Detection of tocopherols in extracts by supercritical carbon dioxide with LC/APCI-MS2

Zhou Quancheng[1], Sheng Guihua[2], Wu Moucheng[3]

(1. *Department of Food Science and Technology, School of Agricultural and Light Industy Engineering, Shandong University of Technology, Zibo* 255049, *China*; 2. *Zibo Mining Group Limited Company, Zibo* 255120, *China*; 3. *College of Food Science and Technology, Huazhong Agricultural University, Wuhan* 430070, *China*)

Abstract: A simple, specific and sensitive high-performance liquid chromatographic method was developed for the determination and detection of tocopherols in extracts by supercritical carbon dioxide. A supercritical carbon dioxide extraction with cosolvents procedure was used to concentrate tocopherols from the rapeseed deodorize distillates. The analyses were separated on a Zorbax C18 reversed-phase column using 98% methanol as mobile phase, UV detection at 292 nm and α-tocopherol as a standard. The calibration graphs of the method were linear. Results by APCI-MS2 experiments were consistent with outcomes from theories.

Key words: supercritical carbon dioxide; tocopherols; LC/APCI-MS2; extraction methods; food analysis; structure identification

CLC number: TS201.2; TS201.1 **Document code**: A **Article ID**: 1002-6819(2004)06-0212-04

0 Introduction

Tocopherols function as the major radical scavenging antioxidant in lipoproteins and efficiently interrupt the chain propagation of lipid oxidation[1]. Different methods were described in the literature for detection of tocopherols by gas chromatography-mass spectrometry (GC-MS) as well as by high-performance liquid chromatography-mass spectrometry (HPLC-MS)[2-10], but these methods were complex and standards used were various and expensive. Furthermore, none of these methods was for detection of tocopherols in extraction by supercritical carbon dioxide with Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Tandom Mass Spectrometry (LC/APCI-MS2). This report describes the development of a method for detection tocopherols in extracts by supercritical carbon dioxide, followed by reversed-phase (HPLC/APCI-MS2).

1 Experiment

1.1 Chemicals and reagents

α-tocopherol was purchased from E. Merck (Germany). Methanol and ethanol (HPLC grade) were obtained from fisher. Water was distillated. All supercritical carbon dioxide extraction experiments were completed with supercritical fluid-grade carbon dioxide. All other reagents were analytical grade. Rapeseed deodorized distillates were kindly supplied by Hubei Tianyi Oil Ltd.

1.2 Liquid chromatography/atmospheric pressure chemical ionization-tandom mass spectrometric (LC/APCI-MS2)

For LC/APCI-MS2 detection, an Agilent (U. S.) Model 1100 Series separation model was used. The unit contained a cooled autosample with a 5 μL sample loop and a degassing unit. Instrument control and acquisition were done by the software LCMSD Agilent 4.1. The column used was a Zorbax C18, Φ21 mm × 50 mm, with 5 μm particle packed. Detections were carried out by measuring the absorbance with diode array detector. An isocratic mobile phase delivery system was 98% methanol delivered at 0.3 mL/min at room temperature with a total run time of 10 min.

Solutions of α-tocopherol were prepared by dissolving 30 mg each analyte in 50 mL ethanol.

MS was quadropole mass spectrometer with an APCI source. Ionization was positive ion and collision-induced dissociation (CID). Thus, Total ions chromatography (TIC) permited coelution of compounds with different masses or product fragment such as necessary for quantification of α-, β+γ- and δ-tocopherol.

The corona discharge electrode was set to 2.5 kV and the APCI probe temperature was 350℃. The flow rate of APCI heater was set to 4.0 L/min and the temperature of nebulizer gas (nitrogen) was 300℃. The sample collision voltage was adjusted to 0.9V.

Received date: 2004-07-16

Foundation item: the "Tenth Five-Year Plan" Science and Technology Key Project (2001BA501A20)

Biography corresponding author: Zhou Quancheng (1977-), PhD, Department of Food Science and Technology, School of Agricultural and Light Industy Engineering, Shandong University of Technology, Zibo 255049. Email: zhouquancheng@hotmail.com

© 1995-2005 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

MS2 experiments were carried out when intensity of target gas reached 2.0×10^5. The range of m/e (matter/electricity) 100～500 was scanned. The dwell time for each of the ions was set up 0.20 s. Tocopherols content in samples was determined from external standard calibration curves, corrected for recovery of added α-tocopherol, which served as an internal standard.

1.3 Sample preparation by supercritical carbon dioxide extraction

Deodorizer distillates (DD) containing 4% (wt) tocopherols were mixed with 3-fold methanol (v/w) and 0.04% sulfuric acid (w/w). The mixture were shakened and refluxed in 65℃ water for 3 h. During reaction, DD were continuously shaken. After reaction, DD were washed to be neutral by 60～70℃ water, and then held at 4℃ for 12 h. The sterol mixture in the DD were removed by filtration under a reduced pressure. The sterol-removed DD were mixed with 100% methanol (v/w) and 0.5% natrium methanol (w/w), then put into 74℃ water to be refluxed for 2 h. During reaction, DD were continuously shaken. After reaction, DD were washed to be neutral by 60～70℃ water, and then held at 4℃ for 12 h. The sterol mixture were then removed from the DD by filtrating under a reduced pressure. The resulting mixture was evaporated under vacuum to remove methanol and salt solution. After removing sterols, the esterified DD contained about 5% (wt) tocopherols.

The supercritical fluid extraction (SFE) system used in this study was purchased from Huaan Supercritical Supplement Ltd. The type is HA 231-50-025. The features of SFE system were as follows: an extraction cell and a separator column with four different temperature levels (2 m length, φ175 mm.)

The esterified DD were put into the extraction cell. A continuous flow of CO_2 was introduced into the whole supercritical system. When the operating pressure and temperature reached planned value and static extraction was performed for 10 min, tocopherol concentration was started. First, fatty acid methyl ester dissolved by CO_2 under 120 bar and 60℃ in the extraction cell was separated in the separator column under 80 bar and 60℃-60℃-60℃-60℃. The CO_2 flow rate was 15.0 kg/h. When the weight of fatty acid methyl ester didn't increased, the pressure of extraction cell increased to 250 bar to concentrate tocopherols. The first procedure of SFE was over. The extraction temperature and the separator column temperature were changed to 40℃ and 40℃-55℃-70℃-80℃ (from bottom to top) respectively, whereas separator pressure was maintained at 8.0 bar. Extraction and separation conditions were kept constant throughout the whole experiments. The resulting concentrate contained about 16% (wt) tocopherols. The resulting concentrates were collected and esterified. When all the working conditions of the second extraction stage were reached, 4% mixed ethanol (methanol∶ethanol = 2∶1) was pumped into the extraction cell and mixed with CO_2. After static extraction for 15 min, the SFE started. The fractionation was collected every 10 min (number 1, 2, 3) untill the weight of all collectors was increased to 12% (wt) of the esterified DD. The CO_2 flow rate was 25.0 kg/h.

2 Results and discussion

SFE is widely perceived as a technique for the extraction of low and moderately polar compounds[9].

The calibration curve for HPLC analysis was obtained by plotting the peak of tocopherols in each tocopherol analyte versus its concentration. The calibration graph for α-tocopherl was linear from 1.0 to 15.0 μg/mL. The equation, obtained through regressional analysis of data for the α-tocopherol standard solutions (each datum is the average of five determinations) was

$$y = 337187x - 546176 \quad (r = 0.9992)$$

Where, y —— the peak area of tocopherols; x —— the tocopherol concentration, μg/mL.

From the equations the concentration of the analyte could be determined. The precision and accuracy of the method were determined by preparing pools of α-tocopherol standard solutions at seven different concentrations. The value for α-tocopherol for each standard concentration were determined by five repeated analyses. The Relative Standard Deviate (RSD) value the slope was 3.4%. The standard errors were comprised between 5.6% and 9.7%. Retention times for α-, β+γ- and δ-tocopherols were 6.484, 5.514 and 4.753 min, respectively. No endogenous matrix components were observed near the retention times corresponding to α-, β+γ- and δ-tocopherols.

It noted that APCI+ typically produces $[M + H]^+$ ions. Thus m/e for α-tocopherol, β+γ-tocopherol and δ-tocopherol should be 431, 417.2 and 403.3 respectively in theroy. In fact, we observed from a, b, c, d of Fig. 1 that molecular ion of α-tocopherol, β+γ-

© 1995-2005 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

tocopherol and δ-tocopherol in the APCI source respectively generated ions at m/e 431.4, 417.2 and 403.3 by MS and at m/e 430.4 and 165.0, 417.3 and 151.0, 403.3 and 137.0 by MS2. Formation of the m/e 430.4 species in the APCI source reflected the facile one-electron oxidation of α-tocopherol by the corona discharge in the APCI source (Fig. 1).

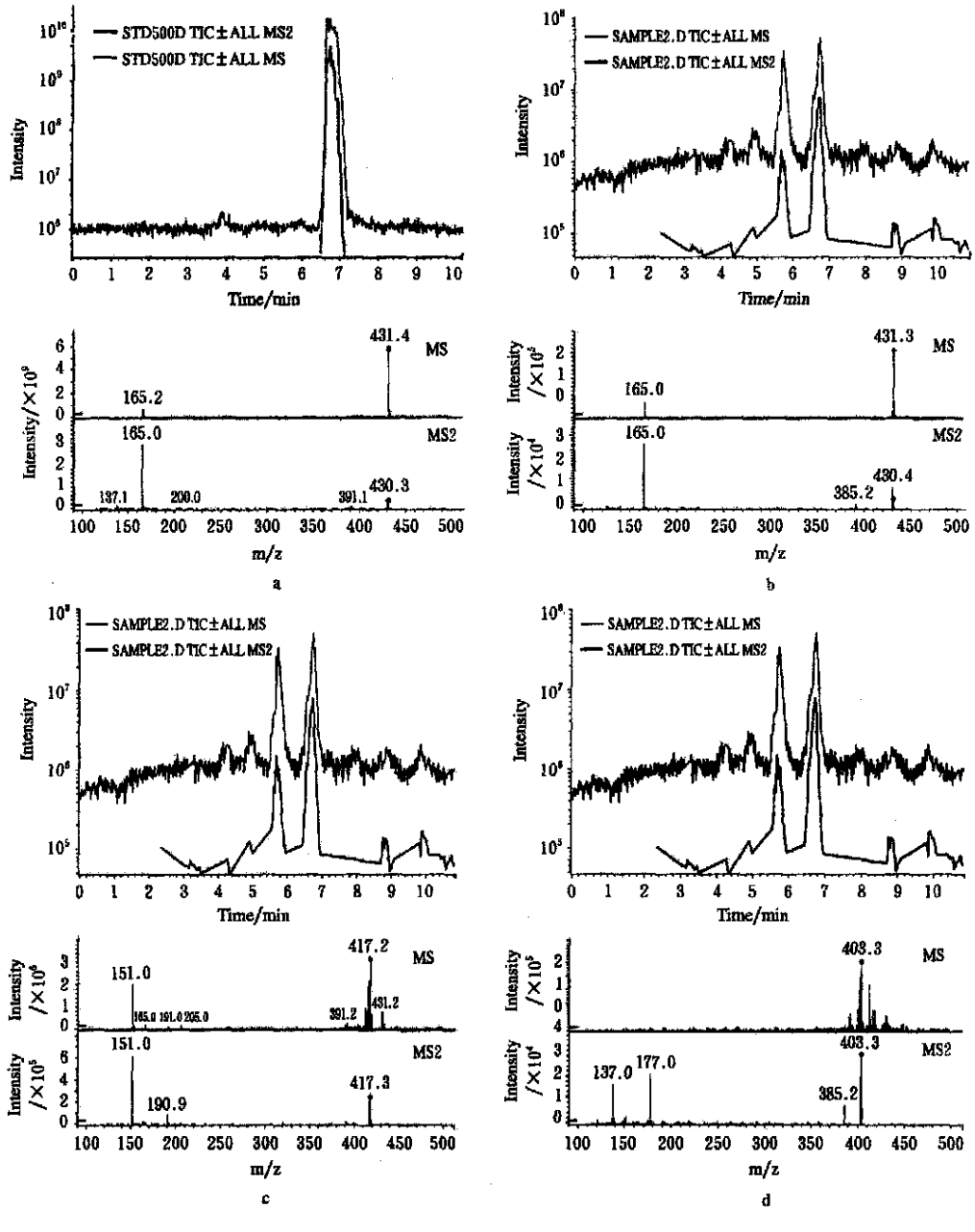

Fig. 1 TIC, MS and MS2 of α-Tocopherol Standard (a), α-Tocopherol in Sample (b), β- or γ-Tocopherol in Sample (c) and δ-Tocopherol in Sample (d)

Formation of m/e 165.0, 151.0 and 137.0 species reflected the loss of the phytyl chain. The results which we observed were consistent with outcomes from theories. We used the method to check extracts from DD with different liquid solvents, i.e. methanol, ethanol. In addition, fewer number of interfering compounds in the supercritical carbon dioxide extracts were found in comparison with the organic solvent extracts and it was possibly due to lack of the saponification step. The procedure described was simple, rapid and accurate and should be of value for the quantity of tocopherols in extracts by supercritical carbon dioxide.

[**References**]

[1] Pascal Mottier, Eric Gremaud, Philippe, et al. Comparison of gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry methods to quantify α-tocopherol and α-tocopherolquinone levels in human plasma [J]. Analytical Biochemistry, 2002, 301: 128- 135.

[2] Melchert, Pabel, Quantitative determination of α-, β-, γ- and δ-tocopherols in human serum by high-performance

© 1995-2005 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.

liquid chromatography and gas chromatography—mass spectrometry as trimethylsilyl derivatives with a two—step sample preparation[J]. Journal of Chromatography A, 2000, 27: 209- 215.

[3] Javier Parcerisa, Isidre Casals, Boatella, et al. Analysis of olive and hazelnet oil mixtures by high-performance liquid chromatography-atmospheric pressure chemical Ionisation mass spectrometry of triacylglycerols and gas-liquid chromatography of non-saponifiable compounds (tocopherols and sterols)[J]. Journal of Chromatography A. 2000, 881: 149- 158.

[4] Melchert, Pollok, Pabel, et al. Determination of tocopherols, tocopherolquinones and tocopherolhydroquinones by gas chromatography-mass spectrometry and preseparation with lipophilic gel chromatography [J]. Journal of Chromatography A, 2002, 8: 215- 220.

[5] Laurdsen, Leonard, et al. Quantitative analysis by liquid chromatography tandom mass spectrometry of deuterium-labeled and unlabeled vitamin E in biological samples[J]. Analytical Biochemistry, 2001, 289: 89- 95.

[6] Vatassery, Smith. Liquid chromatographic method for the simultaneous determination of α-tocopherol and tocopherolquinone in human red blood cells and other biological samples where tocopherol is easily oxidized during sample treatment[J]. Analytical Biochemistry, 1995, 214: 426- 430.

[7] Leray C, Anadriamampandry M, Freund M, et al. Simultaneous determination of homologous of vitamin E and coenzyme Q and products of α-tocopherol oxidation [J]. Journal of Lipid Research, 2001, 39: 2099- 2105.

[8] Roberta Andreoli, Paola Wanini, Diana Poli, et al. Development of a simplified method the simultaneous determination of retionol, α-tocopherol, and β-carotene in serum by liquid chromatography-tandem mass spectrometry with atmospheric pressure chemical ionization [J]. Analytical and Bioanalytical Chemistry, 2004, 378: 987- 994.

[9] Jerry W. King, Janet M Snyder, Hans Frykman, et al. Sterol ester production using lipase-catalyzed reactions in supercritical carbon dioxide[J]. European Food Research and Technology, 2001, 212(5): 566- 569.

超临界二氧化碳提取物中生育酚的LC/APCI-MS2 测定

周泉城[1], 盛桂华[2], 吴谋成[3]

(1. 山东理工大学轻工与农业工程学院食品系, 淄博 250049; 2. 山东淄博矿业集团有限公司, 淄博 255120; 3. 华中农业大学食品科学技术学院, 武汉 430070)

摘　要: 确定了一种简单, 明确和灵敏的高效液相测定和检测超临界二氧化碳萃取物中生育酚的方法。通过加入携带剂, 超临界二氧化碳从菜籽脱臭馏出物提取生育酚浓缩物, 其分析在反向色谱柱 Zorbax C18 上, 用 98% 甲醇作为流动相, UV 检测波长为 292nm, α-生育酚作标准物。此方法线性相关性较高。APCI-MS 和APCI-MS2 检测的各生育酚的 m/e 与理论预测值一致。

关键词: 超临界二氧化碳; 生育酚; LC/APCI-MS2; 提取方法; 食品分析; 结构鉴定

© 1995-2005 Tsinghua Tongfang Optical Disc Co., Ltd. All rights reserved.