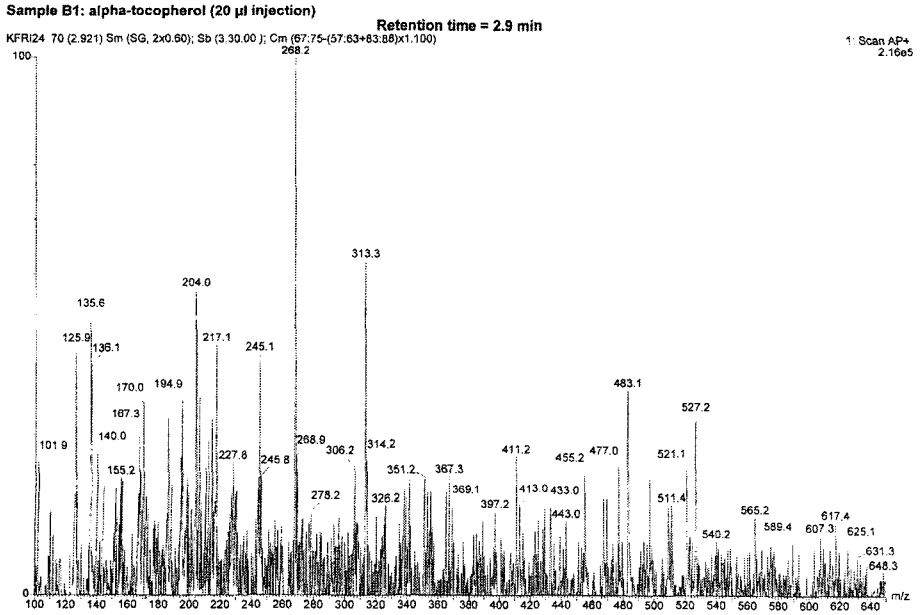

Fig. 2. APcI$^+$-mass spectra of the thermal degradation products of α-tocopherol (retention time=2.9 min).

α-Tocopherol ($C_{29}H_{50}O_2$; Mw 430)

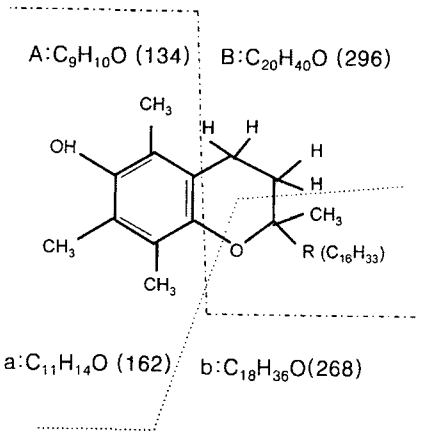

Fig. 3. Proposed fragmentation pattern of α-tocopherol deduced from the mass spectra.

α-Tocopherylquinone ($C_{29}H_{50}O_3$; Mw 446)

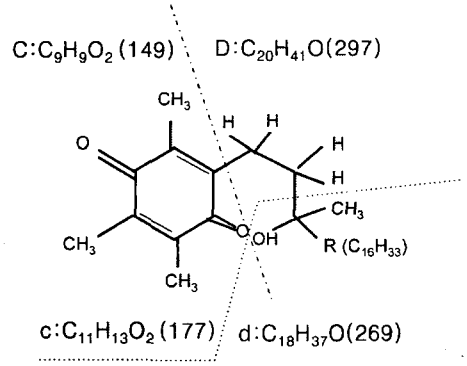

Fig. 4. Proposed fragmentation pattern of α-tocopherylquinone deduced from the mass spectra.

The α-tocopherol (Fig. 3) could be degraded into fragment A (molecular mass 134) and fragment B (molecular mass 296) or into fragment a (molecular mass 162) and fragment b (molecular mass 268). The α-tocopherylquinone (Fig. 4) could be degraded into fragment C (molecular mass 149) and fragment D (molecular mass 297) or into fragment c (molecular mass 177) and fragment d (molecular mass 269).

The *m/z* 577 and 578 (Fig. 5) represent the thermal degradation products from the combination of α-tocopherylquinone (*m/z* 446) and the fragment A of α-tocopherol (*m/z* 134) in Fig. 3. The *m/z* 603, 604 and 605 (Fig. 5) represent the thermal degradation products from the combination of α-tocopherylquinone (*m/z* 446) and the fragment of α-tocopherol (*m/z* 162) in Fig. 3. Strauch et al. (22) reported the formation of dimers and trimers of α-tocopherol in rats. The structure of the dimer of α-tocopherol was synthesized by Csallany and Draper (23).

In summary, major thermal degradation products of tocopherol were identified by LC-MS, and the structures of degradation products were proposed. The α-tocopherol was found to be oxidized to α-tocopherylquinone. Tocopherol and tocopherylquinone were degraded into fragments mainly at non-aromatic parts. Oxidized tocopherol and corresponding tocopherylquinone, and their degraded fragments formed various kinds of thermal degradation products.

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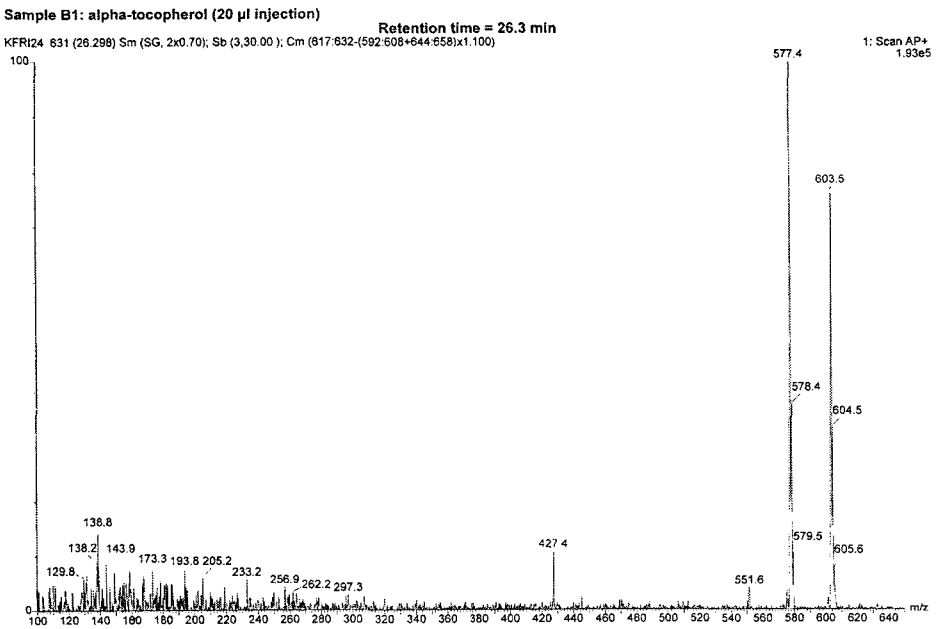

Fig. 5. APcI$^+$-mass spectra of the thermal degradation products of α-tocopherol (retention time=26.3 min).

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(Received April 13, 2004; Accepted November 30, 2004)