Preparation of Conjugated Linoleic Acid from Safflower Oil

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ABSTRACT: Synthetically prepared mixtures of conjugated linoleic acid (CLA) are widely used in animal and cell culture studies to investigate the potential effects of the Δ9*c*,11*t*-18:2 isomer found in food products from ruminant animals. Alkali isomerization of linoleic acid is a common method used in the synthesis of a mixture of CLA isomers containing predominantly the Δ9*c*,11*t*-18:2 and Δ10*t*,12*c*-18:2 isomers. Some biological activity might also be mediated by the Δ10*t*,12*c*-18:2 isomer. Currently few published methodologies exist describing procedures for the enrichment of these two isomers. A method is described herein to take advantage of an inexpensive oil, safflower oil, for use in synthesis of CLA and a procedure to enrich the Δ10*t*,12*c*-18:2 isomer.

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KEY WORDS: Conjugated linoleic acid, safflower oil, urea crystallization.

Conjugated linoleic acid (CLA) refers to a group of geometrical and positional isomers of linoleic acid. CLA is reported to have wide-ranging biological effects such as inhibiting tumor growth (1,2), reducing atherosclerotic risk (3) and reducing body fat (4). Synthetically prepared mixtures containing predominantly the Δ9c,11*t*-18:2 and Δ10*t*,12*c*-18:2 isomers in equal amounts have been widely used to study these effects. It has generally been thought that the Δ9*c*,11*t*-18:2 is the biologically active isomer because of its greater natural abundance in our current food supply (5) and its preferential incorporation into cellular lipids (6). Recently, horse sera was reported to contain significant amounts of the Δ10*t*,12*c*-18:2 isomer, suggesting that this CLA isomer may have biological importance (7).

Alkali isomerization of linoleic acid is a common method used to synthesize100-g quantities of CLA (5). These synthetic mixtures reportedly contain two major isomers, Δ9*c*,11*t*-18:2 and Δ10*t*,12*c*-18:2, accounting for approximately 90% of total CLA content, with the remainder consisting of *cis,cis* (*c,c*) and *trans,trans* (*t,t*) isomers of Δ9,11-, 10,12- and 11,13-18:2 (8). This paper presents methods to utilize safflower oil, high in linoleic acid, to synthesize CLA in inexpensive 100-g quantities and selective enrichment of the Δ10*t*,12*c*-18:2 isomer by urea crystallization.

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EXPERIMENTAL PROCEDURES

Safflower oil was obtained from a local grocery store (Edmonton, Canada). All other chemical reagents were obtained from BDH (BDH Inc., Toronto, Ontario, Canada).

Extraction and purification of linoleic acid (LA). Safflower oil was saponified and saturated, and monounsaturated fatty acids were removed as described by Gunstone *et al.* (9). LA extracted from safflower oil (100 g) was crystallized in urea (120 g) dissolved in warmed MeOH (240 mL; 1 g/2 mL). LA was crystallized overnight at 5°C. Vacuum filtration was used to recover purified LA contained in the mother liquor portion. The mother liquor material was transferred to a separatory funnel (1 L) and acidified to pH <2 with HCl (6 N, 200 mL) and ddH_2O (200 mL). LA was extracted with hexane (100 mL). The aqueous phase was reacidified with sulfuric acid (6 N, 50 mL) and ddH_2O (30 mL), followed by extraction with hexane (100 mL). Hexane fractions were combined. The hexane phase was washed with 30% (vol/vol) methanol/ddH_2O (3 × 50 mL), then with ddH_2O (3 × 50 mL). The washed hexane phase was dried over anhydrous sodium sulfate, and hexane was removed using a rotary evaporator.

Additional LA was recovered from the urea adduct fraction (9). Adduct material was dissolved in minimal warm ddH_2O with stirring. HCl (6 N) was added to acidify the mixture to pH <2. Petroleum ether (2 × 150 mL) was used to extract the oil and then dried over anhydrous sodium sulfate. The organic solvent was removed using a rotary evaporator. The recovered fat and oil were dissolved in acetone (1 g/mL) and crystallized at −20°C. Crude LA was recovered by vacuum filtration, which was subsequently purified again by urea crystallization.

Isomerization of LA. The method described by Chin *et al.* (5) was used to synthesize CLA from LA purified by urea with slight modifications. A lower temperature between 160–180°C reduced the amount of minor isomers formed.

*Enrichment of Δ10*t,*12*c*-18:2.* Equal weight of urea was crystallized with CLA at −25°C overnight and worked up

TABLE 1
Fatty Acid Composition of Safflower Oil, Urea Purified LA, and CLA[a]

	Saturates	Mono/Poly[d]	LA	CLA	*n*	Yield (%)[e]
Safflower oil	10.2 ± 0.1	13.2 ± 1.5	76.4 ± 1.5	0.2 ± 0.01	5	93
Purified LA[b]	0.9 ± 0.8	4.0 ± 2.4	95.1 ± 1.6	0	2	36–58
Purified LA[b]	0.8 ± 0.7	2.3 ± 0.8	96.9 ± 1.5	0	1[f]	21
Isomerized LA[c]	2.0 ± 0.6	4.5 ± 0.4	2.3 ± 0.8	91.2 ± 0.8	6	30–52
Isomerized LA[c]	0.9 ± 0.1	4.6 ± 0.3	0.9 ± 0.02	93.6 ± 0.2	2	17.8

[a]Values represent the percent area count ± SEM from gas chromatographic analysis from either a BP20 or SP2560 gas chromatographic column.
[b]Linoleic acid (LA) recovered from the urea mother liquor and adduct fraction, respectively.
[c]LA from the urea mother liquor and adduct fractions, respectively, isomerized to produce conjugated linoleic acid (CLA).
[d]Monounsaturated/polyunsaturated fatty acids do not include LA.
[e]Yields are with respect to the original starting weight of safflower oil.
[f]The error refers to replicate measurement of the single batch by gas–liquid chromatography.

similarly as described previously in the Experimental Procedures section, under Extraction and Purification of Linoleic Acid.

RESULTS AND DISCUSSION

Using simple methods, procedures described herein have application for the production of CLA and modification of its composition on a small-scale suited for most experimental needs. Oils rich in LA in addition to safflower oil, such as sunflower, corn and soybean oils, can be readily adapted to the procedures described within. Use of these oils provides an inexpensive source of raw materials from which to extract LA by urea crystallization to synthesize CLA (Table 1). Urea crystallization can be used to enrich for Δ10*t*,12*c*-18:2 from a mixture containing equal amounts of Δ9*c*,11*t*-18:2 and Δ10*t*,12*c*-18:2. The enriched mixture contained levels of Δ9*c*,11*t*-18:2, Δ10*t*,12*c*-18:2, and other isomers ranging between 30:60:10 and 30:69:1, respectively.

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REFERENCES

1. Ip, C., S.F. Chin, J.A. Scimeca, and M.W. Pariza, Mammary Cancer Prevention by Conjugated Dienoic Derivative of Linoleic Acid, *Cancer Res. 51*:6118–6124 (1991).
2. Visonneau, S., A. Cesano, S.A. Tepper, J.A. Scimeca, D. Santoli, and D. Kritchevsky, Conjugated Linoleic Acid Suppresses the Growth of Human Breast Adenocarcinoma Cells in SCID Mice, *Anticancer Res. 17*:969–973 (1997).
3. Nicolosi, R.J., E.J. Rogers, D. Kritchevsky, J.A. Scimeca, and P.J. Huth, Dietary Conjugated Linoleic Acid Reduces Plasma Lipoproteins and Early Aortic Atherosclerosis in Hypercholesterolemic Hamsters, *Artery 22*:266–277 (1997).
4. Park, Y., K.J. Albright, W. Liu, J.M. Storkson, M.E. Cook, and M.W. Pariza, Effect of Conjugated Linoleic Acid on Body Composition in Mice, *Lipids 32*:853–858 (1997).
5. Chin, S.F., J.M. Storkson, Y.L. Ha, and M.W. Pariza, Dietary Sources of Conjugated Dienoic Isomers of Linoleic Acid, A Newly Recognized Class of Anticarcinogens, *J. Food Compos. Anal. 5*:185–197 (1992).
6. Liu, K., and M.A. Belury, Conjugated Linoleic Acid Modulation of Phorbol Ester-Induced Events in Murine Keratinocytes, *Lipids 32*:725–730 (1997).
7. Park, Y., and M.W. Pariza, Evidence That Commercial Calf and Horse Sera Can Contain Substantial Amounts of *Trans*-10, *Cis*-12 Conjugated Linoleic Acid, *Ibid. 33*:817–819 (1998).
8. Nichols, P.L.J., S.F. Herb, and R.W. Riemenschneider, Isomers of Conjugated Fatty Acids. I. Alakli-Isomerized Linoleic Acid, *J. Am. Chem. Soc. 73*:247–252 (1951).
9. Gunstone, F.D., J. McLaughlan, C.M. Scrimgeour, and A.P. Watson, Improved Procedures for the Isolation of Pure Oleic, Linoleic, and Linolenic Acids or Their Methyl Esters from Natural Sources, *J. Sci. Fd. Agric. 27*:675–680 (1976).

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