ELSEVIER

Chemistry and Physics of Lipids 119 (2002) 23–31

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

www.elsevier.com/locate/chemphyslip

Production of conjugated linoleic acids through KOH-catalyzed dehydration of ricinoleic acid

Lin Yang [a,b], Yu Huang [c], Han Qing Wang [a], Zhen-Yu Chen [b,*]

[a] *State Key Laboratory of OXO Synthesis & Selective Oxidation, Lanzhou Institute of Chemical Physics, The Chinese Academy of Sciences of Lanzhou, and Department of Chemistry, Henan Normal University, Henan, People's Republic of China*
[b] *Department of Biochemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, People's Republic of China*
[c] *Department of Physiology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, People's Republic of China*

Received 3 December 2001; received in revised form 14 May 2002; accepted 14 May 2002

Abstract

Production of conjugated linoleic acids (CLA) using castor oil as starting material involves conversion of ricinoleic acid to methyl 12-mesyloxy-octadec-9-enoate (MMOE) followed by dehydration. This process usually uses 1,8-diazabicyclo-(5.4.0)-undec-7-ene (DBU) as an expensive dehydrating reagent. The present study reports that potassium hydroxide (KOH) can serve as a dehydrating reagent in replacement of DBU. The results showed that conversion of MMOE to CLA catalyzed by KOH was an efficient reaction, with a 77% conversion efficiency at 80 °C. The CLA isomeric profile produced in KOH-catalyzed dehydration reaction was similar to that catalyzed by DBU. The CLA mixture produced in KOH-catalyzed dehydration of MMOE at 80 °C contained 72% 9*c*,11*t*-18:2 and 26% 9*c*,11*c*-18:2 while in that catalyzed by DBU, 9*c*,11*t*-18:2 and 9*c*,11*c*-18:2 accounted for 78 and 16%, respectively. It was found that the temperature of dehydration was an important factor in the determination of CLA isomer composition and yield of conversion. Elevating the temperature from 78 to 180 °C decreased not only the conversion efficiency but also production of total *c*,*t*-18:2 and *c*,*c*-18:2 isomers regardless of dehydration catalyzed by either DBU or KOH. It is concluded that KOH may replace DBU as a dehydrating reagent in conversion of MMOE to CLA when the reaction conditions are optimized.

Keywords: Alkali isomerization; Conjugated linoleic acids; Linoleic acid; Ricinoleic acid

1. Introduction

There is increasing interest in producing conjugated linoleic acids (CLA) as a food ingredient and health supplement because of its possible health benefits associated with its consumption. CLA has been shown to inhibit tumor growth in experimental animals (Scimeca, 1999), to reduce atherosclerotic plaque (Lee et al., 1994; Nicolosi et al., 1997) and to lower serum cholesterol (Yeung et al., 2000). CLA may boost immune functions (Cook et al., 1993; Miller et al., 1994) and reduce fat accumulation while it increases muscle and bone mass (Dugan and Aalhus, 1999; Li and

* Corresponding author. Tel.: +85-2-2609-6382; fax: +85-2-2603-5123
E-mail address: zhenyuchen@cuhk.edu.hk (Z.-Y. Chen).

0009-3084/02/$ - see front matter
PII: S0009-3084(02)00052-X

Watkins, 1998). However, CLA as an antioxidant is found inconclusive (Chen et al., 1997; Ha et al., 1990; Ip et al., 1991; van den Berg et al., 1995).

High-performance liquid chromatographic analysis has demonstrated that CLA is a mixture of several isomers (Sehat et al., 1998). Dietary CLA predominately originates from dairy products via biohydrogenation of polyunsaturated fatty acids by rumen bacteria (Chin et al., 1992; Ha et al., 1989; Kepler and Tove, 1967). CLA is also biosynthesized from desaturation of *trans*-11 octadecenoic acid catalyzed by delta-9 desaturase (Mahfouz et al., 1980) in mammary tissue (Griinari and Bauman, 1999; Griinari et al., 2000), rodents and humans (Palmquist and Santora, 1999). Commercial production of CLA includes: (1) alkali isomerization of linoleic acid; (2) dehydration of ricinoleic acid methyl ester (Body and Shorland, 1965); and (3) microbial synthesis of 9*c*,11*t*-18:2 from linoleic acid using cultures of different microorganisms (Pariza and Yang, 1999; Jiang et al., 1998). Each of these methods produces a different mixture of CLA isomers. Among these methods, alkali isomerization of linoleic acid is the most commonly method for production of CLA because it is economically viable. In contrast, the production of CLA via dehydration of ricinoleic acid is an efficient reaction but it involves many steps (Berdeaux et al., 1997; Lie Ken Jie et al., 1997), and uses 1,8-diazabicyclo-(5,4,0)-undec-7-ene (DBU), an expensive dehydration reagent. The objectives of the present study were to simplify the dehydration process of ricinioleic acid, and to compare the isomeric distribution of CLA produced by the different dehydrating agents.

2. Materials and methods

2.1. Reagents

Castor oil (89% ricinoleic acid), boron trifluoride in methanol (14%, w/w), and linoleic acid were purchased from Sigma (St. Louis, MO). Methansulfonylchloride was obtained from Merck-Schuchardt (Hohenbrunn, Germany) while DBU was purchased from Fluka Chemie AG (Buchs, Switzerland). CLA standard was obtained from Sigma.

2.2. Conversion of ricinoleic acid from castor oil to CLA

2.2.1. Preparation of ricinoleic acid methyl ester (RAME)

As shown in Fig. 1, ricinoleic acid was converted to RAME by mixing 6 g castor oil, 50 ml of methanol and 12 ml of 14% boron trifluoride in methanol. The mixture was refluxed for 1 h at 65 °C and then cooled to room temperature followed by adding 100 ml of distilled water. The reaction mixture was then extracted three times with 50 ml of diethyl ether, followed by removal of the solvent using a rotary evaporator under vacuum. RAME (4.7 g) was purified on a silica column (60 × 3.4 cm, I.D.) using a mixture of petroleum ether and diethyl ether (6:4, vol./vol.) as an eluting solvent, and then dissolved in $CDCl_3$ for ^{1}H-NMR analysis (Bruker Avance DPX 300 Spectrometer, 300 MHZ, Fällanden, Switzerland). ^{1}H-NMR spectrum showed that RAME had the following characteristics ($CDCl_3$, *d*): 0.76 (*t*, 3H, CH_3); 1.20–1.50 (*m*, 20H, CH_2); 1.93 (*m*, 2H, 8-H); 2.09 (*t*, 2H, 11-H); 2.18 (*t*, 2H, 2-H); 3.48 (*s*, 1H, CH-OH); 3.54 (*s*, 3H, $COOCH_3$); 5.26–5.42 (*m*, 2H, 9H and 10H).

2.2.2. Preparation of methyl 12-mesyloxy-octadec-9-enoate (MMOE)

As shown in Fig. 1, MMOE was prepared by mixing RAME (2 g), methanesulfonyl chloride (1.6 g, 1.4 mmol), triethylamine (2 ml) and dichloromethane (20 ml), and stirring it for 45 min on an ice water bath. The reaction mixture was diluted with 50 ml of dichloromethane and successively washed with 10 ml of HCl solution (2 N) and twice with 20 ml of distilled water. The mixture was then dried over anhydrous sodium sulfate, filtered, and the solvents were removed in a rotary evaporator to obtain 2.2 g MMOE. ^{1}H-NMR spectrum showed that MMOE had the following characteristics ($CDCl_3$, *d*): 0.86 (*t*, 3H, CH_3); 1.25–1.63 (*m*, 20H, CH_2), 1.98 (*m*, 2H, 8-H), 2.26 (*t*, 2H, 2-H), 2.56 (*m*, 2H, 11-H), 2.95 (*s*, 3H, CH_3 of OMS), 3.65 (*s*, 3H, $COOCH_3$), 4.66 (*quintet*, 1H, CH-OMs), 5.33 (*m*, 1H, 9-H), 5.51 (*m*, 1H, 10-H).

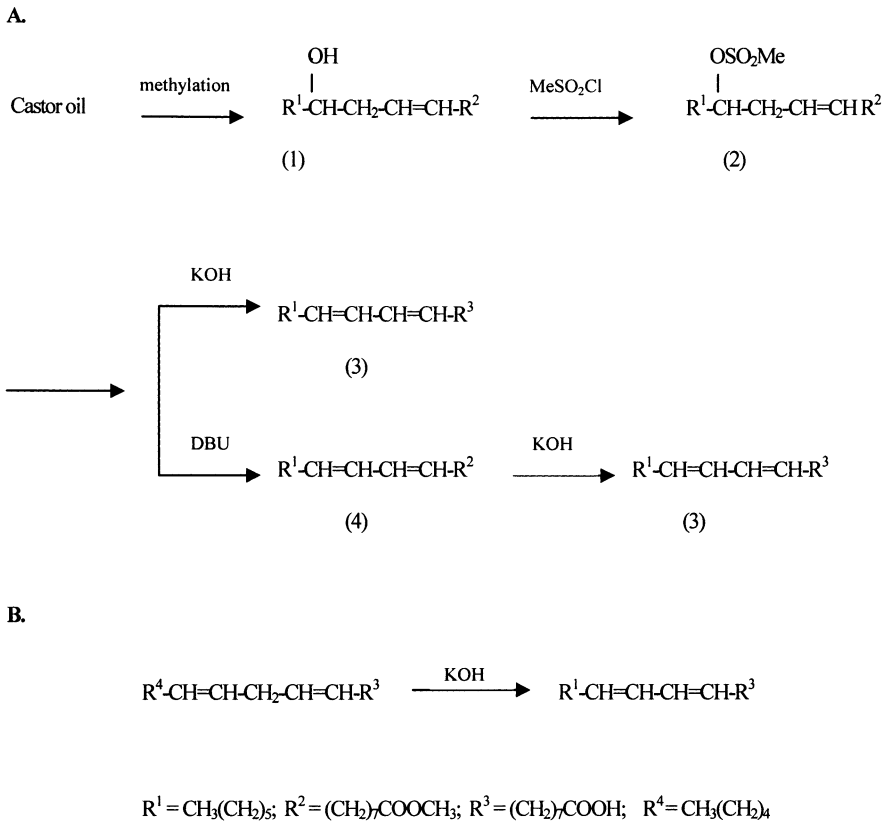

Fig. 1. Production of conjugated linoleic acids. (A) Castor oil was converted to fatty acid methyl esters followed by isolation of ricinoleic acid methyl ester (RAME, 1) which was then used for preparation of methyl 12-mesyloxy-octadec-9-enoate (MMOE, 2); conversion of MMOE to conjugated linoleic acids was catalyzed by 1,8-diazabicyclo-(5.4.0)-undec-7-ene (DBU) or potassium hydroxide (KOH). (B) Alkali isomerization of linoleic acids.

2.2.3. Production of CLA via dehydration of MMOE catalyzed by KOH

KOH-dependent dehydration of MMOE was accomplished in either absolute ethanol or ethylene glycol (50, 80, 120, 160, 180 °C). In brief, 20 ml of absolute ethanol (or ethylene glycol) and 1.4 g KOH were placed in a two-neck round-bottom flask (100 ml) equipped with a reflux condenser and a stream of nitrogen gas was bubbled through the solution for 5 min followed by adding 0.5 g MMOE. Then reaction mixture was refluxed with constant stirring for 8 h under a gentle stream of nitrogen gas. The flask was removed from the oil bath and cooled to room temperature. To the flask, 10 ml of distilled water was added and the solution was then transferred to a 250 ml separation funnel. The mixture was acidified with 10 ml of HCl solution (6 N) and extracted three times with 40 ml of diethyl ether. The combined ether extract was then washed with 15 ml of NaCl solution (0.9%) and dried over anhydrous sodium sulfate. Diethyl ether was removed in a rotary evaporator and the product was purified on a silica column (30 × 2.5 cm, I.D.) and eluted using the solvent mixture of petroleum ether and diethyl ether (6:4, vol./vol.). The fractions containing CLA were pooled. Individual CLA isomers were identified according to the eluting pattern of silver-ion high performance liquid chromatography (Ag-HPLC) previously described (Sehat et al., 1998; Ostrowska et al., 2000). The structure of the CLA was further confirmed by comparing its NMR spectra with those published previously (Lie Ken Jie et al., 1997). ^{1}H-NMR spectrum showed that

CLA prepared at 78 °C had the following characteristics ($CDCl_3$, *d*): 0.89 (*t*, 3H, CH_3), 1.20–1.40 (*m*, 18H, CH_2), 2.00–2.30 (*m*, 4H, 8-H_2 and 13-H_2), 2.28 (*t*, 2H, 2-H), 5.27–6.28 (*m*, 4H, 9-, 10-, 11-, 12-H). The CLA mixture was also subjected to Ag-HPLC analysis.

2.2.4. Production of CLA via dehydration of MMOE catalyzed by DBU

The method previously described by Lie Ken Jie et al. (1997) was adopted for dehydration of MMOE. In brief, 0.5 g MMOE was mixed with 1.3 g DBU in 4 ml of dimethylsulfoxide in a two-neck round-bottom flask. The reaction mixture was refluxed at different temperatures under nitrogen gas for 12 h. The reaction mixture was purified on a silica column (30 × 2.5 cm, I.D.) using the solvent mixture of *n*-hexane and diethyl ether (9:1, vol./vol.). The CLA methyl ester fractions were hydrolyzed with 10 ml of ethanolic KOH solution (5%) at room temperature for 1 h. After the reaction, 12 ml of distilled water was added and the solution was acidified with 10 ml of 1N HCl solution followed by three extractions with 20 ml of diethyl ether. The ether extract was washed twice with 15 ml of distilled water and dried over anhydrous sodium sulfate. After removal of ether, the crude CLA mixture was crystallized three times in 10 ml of ethanol. The resulting CLA had the following ^{1}H-NMR characteristics ($CDCl_3$, *d*): 0.88 (*t*, 3H, CH_3);1.20–1.62 (*m*, 18H, CH_2); 2.00–2.09 (*m*, 4H, 8-H_2 and 13-H_2); 2.33 (*t*, 2H, 2-H_2); 5.36–6.28 (*m*, 4H, 9-H, 10-H, 11-H and 12-H).

2.3. Production of CLA by alkali isomerization of linoleic acid

Linoleic acid was isomerized to CLA as previously described by Chin et al. (1992). In brief, linoleic acid (1 g) was mixed with 2.6 g KOH in 10 ml of ethylene glycol and refluxed at different temperatures under nitrogen for 4 h. To the reaction mixture, 20 ml of methanol was added followed by acidification with 40 ml of 3 N HCl. The reaction mixture was then extracted three times with 20 ml hexane. The hexane extract was washed thrice with 15 ml of 30% methanol in water, three times with 15 ml of distilled water, and then dried over anhydrous sodium sulfate. Hexane was removed in a rotary evaporator under vacuum. The crude CLA produced the following ^{1}H- NMR characteristics ($CDCl_3$, *d*): 0.89 (*t*, 3H, CH_3); 1.30–1.40 (*m*, 18H, CH_2); 2.08–2.17 (*m*, 4H, 8-H_2 and 13-H_2), 2.35 (*t*, 2H, 2-H_2); 5.28-6.30 (*m*, 4H, 9/10-H, 10/11-H, 11/12-H and 12/13-H).

2.4. Ag-HPLC analysis

Individual CLA isomers were quantified according to Ostrowska et al. (2000). In brief, the above mentioned CLA preparations (8 mg/ml) were separated using an Alltech Model 525 HPLC equipped with a ternary pump delivery system, a stainless steel silver-ion impregnated column (250 × 4.6 mm, I.D., 5 μm, Chrompack, Bridgewater, NJ), a rheodyne valve injector. Hexane containing 1.4% acetic acid and 0.014% acetonitrile was chosen as a mobile phase at a flow rate of 1.0 ml/min. The separated individual CLA isomers were quantified at 233 nm using a UV detector (UVIS-205, Alltech, Deerfield, IL). The Ag-HPLC is not capable of separating a *cis,trans*-18:2 species with a *trans,cis* species with same conjugated double bond positions. For example, 9*c*,11*t*-18:2 and 9*t*,11*c*-18:2 co-elute as a single peak. To simplify the presentation, the two isomers were hereafter designated as 9*c*,11*t*-18:2 only. This will apply for 8*c*,10*t*-18:2, and 10*c*,12*t*-18:2.

3. Results

Castor oil was used as a source of ricinoleic acid. Transesterification process led to produce a mixture of fatty acid methyl esters in which 89% was RAME. A silica column was used to isolate RAME with purity of 99%. RAME was subsequently used to prepare MMOE. Potassium hydroxide was evaluated in this study as a dehydrating reagent to produce CLA. As shown in Fig. 2, only two CLA isomers, namely 9*c*,11*t*–18:2 and 9*c*,11*c*-18:2, were obtained at 50 °C. When the temperature was increased to 120 °C, two additional isomers namely 10*t*,12*t*-18:2 and 9*t*,11*t*-18:2 were also produced. At 160 °C, *t*,*t*-

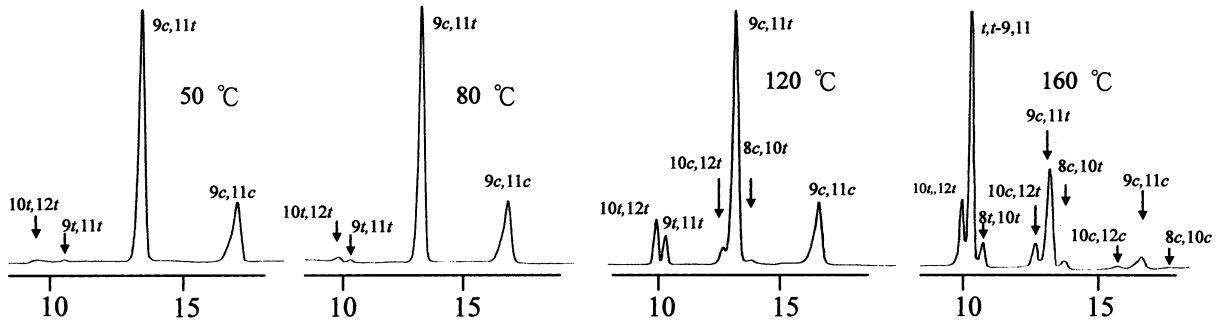

Fig. 2. Typical silver-ion high-performance liquid chromatograms of conjugated linoleic acids as free fatty acids produced by KOH-catalyzed dehydration of methyl 12-mesyloxy-octadec-9-enoate (MMOE). Separation was performed on a silver-ion impregnated Chrompack analytical column (250 × 4.6 mm. I.D.) using hexane/1.4% acetic acid/0.014% acetonitrile as mobile phase at a flow rate of 1 ml/min.

18:2 including 9*t*,11*t*-18:2, 10*t*,12*t*-18:2 and 8*t*,10*t*-18:2 became the major products followed by total *c*,*t*-18:2 isomers, while only minor amounts of total *c*,*c*-18:2 isomers were formed during the dehydration process. The change in CLA profile with increasing temperature is best illustrated in Fig. 3. Elevation of temperature increased production of total *t*,*t*-18:2 isomers while it decreased both total *c*,*t*-18:2 and *c*,*c*-18:2 products.

When DBU was used as a dehydrating reagent, the CLA profile was similar to that when MMOE was dehydrated by KOH (Fig. 4). At less than 80 °C, the major product was 9*c*,11*t*-18:2 followed by 9*c*,11*c*-18:2 with production of a trace amount of 9*t*,11*t*-18:2. When the temperature was elevated to 120 °C, the amount of 9*t*,11*t*-18:2 was increased to a level similar to that of 9*c*,11*c*-CLA, and at 180 °C, there were total 11 CLA isomers formed with *t*,*t*-18:2 being the major isomers. Among these *t*,*t*-18:2 isomers, 9*t*,11*t*-18:2 was most abundant. Similar to KOH as a dehydrating reagent, the profile of CLA isomers was a function of temperature. At a lower temperature, *c*,*t*-18:2 isomers were predominant while total *t*,*t*-18:2 isomers became the major CLA isomers at a higher temperature (Fig. 3).

The profile in alkali isomerization of linoleic acid was different from that of the CLA mixture produced by dehydration of MMOE with DBU or KOH. As shown in Fig. 5, 10*c*,12*t*-CLA and 9*c*,11*t*-CLA were the two major isomers with production of trace amounts of 9*c*,11*c*-18:2, 10*c*,12*c*-18:2, and 10*t*,12*t*-18:2. The temperature had less influence on the profile of CLA isomers formed. In the absence of oxygen, 94% of total CLA formed was *c*,*t*-18:2 isomers.

The conversion efficiency was different among the three methods. As shown in Fig. 6, the yield of alkali isomerization could reach 90% when the temperature was set at 160 °C or above, but below 120 °C, the conversion was 20% or less. In contrast, when KOH was used to dehydrate MMOE, the conversion was most efficient at 80 °C (77%). When the temperature of dehydration was increased to 160 °C, the conversion efficiency decreased gradually to 34%. The yield of CLA production by dehydration of MMOE using DBU reached 88% at 80 °C similar to KOH. At higher reaction temperatures, the conversion efficiency was less (60–63%) but relatively consistent. Among the three methods, the conversion efficiency of alkali isomerization was most temperature-dependent, while the change in the CLA profile was most significant with increasing temperature when MMOE was dehydrated by either DBU or KOH.

4. Discussion

Dehydration of MMOE is the most attractive alternative to produce CLA by alkali isomerization of linoleic acid. The starting material, castor

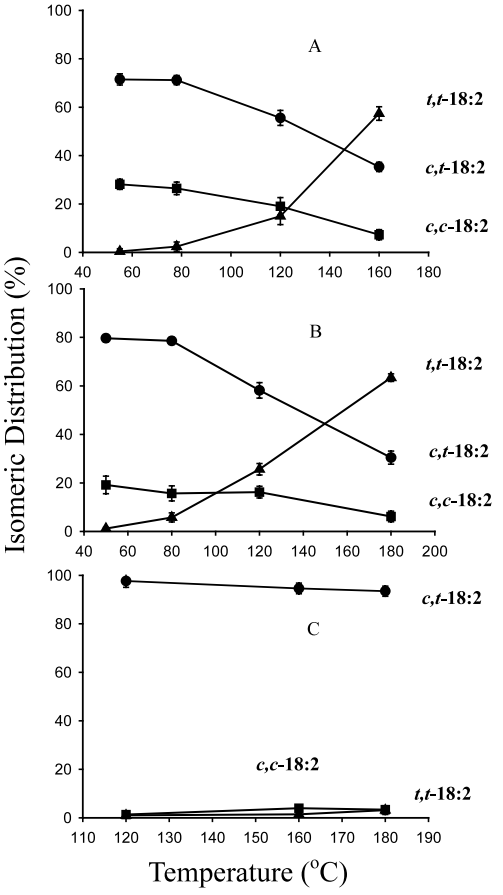

Fig. 3. Effect of temperature on isomeric distribution of total *t*,*t*-18:2, *c*,*t*-18:2 and *c*,*c*-18:2. (A) KOH-catalyzed dehydration of methyl 12-mesyloxy-octadec-9-enoate (MMOE). (B) 1,8-diazabicyclo-(5.4.0)-undec-7-ene (DBU)-catalyzed dehydration of MMOE. (C) Alkali-isomerization of linoleic acid.

oil, is relatively inexpensive. However, DBU, the previously reported (Lie Ken Jie et al., 1997), is six times as expensive as KOH. The present study was to seek an alternative dehydrating reagent to replace DBU without comprising the conversion efficiency. This is the first to show that KOH served as an efficient dehydrating reagent of MMOE. This method has the potential of being scaled up to produce an inexpensive and efficient CLA product.

The result clearly demonstrated that conversion of MMOE to CLA by KOH was an efficient reaction with a 77% conversion efficiency compared to 88% conversion efficiency using DBU at 80 °C (Fig. 3). The present results using DBU are in agreement with those reported by Berdeaux et al. (1997) to produce pure 9*c*,11*t*-18:2 at a conversion efficiency of 87% (sum of 9*c*,11*t*-18:2, 9*c*,11*c*-18:2 and 9*t*,11*t*-18:2). Lie Ken Jie et al. (1997) showed a similar conversion efficiency of 91% (sum of 9*c*,11*c*-18:2 and 9*c*,11*t*-18:2) for dehydration of MMOE using DBU. The present study found that replacement of DBU with KOH was chemically viable without significantly comprising the yield of CLA produced.

The CLA composition produced by KOH dehydration was similar to that produced by DBU dehydration. In KOH dehydration at 80 °C, 9*c*,11*t*-18:2 was predominant, accounting for 72% of total CLA, followed by 9*c*,11*c*-18:2 (26%). Similarly, DBU dehydration led to production of a CLA mixture in which 9*c*,11*t*-18:2

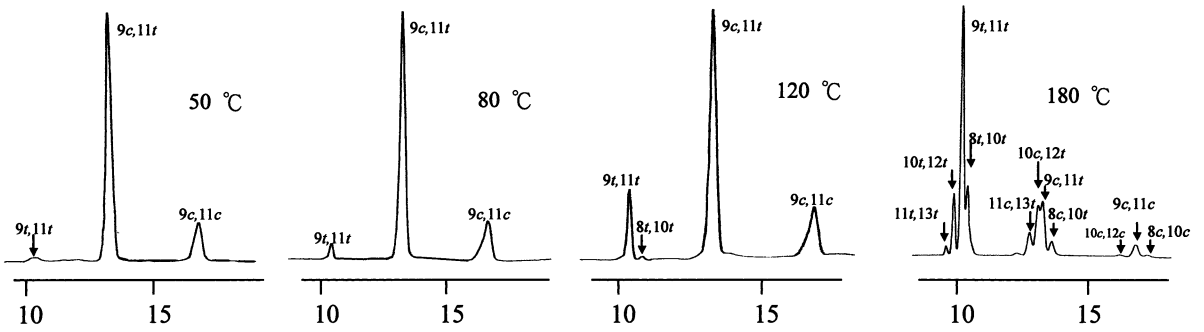

Fig. 4. Typical silver-ion high-performance liquid chromatograms of conjugated linoleic acids as free fatty acids produced by1,8-diazabicyclo-(5.4.0)-undec-7-ene (DBU)-catalyzed dehydration of methyl 12-mesyloxy-octadec-9-enoate (MMOE). Separation was performed on a silver-ion impregnated Chrompack analytical column (250 × 4.6 mm. I.D.) using hexane/1.4% acetic acid/0.014% acetonitrile as mobile phase at a flow rate of 1 ml/min.

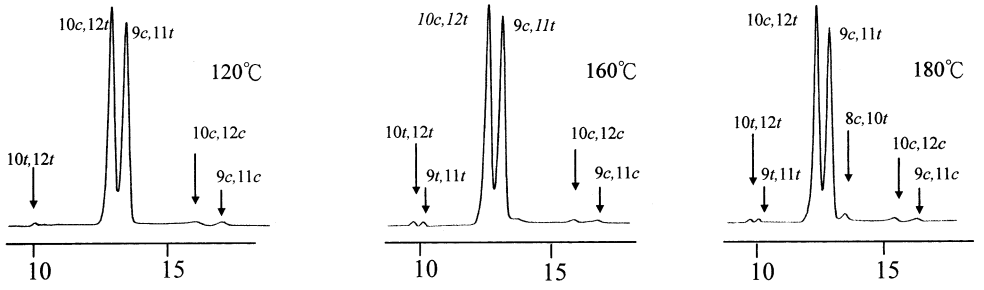

Fig. 5. Typical silver-ion high-performance liquid chromatograms of conjugated linoleic acids as free fatty acids produced by alkali-isomerization of linoleic acids. Separation was performed on a silver-ion impregnated Chrompack analytical column (250 × 4.6 mm. I.D.) using hexane/1.4% acetic acid/0.014% acetonitrile as mobile phase at a flow rate of 1 ml/min.

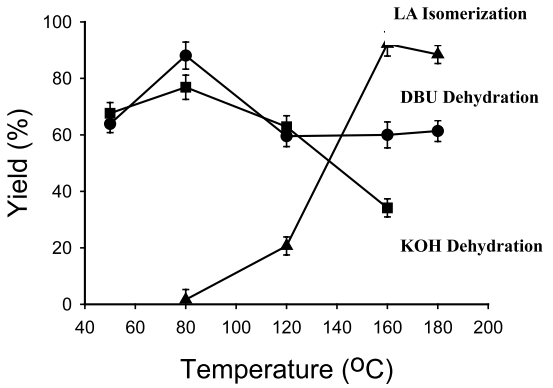

Fig. 6. Effect of temperature on production of conjugated linoleic acids. ■, KOH-catalyzed dehydration of methyl 12-mesyloxy-octadec-9-enoate (MMOE). ●, 1,8-diazabicyclo (5.4.0)undec-7-ene (DBU)-catalyzed dehydration of MMOE. ▲, alkali-isomerization of linoleic acid (LA).

accounted for 78% followed by 9*c*,11*c*-18:2, which accounted for 16% of total CLA at 80 °C. The data were in agreement with that of Berdeaux et al. (1997), who showed that DBU dehydration of MMOE produced 66% 9*c*,11*t*-18:2, 20.7% 9*c*,11*c*-18:2 and 0.6% 9*t*,11*t*-18:2. However, the profile of CLA isomers produced by KOH dehydration was markedly different from that of alkali isomerization of linoleic acid, in which two CLA isomers were produced (*c*,*t*-10,12-18:2 and 9*c*,11*t* –18:2) in about equal abundance (Fig. 5).

The temperature of the dehydration reaction was an important factor in the CLA isomer composition and yield of conversion. When the temperature was elevated to 180 °C, 9*t*,11*t*-18:2 was predominant for both DBU and KOH dehydration, because the *trans* configuration is chemically more stable than *cis* configuration. In contrast, the isomer distribution during alkali isomerization of linoleic acid was less influenced by temperature. The conversion of MMOE to CLA was most efficient at 80 °C using either DBU or KOH as dehydrating agent. The conversion rate declined at 180 °C probably due to thermal oxidation of CLA (Eulitz et al., 1999; Yang et al., 2000). The lower temperature (< 80 °C) for conversion of MMOE to CLA using KOH dehydration has an advantage over CLA produced by alkali isomerization of linoleic acid because of possible double bond migrating (> 160 °C).

We are unaware of any studies that have compared the isomeric distribution of CLA produced either by DBU dehydration of MMOE or alkali isomerization of linoleic acid. In the present study, the dehydration reaction of MMOE catalyzed by both KOH and DBU produces mainly one positional isomer (9*c*,11*t*-18:2) at temperature below 120 °C (Figs. 2 and 4). In contrast, alkali isomerization reaction of linoleic acid produces mainly two CLA isomers (9*c*,11*t*-18:2 and 10*c*,12*t*-18:2) at the three temperatures studied (Fig. 5). This can be explained by the different natures of the two reaction mechanisms. The dehydration of MMOE tends to occur between positions 12 and 11, leading to production of 9*c*,11*t*-18:2 with the lowest free energy due to resonance conjugated structure. However, the isomerization of linoleic acid at high temperature involves migration of the two double bonds. Thus,

the double bond at position 9 migrates towards position 10 to produce 9*c*,11*t*-18:2, and the double bond at position 12 migrates towards position 11 to form 9*c*,11*t*-18:2.

The present study examined the effects of temperature and dehydration reagents on the CLA production and when ricinoleic acid and linoleic acid were used as starting materials. The results clearly demonstrated that dehydration of MMOE and alkali isomerization of linoleic acid produced different CLA profiles. It is premature to assess merits of one method over another at the present time because the information on biological activities of each CLA isomer is still limited. Some evidences suggested that 9*c*,11*t*-18:2 was the active isomer (Ip et al., 1991; Knekt et al., 1996) while several recent reports showed that 10*c*,12*t*-18:2 was biologically more potent than 9*c*,11*t*-18:2 (Leung and Liu, 2000; Lin et al., 2001; Park et al., 1999). If 9*c*,11*t*-18:2 were the only active isomer, dehydration of MMOE catalyzed by KOH would be a preferred method to produce CLA. If 9*c*,11*t*-18:2 and 9*c*,11*t*-18:2 were both biologically active, alkali isomerization would be preferred to synthesize the CLA because it produced equal amounts of these two isomers.

References

Berdeaux, O., Christie, W.W., Gunstone, F.D., Sebedio, J.L., 1997. Large-scale synthesis of methyl *cis*-9, *trans*-11-octadecadienoate from methyl ricinoleate. J. Am. Oil Chem. Soc. 74, 1011–1015.

Body, D.R., Shorland, F.B., 1965. The geometric isomers of conjugated octadecadienonates from dehydration of methyl ricinoleate. J. Am. Oil Chem. Soc. 42, 5–8.

Chen, Z.Y., Chan, P.T., Kwan, K.Y., Zhang, A., 1997. Reassessment of the antioxidant activity of conjugated linoleic acids. J. Am. Oil Chem. Soc. 74, 749–753.

Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L., Pariza, M.W., 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Anal. 5, 185–197.

Cook, M.E., Miller, C.C., Park, Y., Pariza, M.W., 1993. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poultry Sci. 72, 1301–1305.

Dugan, M.E.R., Aalhus, J.L., 1999. Feeding CLA to pigs. In: Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., Nelson, G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, vol. 1. AOCS Press, Champaign, IL, USA, pp. 354–368.

Eulitz, K., Yurawecz, M.P., Ku, Y., 1999. The oxidation of conjugated linoleic acid. In: Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., Nelson, G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, vol. 1. AOCS Press, Champaign, IL, USA, pp. 55–63.

Griinari, J.M., Bauman, D.E., 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., Nelson, G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, vol. 1. AOCS Press, Champaign, IL, USA, pp. 180–200.

Griinari, J.E., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V., Bauman, D.E., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta(9)-desturase. J. Nutr. 130, 2285–2291.

Ha, Y.L., Grimm, N.K., Pariza, M.W., 1989. Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. J. Agric. Food Chem. 37, 75–81.

Ha, Y.L., Storkson, J., Pariza, M.W., 1990. Inhibition of benz(a)pyrene-induced mouse forstomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res. 50, 1097–1101.

Ip, C., Chin, S.F., Scimeca, J.A., Pariza, M.W., 1991. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res. 51, 6118–6124.

Jiang, J., Bjorck, L., Fonden, R., 1998. Production of conjugated linoleic acid by dairy starter cultures. J. Appl. Microbiol. 85, 95–102.

Kepler, C.R., Tove, S.B., 1967. Biohydrogenation of unsaturated fatty acids. J. Biol. Chem. 242, 5606–5612.

Knekt, P., Jarvinen, R., Seppanen, R., Pukkala, E., Aromaa, A., 1996. Intake of dairy products and the risk of breast cancer. Br. J. Cancer 73, 687–691.

Lee, K.N., Kritchevsky, D., Pariza, M.W., 1994. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 108, 19–25.

Leung, Y.H., Liu, R.H., 2000. *Trans*-10, *cis*-12-conjugated linoleic acid isomer exhibits stronger oxyradical scavenging capacity than *cis*-9, *trans*-11 conjugated linoleic acid isomer. J. Agric. Food Chem. 48, 5469–5475.

Li, Y., Watkins, B.A., 1998. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo bone prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 33, 417–425.

Lin, Y., Kreeft, A., Schuurbiers, J.A., Draijer, R., 2001. Different effects of conjugated linoleic acid isomers on lipoprotein lipase activity in 3T3 adipocytes. J. Nutr. Biochem. 12, 183–189.

Lie Ken Jie, M.S.F., Pasha, M.K.&, Alam, M.S., 1997. Synthesis and nuclear magnetic resonance properties of all geometrical isomers of conjugated linoleic acids. Lipids 32, 1041–1044.

Mahfouz, M.M., Valicenti, A.J., Holman, R.T., 1980. Desaturation of isomeric *trans*-octadecenoic acids by rat liver microsomes. Biochim. Biophys. Acta 618, 1–12.

Miller, C.C., Park, Y., Pariza, M.W., Cook, M.E., 1994. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem. Biophys. Res. Commun. 198, 1107–1112.

Nicolosi, R.J., Rogers, E.J., Kritchevsky, D., Scimeca, J.A., Huth, P.J., 1997. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 22, 266–277.

Ostrowska, E., Dunshea, F.R., Muralitharan, M., Cross, R.E., 2000. Comparison of silver-ion high-performance liquid chromatographic quantification of free and methylated conjugated linoleic acids. Lipids 35, 1147–1153.

Palmquist, D.L., Santora, J.E., 1999. Endogenous synthesis of rumenic acid in rodents and humans. In: Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., Nelson, G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, vol. 1. AOCS Press, Champaign, IL, USA, pp. 201–208.

Pariza, M.W., Yang, X.Y., 1999. Method of producing conjugated linoleic acids, US Patent 5,856,149.

Park, Y., Albright, K.L., Storkson, J.M., Liu, W., Pariza, M.W., 1999. Evidence that the *trans*-10, *cis*-12 isomers of conjugated linoleic acid induces body composition in mice. Lipids 34, 235–241.

Scimeca, J.A., 1999. Cancer inhibition in animals. In: Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., Nelson, G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, vol. 1. AOCS Press, Champaign, IL, USA, pp. 420–443.

Sehat, N., Yurawecz, M.P., Roach, J.A.G., Mossoba, M.M., Kramer, J.K.G., Ku, Y., 1998. Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. Lipids 33, 217–221.

van den Berg, J.J.M., Cook, N.E., Tribble, D.L., 1995. Reinvestigation of the antioxidant properties of conjugated linoleic acid. Lipids 30, 599–605.

Yeung, C.H., Yang, L., Huang, Y., Wang, J., Chen, Z.Y., 2000. Dietary conjugated linoleic acid mixture affects. The activity of intestinal acyl coenzyme A:cholesterol acyltransferase in hamsters. Br. J. Nutr. 84, 935–941.

Yang, L., Leung, L.K., Huang, Y., Chen, Z.Y., 2000. Oxidative stability of conjugated linoleic acid isomers. J. Agric. Food Chem. 48, 3072–3076.