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Identification of Conjugated Linoleic Acids in Hydrogenated Soybean Oil by Silver Ion-Impregnated HPLC and Gas Chromatography-Ion Impacted Mass Spectrometry of Their 4,4-Dimethyloxazoline Derivatives

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Hydrogenated soybean oil was obtained after 10 min of hydrogenation with 0.5% selective type Ni catalyst at 230 °C, a hydrogenation pressure of 0.049 MPa, and an agitation rate of 300 rpm. The conjugated linoleic acid isomers in the hydrogenated soybean oil were isolated by using a silver ion-impregnated HPLC. Gas chromatography-mass spectrometry of 4,4-dimethyloxazoline (DMOX) derivatives of the isolated conjugated linoleic acid isomers were carried out for the identification of their chemical structures. By interpreting the mass spectra of the DMOX derivatives of conjugated linoleic acid isomers isolated by silver ion-impregnated HPLC, 20 different conjugated linoleic acid isomers present in hydrogenated soybean oil were identified. This is the first report for the mass spectrometric identification of the conjugated linoleic acid isomers present in hydrogenated vegetable oil.

KEYWORDS: Conjugated linoleic acids (CLA); hydrogenated soybean oil; DMOX derivatives; mass spectrometry

INTRODUCTION

Conjugated linoleic acid is a term for a mixture of positional and geometric isomers of linoleic acid with a conjugated double bond system. Conjugated linoleic acids have been recognized for their ability to prevent cancer (*1*, *2*), atherosclerosis (*3*), and NIDDM (Type II diabetes) (*4*). It also has been reported that conjugated linoleic acids are involved in the regulation of cytokines production, resulting in muscle and bone strengthening activity (*5*). Fat partitioning activity of conjugated linoleic acids also has been reported, resulting in fat reduction in pigs and humans (*6*). Conjugated linoleic acids are abundant in dairy products and meats from ruminant animals (*7*−*9*). Ha et al. (*7*) originally reported high levels of conjugated linoleic acids in cheeses and milk. Chin et al. (*8*) reported that dairy products (milk, butter, cheese, and yogurt) and meats from ruminant animals contained a large quantity of conjugated linoleic acids (ca. 3−8 mg total of conjugated linoleic acids/g of fat).

Jung and Ha (*9*) studied the formation of conjugated linoleic acids during selective and nonselective hydrogenation processes, and they reported that the large quantity of conjugated linoleic acids was formed during selective hydrogenation of soybean oil. Jung et al. (*10*) also reported that the hydrogenation conditions such as temperature and agitation rate greatly affected the quantity and composition of conjugated linoleic acids, and the time to reach the maximum quantity of conjugated linoleic acids during hydrogenation of soybean oil. The functionalities of conjugated linoleic acids reportedly differ greatly with isomers of different chemical structure. Thus, the elucidation of chemical structures of individual conjugated linoleic acid isomers in hydrogenated soybean oil is of great importance. In a previous report (10), however, the conjugated linoleic acids present in hydrogenated vegetable oils were tentatively identified by comparing those with authentic conjugated linoleic acid standard and conjugated linoleic acid isomers extracted from a cream cheese by silver ion-impregnated HPLC. Thus the systematic analysis for the elucidation of the chemical structure of CLA in hydrogenated soybean oil should be followed to correctly identify these isomers.

It has been reported that the derivatization of the fatty acid carboxylic acid group to pyrrolidides, picolinyl, piperidyl, morpholinyl esters, triazolopyridines, and 2-alkenylbenzoxanoles makes it possible to determine the double bond positions with mass spectrometry. However, these derivatives have low volatility, especially with long- chain fatty acids, and their gas chromatographic behavior is not good enough to permit the separation of the mixture of fatty acid derivatives (*11*). Zhang et al. (*12*) successfully introduced a derivatization technique for converting the carboxylic acid to the corresponding 2-substituted 4,4-dimethyloxazoline (DMOX). These derivatives markedly improved volatility, comparable to that of simple esters, and are well-suited for the location of olefinic linkage in polyunsaturated fatty acids with mass spectrometry. The structural identification of CLA in milks, cheeses, authentic CLA

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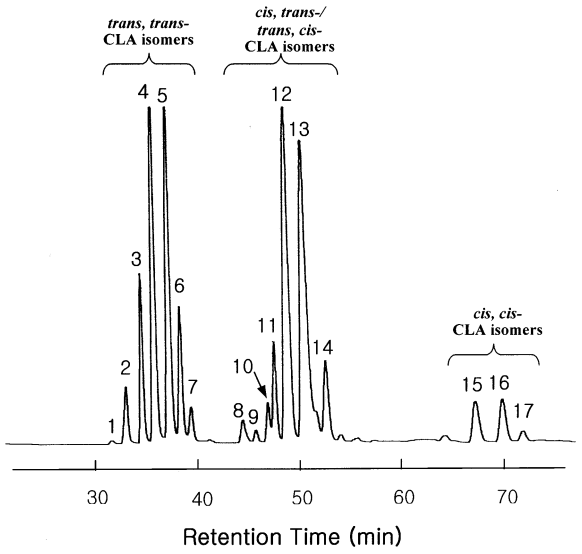

Figure 1. Silver ion-impregnated HPLC chromatogram of partially hydrogenated soybean oil fatty acid methyl esters.

samples, animal meats and organs, and human adipose tissue has been successfully carried out in previous work (*13*−*15*), using a silver ion-impregnated HPLC and gas chromatography−mass spectrometry with DMOX derivatives of CLA. However, the systematic study on the elucidation of individual CLA chemical structures in hydrogenated vegetable oils by gas chromatography-mass spectrometry (GC-MS) has never been previously carried out.

The objective of this research was to identify the individual CLA isomers present in hydrogenated soybean oil by using a silver ion (Ag^+) impregnated HPLC and gas chromatography-mass spectrometry of conjugated linoleic acid DMOX derivatives.

EXPERIMENTAL PROCEDURES

Materials. HPLC grade hexane and acetonitrile were purchased from Mallinckrodt Specialty Chem. Co. (Paris, KY). Sodium methoxide in methanol was obtained from Aldrich Chemical Co. (Milwaukee, WI). Refined, bleached, and deodorized soybean oil without any additive were obtained from Heinz Korea Ltd. (Seoul, Korea). The peroxide value of the oil was less than 0.3 meq/kg oil. Selective nickel catalyst (Pricat 9908) was obtained from Synetix (Emmerich, Germany).

Hydrogenation Conditions for the Preparation of Partially Hydrogenated Soybean Oils. The hydrogenation was performed with a 1-L-capacity hydrogenation reactor (Next Instrument, Hwa-sung, Korea) equipped with hydrogen pressure, temperature, and agitation rate controller. The hydrogenation was carried out for 10 min with 0.5% of a commercially available selective nickel catalyst (Pricat 9908, Synetix) and 0.049 MPa hydrogen pressure. The temperature and agitation rate used were 230 ± 2 °C and 300 rpm, respectively, during the hydrogenation process.

Preparation of Fatty Acid Methyl Esters. Oil samples were esterified with sodium methoxide (0.25 N) in methanol at 70 °C for 20 min. The fatty acid methyl esters (FAME) were extracted with 2,2,4-trimethylpentane containing an internal standard (heptadecanoic acid). It has been reported that the method of sodium methoxide−methanol does not induce the isomerization of conjugated linoleic acids (*16*).

Gas Chromatography. The separation of conjugated linoleic acids was carried out by gas chromatography. FAME (fatty acid methyl esters) samples of 2 μL were injected into a gas chromatograph equipped with a flame ionization detector. The column used was a highly polar (cyanopropylsiloxane phase, SP2380 100 m × 0.25 mm i.d., 0.25 μm thickness) fused silica capillary column (Supelco Inc., Bellefonte, PA). A 100:1 split injection was used for sample injection. Helium was used as a carrier gas with a head pressure of 300 kPa. Temperatures of injector and detector were 230 and 250 °C, respectively. The initial oven temperature was held at 170 °C for 1 min and then increased at 0.8 °C/min to 200 °C. The conjugated linoleic acid content was calculated according to the previously reported method (*10*, *16*), to express the results as mg of fatty acid/g of fat rather than as methyl esters.

Silver Ion High-Performance Liquid Chromatography. Silver ion high-performance liquid chromatographic separation of conjugated linoleic acid methyl esters was carried out with an HPLC (Shimadzu, Tokyo, Japan) equipped with a 20 μL injection loop (Waters) and UV detector operated at 233 nm (*13*, *14*). Three 250 × 4.6 mm i.d., 5 μm, ChromSpher 5 Lipids analytical silver-impregnated columns (Chrompack, Bridgewater, NJ) were used in series. The mobile phase was 0.1% acetonitrile in hexane, operated isocratically at a flow rate of 1.0 mL/min.

Preparation of 4,4-Dimethyloxazoline (DMOX) Derivatives. Individual isomers of conjugated linoleic acid methyl esters were collected from the silver ion-impregnated HPLC and were converted into DMOX derivatives (*11*) and analyzed by a GC-MS to identify the position of double bonds in conjugated linoleic acid isomers (*16*). Small portions (5−10 mg) of FAMEs were placed in microscale reaction vials, an amout of 2-amino-2-methyl-1-propanol equal to ca. 5 times the weight of the FAMEs was added, and the vial purged with argon and kept in a 170 °C dry oven for 6 h. On cooling diethyl ether/hexane (1:1, vol/vol; 5 mL) was added to the tube, followed by water (5 mL). The organic layer was washed with distilled water (3 mL) and dried carefully over anhydrous sodium sulfate. Then the organic solvents were taken to dryness by a gentle stream of nitrogen. The obtained DMOX derivatives of fatty acids were redissolved in a small volume of 2,2,4-trimethylpentane for GC-MS analysis.

Gas Chromatography-Mass Spectrometry. The gas chromatography-mass spectrometry was carried out with a Perkin-Elmer Auto System XL to identify the chemical structures of conjugated linoleic acids by injecting their DMOX derivatives. The same column was used in GC-MS as in GC. However, the oven temperature program was slightly different due to the slightly lower volatility of DMOX derivatives than the methyl esters of conjugated linoleic acids. The oven temperature was initially 170 °C, after injection, then increased 0.8 °C/min to 210 °C, and held for 10 min.

Iodine Value. The iodine value of the partially hydrogenated soybean oil was determined by AOCS official method Cd 1c-85 (*17*).

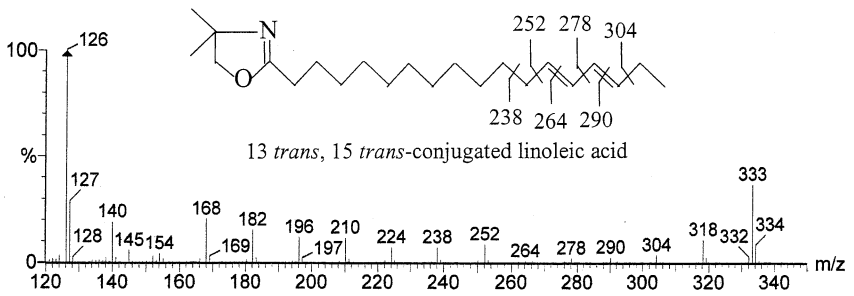

Figure 2. Mass spectrum of the DMOX derivative of peaks 1.

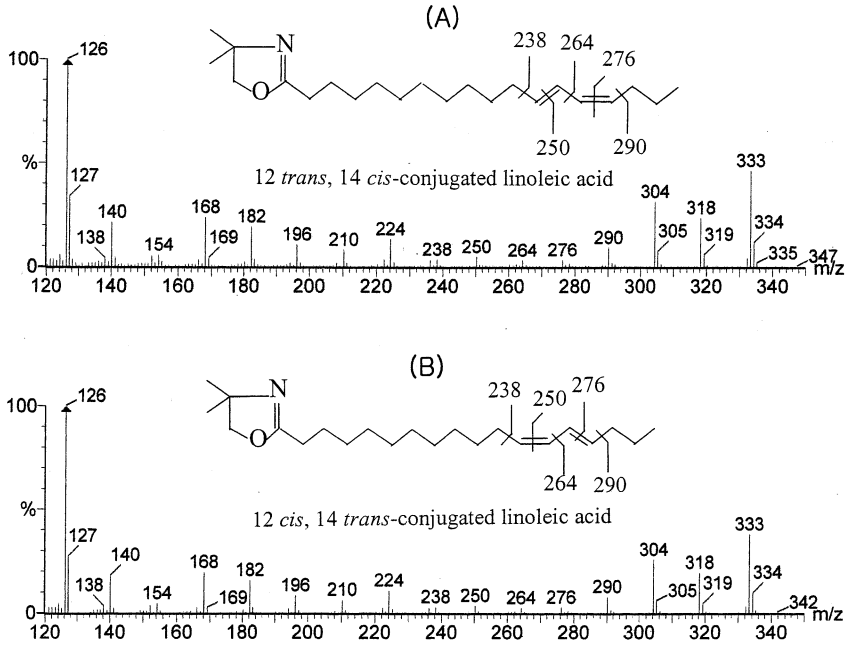

Figure 3. Mass spectra of the DMOX derivative of peaks 8 (A) and 9 (B).

RESULTS AND DISCUSSION

Silver Ion-Impregnated HPLC. The hydrogenated soybean oil was obtained after 10 min of hydrogenation. The obtained oil contained 156 mg of conjugated linoleic acids/g of oil. The iodine value of the oil was 118.7. The fatty acid composition of the oil was 11.21% palmitic acid, 6.17% stearic acid, 3.87% trans C18:1, 22.86% oleic acid, 1.75% cis C18:1, 6.54% unconjugated linoleic acid isomers, 26.83% linoleic acid, 0.49% arachidic acid, 1.13% C18:3 isomers, 1.45% linolenic acid, 0.56% behenic acid, and 17.16% conjugated linoleic acids. The obtained hydrogenated soybean oil was methylated with sodium methoxide, and the fatty acid methyl esters were subjected to silver ion (Ag^+) impregnated HPLC to isolate the individual conjugated linoleic acid isomers. **Figure 1** shows the partial Ag^+-HPLC chromatograms of the fatty acid methyl esters of hydrogenated soybean oil. The retention times of peaks 1−17 were 32.42, 33.49, 34.72, 35.71, 36.91, 37.97, 38.93, 45.12, 46.37, 47.57, 48.22, 49.24, 50.87, 53.12, 67.31, 69.98, and 72.87 min, respectively. The elution pattern of chromatographic peaks for the hydrogenated soybean oil methyl esters was exactly the same as that reported previously (*10*). In the Ag^+-HPLC chromatogram, the conjugated linoleic acid isomers were separated into three isomer groups (trans, trans; cis, trans and trans, cis; and cis, cis conjugated linoleic acids) (*13*−*15*). In a previous report (*10*), the individual Ag^+-HPLC peaks of the hydrogenated soybean oil were tentatively identified by comparing with those of authentic conjugated linoleic acid standard and conjugated linoleic acid isomers extracted from cream cheese. Moreover, the identification of some peaks (2, 8, and 9), which were not present in authentic conjugated linoleic acids and the isomers extracted from cheese, were assumed based on their expected elution order in Ag^+-HPLC (*10*).

Identification of Trans,Trans-Conjugated Linoleic Acid Isomers. In this research, to identify the individual conjugated linoleic acid isomers present in hydrogenated soybean oil, individual fractions of Ag^+-HPLC chromatographic peaks of hydrogenated soybean oil were collected by repeated Ag^+-HPLC runs. The collected conjugated linoleic acid fractions were converted into their DMOX derivatives. The DMOX derivatives of the individual fractions were injected into a GC-MS. The

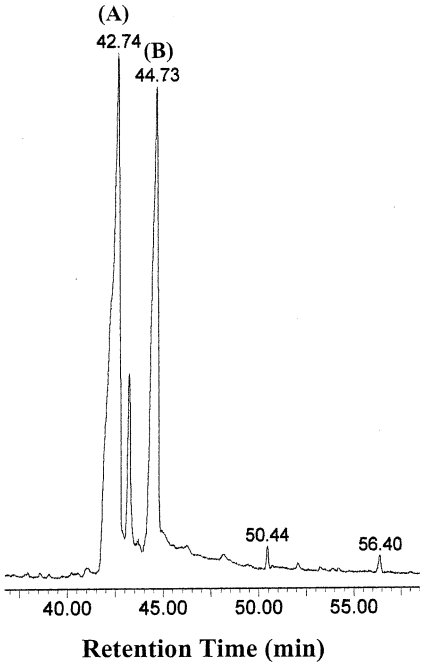

Figure 4. Partial GC-MS chromatogram of the DMOX derivative of peak 12.

MS spectrum of the DMOX derivative of peak 1 is shown in **Figure 2**. The fragment at *m*/*z* 126 was due to a cyclic ion formed by cleavage between carbons 4 and 5 of the 4,4-dimethyloxazoline derivative of fatty acid. The peak at *m*/*z* 333 was easily identified as a molecular ion peak due to the presence of one nitrogen atom in its structure. The molecular ion peak showed that the fatty acid contained 18 carbon atoms and 2 double bonds. The increment of 14 amu from 126 was observed in the mass spectrum as shown in **Figure 2**. The 14-amu increment was interrupted at the values of *m*/*z* 252, 264 and *m*/*z* 278, 290. The differences of 12 amu, instead of the usual 14, between *m*/*z* 252 and 264 and between *m*/*z* 278 and 290 were the indication of double bond presence between carbons positioned at 13 and 14, and 15 and 16, respectively. Since peak 1 was eluted in the trans/trans conjugated linoleic acid isomer

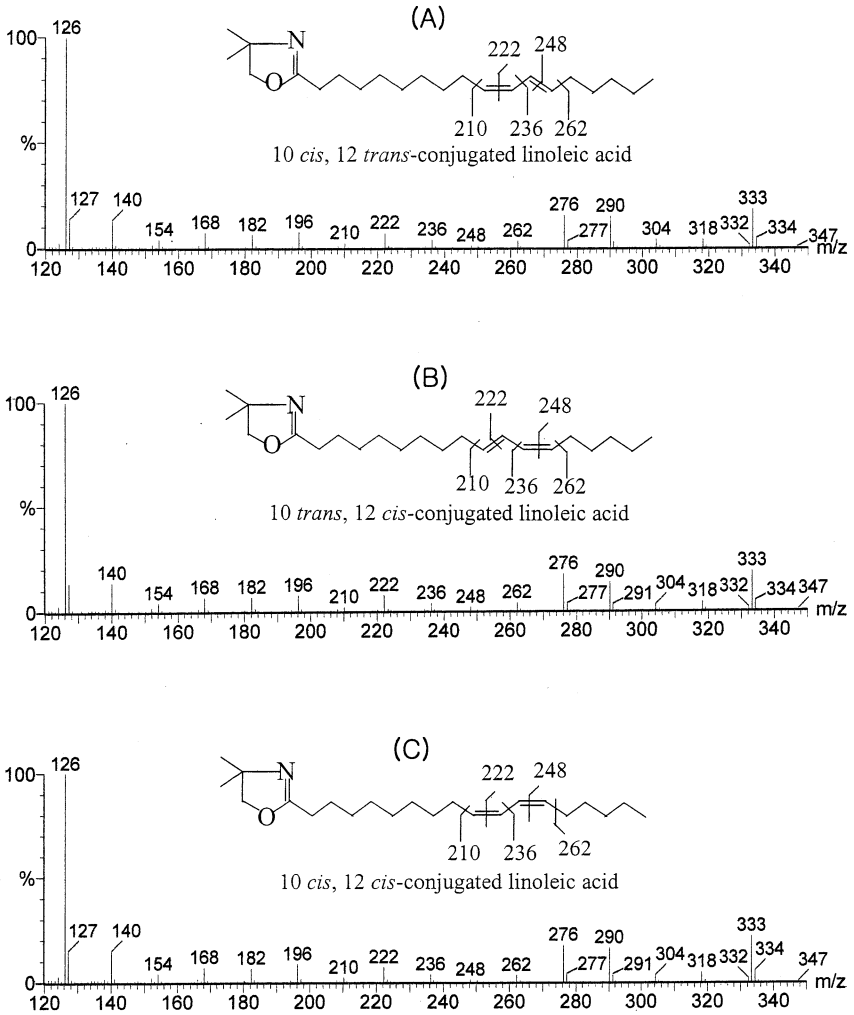

Figure 5. Mass spectra of the DMOX derivative of peaks 12-A (A), 12-B (B), and 15 (C).

region in Ag^+-HPLC, peak 1 could be easily identified as 13-*trans*,15-*trans*-octadecadienoic acid (13-trans,15-trans-conjugated linoleic acid). To our knowledge, the presence of 13-trans,15-trans-conjugated linoleic acid in foods has never been reported. This is also the first report on the elucidation of 13-trans,15-trans-conjugated linoleic acid by using mass spectrometry of its DMOX derivatives. By interpreting the mass spectra of DMOX derivatives of peaks 2−6 in the same way as peak 1, peaks 2−7 were identified as 12-trans,14-trans-, 11-trans,-12-trans-, 10-trans,12-trans-, 9-trans,11-trans-, 8-trans,10-trans-, and 7-trans,9-trans-conjugated linoleic acid isomers, respectively (mass spectra of DMOX derivatives of peaks 2−7 not shown). The mass spectrometric identification of these isomers (peaks 2−7) confirmed the previously reported identification of conjugated linoleic acid isomers in hydrogenated vegetable oil (*10*). The elution order of the trans,trans-conjugated linoleic acid isomers in Ag^+-HPLC in this report was also consistent with those previously reported (*13*, *14*).

Identification of Cis,Trans- or Trans,Cis-Conjugated Linoleic Acid Isomers. The mass spectra of the DMOX derivatives of peaks 8 and 9 are shown in **Figure 3**. The mass spectra of the DMOX derivatives of peaks 8 and 9 were identical, indicating that these two conjugated linoleic acids were isomers with the same double bond positions but with different geometrical configuration of their double bonds. The two mass spectra had same molecular ion peak at *m*/*z* 333, showing the fatty acid structure of 18-carbon atoms and 2 double bonds. The 14-amu increment from 126 was interrupted at the values of *m*/*z* 238, 250 and 264, 276. The gaps of 12 amu between *m*/*z* 238 and 250 and *m*/*z* 264 and 276 were the indication of double bonds present between carbons positioned at 12 and 13, and 14 and 15, respectively. Since these two peaks were eluted in the region of cis,trans- and trans,cis-conjugated linoleic acid isomers of Ag^+-HPLC, these isomers must be 12-cis,14-trans- and 12-trans,14-cis-conjugated linoleic acids. In the GC-MS chromatogram, the DMOX derivative of peak 9 was eluted before that of peak 8 (chromatogram not shown). It has been reported that the DMOX derivative of cis,trans-isomer eluted consistently earlier than that of trans,cis-isomer for all geometric pairs on cyanopropylsiloxane-phase column gas chromatograph (*14*, *18*). In a previous report (*14*), the gas chromatographic elution order of the conjugated linoleic acid isomers was studied by a combination of gas chromatography-mass spectrometry, and gas chromatography-direct deposition-Fourier transform infrared spectroscopy. On the basis of this previously reported elution order in a gas chromatograph, peaks 8 and 9 shown in **Figure 1** could be easily identified as 12-trans,14-cis- and 12-cis,14-trans-conjugated linoleic acid isomers, respectively. Peaks 10 and 11 also had identical mass spectra (data not shown). In a similar way as with the peaks 8 and 9, the mass spectra of DMOX derivatives of peak 10 and 11 were interpreted. On the basis of the reported gas chromatographic elution order (*14*), peaks 10 and 11 were identified as 11-trans,13-cis- and 11-cis,-13-trans-conjugated linoleic acid isomers, respectively. The identification of these two isomers was further confirmed by

the previously reported elution order of these two isomers on Ag^+-HPLC (*13*, *18*).

The GC-MS chromatograms of peaks 12−14 obtained from Ag^+-HPLC revealed that each of these peaks contained two major conjugated linoleic acid isomers. All the other peaks showed only one major peak in the conjugated linoleic acid region on the GC-MS chromatogram. The results suggested that no considerable isomerization of conjugated linoleic acid took place under the tested conditions (170 °C, 6 h under argon) of DMOX derivatization. It also has been reported that under the condition of the DMOX derivatization of fatty acids with 2-amino-2-methylpropanol heating overnight at 180 °C, no discrimination effect and no decomposition products of fatty acids have been detected (*11*).

Figure 4 shows the partial GC-MS chromatogram of DMOX derivatives of peak 12 obtained from Ag^+-HPLC shown in **Figure 1**. The GC-MS chromatogram clearly showed that there were two major conjugated linoleic acid isomers (A and B, retention times of 42.74 and 44.73 min, respectively) in peak 12. The mass spectra of these two conjugated linoleic acid isomers (A and B) are shown in **Figure 5**. These two conjugated linoleic acids had exactly the same mass spectra, indicating the same positions of double bonds in their molecules. The 14-amu increment was interrupted at the values of *m*/*z* 210, 222 and *m*/*z* 236, 248. The gaps of 12 amu between *m*/*z* 210 and 222 and *m*/*z* 236 and 248 were the indication of double bonds present between carbons positioned at 10 and 11, and 12 and 13, respectively. Since peak 12 eluted in the region of trans,-cis- or cis,trans-conjugated linoleic acid isomers in Ag^+-HPLC, the two isomers in peak 12 must be 10-trans,12-cis- and 10-cis,12-trans-conjugated linoleic acids. On the basis of the previously reported gas chromatographic elution order (*14*), the isomers A and B in **Figure 5** were also identified as 10-cis,-12-trans- and 10-trans,12-cis-conjugated linoleic acids, respectively. In a similar way, the mass spectra of all the individual conjugated linoleic acid isomers in peaks 13 and 14 were interpreted. It was found that peak 13 contained 9-cis,11-trans- and 9-trans,11-cis-conjugated linoleic acids. It was also found that peak 14 contained both 8-cis,10-trans- and 8-trans,10-cis-conjugated linoleic acids. It has been reported in a previous report (*18*) that these two isomers (8-cis,10-trans- and 8-trans,-10-cis-isomers) were separated on silver ion HPLC. The peak assignments of 8-cis,10-trans- and 8-trans,10-cis-isomers in a previous report were based on the assumption of equal distribution of these two paired isomer contents. However, the peak assignment in this research was based on fraction collection of peak 14 and synthesis of DMOX derivatives, and followed by GC-MS analysis.

Identification of Cis,Cis-Conjugated Linoleic Acid Isomers. The GC-MS chromatograms revealed that peaks 15−17 had one major conjugated linoleic acid isomer each (chromatograms not shown). The mass spectrum of the DMOX derivative of peak 15 is shown in **Figure 5C**. The interpretation of the mass spectra indicated that peak 15 has 18 carbons with two double bonds at the positions of carbons 10 and 12. Since peak 15 was placed in the region of cis,cis-conjugated linoleic acid isomers in the Ag^+-HPLC chromatogram shown in **Figure 1**, peak 15 was identified as a 10-cis,12-cis-conjugated linoleic acid. In a previous report (*10*), the peak eluted immediately before peak 15 was reported as a 11-cis,13-cis-conjugated linoleic acid isomer by comparing the retention time with authentic conjugated linoleic acid isomers. A careful examination of the mass spectrum of the peak eluting before peak 15 did not show the 11-cis,13-cis-conjugated linoleic acid to be present. Instead, the peak consisted of two unconjugated fatty acids (oleic acid and linoleic acid). By interpreting the mass spectra of the DMOX derivatives of peaks 16 and 17, these isomers were confirmed as 9-cis,11-cis- and 8-cis,10-cis-conjugated linoleic acids, respectively. The identification of the 8-cis,10-cis-conjugated linoleic acid isomer in hydrogenated vegetable oil has also never been previously reported. The composition of each of the CLA isomer peaks 1−17 shown in **Figure 1** was 0.24, 2.46, 5.49, 14.05, 13.76, 5.1, 1.76, 1.95, 0.7, 1.53, 3.74, 18.35, 20.54, 4.22, 2.71, 2.65, and 0.75%, respectively.

In summary, 20 different CLA isomers in hydrogenated vegetable oils were identified by a combination of silver ion-impregnated HPLC and GC-MS of DMOX derivatives of their isomers. The presence of 13-trans,15-trans-conjugated linoleic acid in foods and the structural elucidation of 8-cis,10-cis-conjugated linoleic acid in hydrogenated vegetable oil by using mass spectrometry of its DMOX derivatives are reported for the first time.

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LITERATURE CITED

(1) Ha, Y. L.; Grimm, N. K.; Pariza, M. W. Anticarcinogens from fried ground beef: Heat-altered derivaties of linoleic acid. *Carcinogenesis* **1987**, *8*, 1881−1887.

(2) Liew, C.; Schut, H. A. J.; Chin, S. F.; Pariza, M. W.; Dashwood, R. H. Protection of conjugated linoleic acids against 2-amino-3-methylimidazo[4,5-*f*]quinoline-induced colon carcinogenesis in F344 rat; a study of inhibitory mechanisms. *Carcinogenesis* **1995**, *16*, 3037−3043.

(3) Lee, K. N.; Kritchevsky, D.; Pariza, M. W. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **1994**, *108*, 19−25.

(4) Houseknecht, K. L.; Vanden Heuvel, J. P.; Moya-Camarena, S. Y.; Portocarrero, C. P.; Peck, L. W.; Nickel, K. P.; Belury, M. A. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker Diabetic Fatty fa/fa Rat. *Biochem. Biophys. Res. Commun.* **1998**, *244*, 678−682.

(5) Cook, M. E.; Miller, C. C.; Park, Y.; Pariza. M. W. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poult. Sci.* **1993**, *72*, 1301−1305.

(6) Park, Y.; Storkson, J. M.; Albright, K. J.; Liu, W.; Pariza, M. W. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* **1999**, *34*, 235−241.

(7) Ha, Y. L.; Grimm, N. K.; Pariza, M. W. Newly recognized anticarcinogenic fatty acids: Identification and quantification in natural and processed cheeses. *J. Agric. Food Chem.* **1989**, *37*, 75−81.

(8) Chin, S. F.; Liu, W.; Storkson, J. M.; Ha, Y. L.; Pariza, M. W. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Compos. Anal.* **1992**, *5*: 185−197.

(9) Jung, M. Y.; Ha, Y. L. Conjugated linoleic acid isomers in partially hydrogenated soybean oil obtained during nonselective and selective hydrogenation processes. *J. Agric. Food Chem.* **1999**, *47*, 704−708.

(10) Jung, M. O.; Yoon, S. H.; Jung, M. Y. Effects of temperature and agitation rate on the formation of conjugated linoleic acids in soybean oil during hydrogenation process. *J. Agric. Food Chem.* **2001**, *49*, 3010−3016.

(11) Fay, L.; Richli, U. Location of double bonds in polyunsaturated fatty acids by gas chromatography-mass spectrometry after 4,4-dimethyloxazoline derivatization. *J. Chromatogr.* **1991**, *541*, 89−98.

(12) Zhang, J. Y.; Yu, Q. T.; Liu, B. N.; Hunag, Z. H. Chemical modification in mass spectrometry IV: 2-alkenyl-4,4-dimethyloxazolines as derivatives for double bond location of long chain olefinic acids. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 33−44.

(13) Sehat, N.; Rickert, R.; Mossoba, M. M.; Kramer, J. K. G.; Yurawecz, M. P.; Roach, J. A. G.; Adlof, R. O.; Morehouse, K. M.; Fritsche, J.; Eulitz, K. D.; Steinhart, H.; Ku, Y. Improved separation of conjugated fatty acid methyl esters by silver ion-high performance liquid chromatography. *Lipids* **1999**, *34*, 407−413.

(14) Sehat, N.; Kramer, J. K. G.; Mooba, M. M.; Yurawecz, M. P.; Roach, J. A. G.; Eulitz, K.; Morehouse, K. M.; Ku, Y. Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. *Lipids* **1998**, *33*, 963−971.

(15) Yurawecz, M. P.; Roach, J. A. G.; Sehat, N.; Mossoba, M. M.; Kramer, J. K. G.; Fritsche, J.; Steinhart, H.; Ku, Y. A new conjugated linoleic acid isomer, 7 *trans*, 9 *cis* octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue. *Lipids* **1998**, *33*, 803−809.

(16) Shantha, N. C.; Decker, E. A.; Henning, B. Comparison of methylation methods for the quantification of conjugated linoleic acid isomer. *J. AOAC Int*. **1993**, *76*, 644−649.

(17) AOCS. *Official Methods and Recommended of the American Oil Chemists Society*, 4th ed.; Firestone, D., Ed.; American Oil Chemists Society: Champaign, IL, 1990.

(18) Eluitz, K.; Yurawecz, M. P.; Sehat, N.; Fritsche, J.; Roach, J. A. G.; Mossoba, M. M.; Kramer, J. K. G.; Adolf, R.; Ku, Y. Preparation, separation, and confirmation of the eight geometrical cis/trans conjugated linoleic acid isomers 8, 10- through 11, 13−18: 2. *Lipids* **1999**, *34*, 873−877.

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