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Short communication

Separation of conjugated linoleic acid isomers by cyclodextrin-modified micellar electrokinetic chromatography

Xiaohua Liu [a,b], Yusheng Cao [a,b,*], Yan Chen [a,b]

[a] *Sino-German Joint Research Institute, Nanchang University, Nanchang 330047, China*
[b] *The Key Laboratory of Food Science (Nanchang University), Ministry of Education, Nanchang University, Nanchang 330047, China*

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Abstract

A cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) method was developed for separating conjugated linoleic acid (CLA) isomers. All the seven CLA isomers (9*cis*,11*cis*-CLA, 9*cis*,11*trans*-CLA, 9*trans*,11*trans*-CLA, 10*trans*,12*cis*-CLA, 11*cis*,13*cis*-CLA, 11*cis*,13*trans*-CLA and 11*trans*,13*trans*-CLA) were completely separated in the optimized conditions (4% (w/v) β-cyclodextrin (β-CD), 54 mM sodium dodecyl sulphate (SDS), 80 mM borate (pH 9.0), 8 M urea, 4% (v/v) ethanol, 30 kV and 15 °C). The CD-MEKC method was superior to the gas chromatographic (GC) and silver-ion high-performance liquid chromatographic (Ag^+-HPLC) methods that were generally used in analyzing CLA isomers.

Keywords: Micellar electrokinetic chromatography (MEKC); Conjugated linoleic acid; Isomers; CD-MEKC

1. Introduction

Conjugated linoleic acid (CLA) is a collective term for geometric and positional isomers of linoleic acid (*cis*,*cis*-9,12-octadecadienoic acid) with conjugated double bonds. CLA occurs naturally in fat of dairy products and meat from ruminants. Conjugated dienoic acids have been found to be responsible for many biological properties that relate to human health. 9*cis*,11*trans*-CLA has the anticarcinogenic activity in vivo and in vitro [1–3], and 10*trans*,12*cis*-CLA seems to be specifically responsible for enhancement of energy metabolism and reducing body fat [4,5]. The level of CLA in dairy products and natural food is relatively low. It can be produced by chemical or enzymatic isomerization in large scale [6–9].

It is important to have available analytical methods for determination of the composition of CLA isomers in research and routine inspection. Gas chromatographic (GC) was one of the methods of analyzing CLA isomers, which produced overlapped peaks of some CLA isomers, such as 9*cis*,11*trans* and 8*trans*,10*cis* and their geometric isomers (*cis*,*cis* and *trans*,*trans*). Long column (CP-Sil 88, 100 m × 0.25 mm i.d. × 0.2 μm film thickness, Chrompack Inc., Raritan, NJ, USA) and more than 50 min retention time was needed in this method [10]. Furthermore, fatty acids needed to be methylated for GC method. Methylating of fatty acids was time-consuming, and *cis*,t*rans* or *trans*,*cis* isomers of CLA could be converted to *trans*,*trans* isomers [11]. Another method, Ag^+-HPLC, allowed for well-resolved separation of three groups of geometric isomers (*trans*,*trans*, *cis*/*trans*, and *cis*,*cis*) of a commercial CLA mixtures, and each group could be further separated into positional isomers, such as 11,13-CLA; 10,12-CLA; 9,11-CLA; and 8,10-CLA. The four peaks of each group were partial overlapped by single-column Ag^+-HPLC (ChromSpher 5 Lipids analytical silver-impregnated column, 4.6 mm i.d. × 250 mm stainless steel, 5 μm particle size, Chrompack, Bridgewater, NJ, USA) [12]. The four peaks of each group were separated using three columns in series, while the retention time was prolonged up to 60 min [10].

CE could separate isomers, such as nitrophenols, phenyl butyrates and naphthalenesulfonate isomers [13,14]. However, there were only a few reports related to separation of conjugated fatty acids by CE. Bohlin et al. separated conjugated trienoic fatty acid isomers by CE with a dual cyclodextrin system [15]. Öhman et al. explored the potential of CE for the

* Corresponding author. Tel.: +86 791 8327754.
E-mail address: yyssccc@hotmail.com (Y. Cao).

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separation of CLA isomers. It was reported that 9*trans*,11*trans*; 9*cis*,11*trans* and 9*cis*,11*cis* isomers could be separated with a buffer system contained heptakis-(2,3-dimethyl-6-sulfo)-β-CD (HDMS-β-CD), β-CD and (*R*)-*N*-dodecoxycarbonylvaline ((*R*)-*N*-DOCV), while 9*trans*,11*trans* and 8*trans*,10*trans*, and 9*cis*,11*trans* and 10*trans*,12*cis* were overlapped [16]. The present paper developed a cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) method for separating CLA isomers. CLA isomers tested were completely separated in 15 min. The effects of various parameters on the separation were investigated.

2. Experimental

2.1. Instrumentation

All experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system (Beckman, Fullerton, CA, USA) equipped with a photodiode array detection system and a coolant-cooled capillary cartridge. Analyses were carried out on untreated fused-silica capillaries of 61 cm (50 cm effective length) × 100 μm i.d. (Yongnian Optical Fibre Factory, Hebei, China). The electropherograms were recorded and integrated by a personal computer with 32 Karat software version 4.0 (Beckman).

2.2. Chemicals

Sodium dodecyl sulphate (SDS), 99.99%, was purchased from H&Y Bio. (Tianjin, China). β-CD was from Yunan Cyclodextrin Company (Guangdong, China). $Na_2B_4O_7{\cdot}10H_2O$, H_3BO_3, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, methanol, ethanol, acetonitrile and urea were from Shanhai Reagent (Shanhai, China). All reagents were of analytical grade. Pure water was made in a Milli-Q system (Millipore, France). CLA mixture was from Sigma (St. Louis, MO, USA). 9*cis*,11*cis*-CLA, 9*cis*,11*trans*-CLA, 9*trans*,11*trans*-CLA, 10*trans*,12*cis*-CLA and 11,13-CLA (80% *cis*, *trans*; 2% *cis*, *cis*; 6% *trans*, *trans*) were from Matreya (Pleasant Gap, PA, USA).

2.3. Electrophoretic procedures

The new capillary was rinsed in turn with methanol, water, 1 M HCl and water for 5 min, and then activated with 1 M NaOH and water for 30 min, respectively. Direct UV detection was performed at a single wavelength of 231 nm or scanning mode (190–300 nm). Pressure injection was performed using 0.5 p.s.i. for 5 s (1 p.s.i. = 6894.76 Pa).

2.4. Electrolyte preparation

Stock solutions of SDS and borate were prepared by adding appropriate aliquots to pure water, respectively. Additives, such as β-CD, urea and organic modifier were mixed with the buffer directly. The buffer solutions were filtered through a 0.45 μm membrane and degassed by sonication prior to use.

2.5. Sample preparation

Stock solutions of CLA mixture and CLA isomers were prepared in anhydrous ethanol. The solutions were kept at −20 °C under nitrogen. Samples were prepared daily by dissolving appropriate aliquots of the stock solution in running buffer. The sample solutions were degassed by sonication before use.

3. Results and discussion

3.1. Optimization of the separation parameters

In preliminary experiments, we found that it was difficult to separate 9*cis*,11*trans*-CLA and 11*cis*,13*trans*-CLA isomers among the standard CLA isomers. So we choose the R_s value of 9*cis*,11*trans*-CLA and 11*cis*,13*trans*-CLA for the evaluation of the resolution during method development. It was reported that six CLA isomers were not successfully separated when a dual CD system containing 10 mM β-CD and 5 mM HDMS-β-CD was used [16]. We found that all the seven CLA isomers tested in this study were completely separated using a unitary CD system. When β-CD was not used, all the CLA isomers coeluted. In case of the concentration of β-CD was 1% (w/v), all the other CLA isomers were separated except 9*cis*,11*trans*-CLA and 11*cis*,13*trans*-CLA coeluted. When the concentration increased from 2% to 4% (w/v), all the isomers were completely separated, and the resolution increased simultaneously. However, the 9*cis*,11*trans*-CLA and 11*cis*,13*trans*-CLA coeluted as the β-CD concentration increased to 5% (w/v). Furthermore, when the concentration increased up to 6% (w/v), 9*cis*,11*trans*-CLA, 10*trans*,12*cis*-CLA and 11*cis*,13*trans*-CLA overlapped and the resolution decreased quickly. The *cis*,*cis* isomers eluted before the *cis*/*trans* isomers, and the *cis*/*trans* isomers eluted before the *trans*,*trans* isomers.

9*cis*,11*trans*-CLA and 11*cis*,13*trans*-CLA coeluted when the concentration of SDS was 36 mM. The resolution increased from 1.0 to 1.37 as the SDS concentration increased from 42 mM to 54 mM. There was also a significant increase in migration time with the increase of SDS concentration. Varying the separation voltage produced slight changes in resolution, while the migration time decreased more than half (from 31.0 min in 15 kV to 13.2 min in 30 kV). 9*cis*,11*trans*-CLA and 11*cis*,13*trans*-CLA were somewhat overlapped when the concentration of urea was lower than 2 M. As urea concentration increased up to 4 M, the two isomers were fully separated. The resolution was increased from 1.35 to 2.10 with the urea concentration increased from 4 M to 8 M. The migration time was increased only 1 min when urea concentration increased from 4 M to 8 M. It was found that the resolution and migration time changed only a little as the pH varied from 9.0 to 5.8. The peaks started to be somewhat dissymmetric when the pH lower than 7.4. It was suggested that a higher pH might be the best choice.

Both migration time and resolution were increased with the increasing of borate concentration, because the increase in ionic strength reduced electroosmotic flow (EOF) and electromigration dispersion. The resolution increased from 1.51 to 2.05 as the concentration of borate increased from 20 mM to 80 mM. How-

Table 1
Linear range, limits of detection and relative standard deviation

Isomer	Linear range (γ^2) (μg/ml)	LOD (μg/ml)	RSD (%)[a]	
			Migration time	Peak area
9*cis*,11*cis*-CLA	35–560 (0.9927)	3.8	0.4	5.5
11*cis*,13*cis*-CLA			0.4	7.3
10*trans*,12*cis*-CLA	35–560 (0.9934)	3.4	0.4	4.5
11*cis*,13*trans*–CLA			0.4	5.5
9*cis*,11*trans*-CLA	35–560 (0.9933)	3.4	0.4	6.7
11*trans*,13*trans*-CLA			0.6	5.5
9*trans*,11*trans*-CLA	35–560 (0.9928)	3.8	0.4	5.7

[a] 140 μg/ml of 9*cis*,11*cis*-CLA, 10*trans*,12*cis*-CLA, 9*cis*,11*trans*-CLA and 9*trans*,11*trans*-CLA, $n = 5$.

ever, the resolution declined a little when the concentration of borate increased from 80 mM to 100 mM. The current increased simultaneously with increasing of borate concentration. Even then the higher ionic strength reduced electromigration dispersion, Joule heating still gave a relatively large contribution to band broadening, which might account for the decrease of resolution. We found that the resolution decreased, especially the resolution of 11*cis*,13*trans*-CLA and 10*trans*,12*cis*-CLA, when the capillary temperature was increased from 15 °C to 25 °C. 11*cis*,13*trans*-CLA and 10*trans*,12*cis*-CLA were somewhat overlapped as the capillary temperature was 20 °C, and coeluted at 25 °C. The peaks started to be dissymmetric as the increase of capillary temperature. The migration time decreased only a little with the increase of capillary temperature. When 4% (v/v) ethanol was added, the resolution was improved greatly. The resolution and migration time changed only a little when the concentration of ethanol increased from 4% to 16% (v/v). Methanol and acetonitrile were also investigated. We found that ethanol was the best organic modifier in this study.

3.2. Method validation

The assay was optimized by investigating factors affected the separation. The optimized conditions were 4% (w/v) β-CD, 54 mM SDS, 80 mM borate (pH 9.0), 8 M urea, 4% (v/v) ethanol, 30 kV and 15 °C for the separation of standard CLA isomers by CD-MEKC method. The result of assay in the optimized conditions was showed in Fig. 1.

Table 1 showed the linear range, limits of detection (LOD) and relative standard deviation. The limits of detection were measured when the signal-to-noise ratio (S/N) is 3. The relative standard deviation (RSD) of migration time and peak area were calculated based on five duplicated injection of mixture of standard CLA isomers.

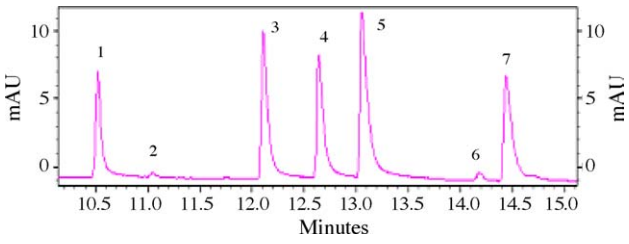

Fig. 1. Separation of CLA isomers. Conditions: 4% (w/v) β-CD, 54 mM SDS, 80 mM borate (pH 9.0), 8 M urea, 4% (v/v) ethanol, 30 kV and 15 °C. Peaks 1–7 are 9*cis*,11*cis*-CLA, 11*cis*,13*cis*-CLA, 10*trans*,12*cis*-CLA, 11*cis*,13*trans*-CLA, 9*cis*,11*trans*-CLA, 11*trans*,13 *trans*-CLA and 9*trans*,11*trans*-CLA, respectively.

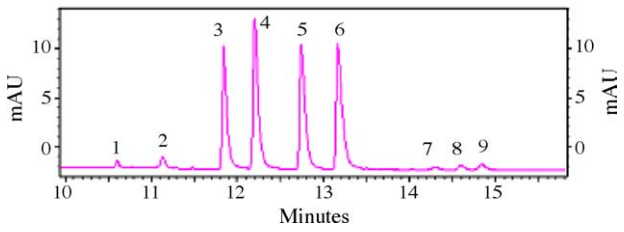

Fig. 2. Separation of a commercial CLA mixture. Conditions: 4% (w/v) β-CD, 54 mM SDS, 80 mM borate (pH 9.0), 8 M urea, 4% (v/v) ethanol, 30 kV and 15 °C. The individual isomers are: 1, 9*cis*,11*cis*-CLA; 2, 11*cis*,13*cis*-CLA; 3, *cis*/*trans*-8,10-CLA; 4, *cis*/*trans*-10,12-CLA; 5, *cis*/*trans*-11,13-CLA; 6, *cis*/*trans*-9,11-CLA; 7, 11*trans*,13 *trans*-CLA; 8, 9*trans*,11*trans*-CLA; and 9, an unidentified *trans*,*trans* isomer.

3.3. Analysis of CLA mixture sample

The feasibility of CD-MEKC method was tested in practice assay of a CLA mixture purchased from Sigma. It was found that four *cis*/*trans* isomers (peaks 3–6) were completely separated (Fig. 2), the maximum UV absorbance was 231.5 nm. Peak 3 was confirmed to be *cis*/*trans* 8,10-CLA by comparison with the result of Ag^+-HPLC [10]. As the concentration of CLA mixture was up to 40 mM, the four *cis*/*trans* isomers could be baseline separated. In spite of the content of *cis*,*cis* and *trans*,*trans* isomers were very low in the CLA mixture, they could also be detected. The maximum UV absorbance of peaks 1 and 2 was 234.5 nm, and peaks 7 to 9 was 228.5 nm. Therefore, they were *cis*,*cis* and *trans*,*trans* isomers, respectively.

4. Conclusion

A CD-MEKC method was developed for separating CLA isomers. There was a good agreement between the results of Ag^+-HPLC and CD-MEKC. But the assay time of CD-MEKC method was shortened to 15 min. The CD-MEKC method was simpler, safer and more economical than Ag^+-HPLC and GC methods, and it could be used in analyzing CLA isomers.

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