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(72) Inventeurs/Inventors:
SAEBO, ASGEIR, NO;
SAEBO, PER CHRISTIAN, NO
(73) Propriétaire/Owner:
AKER BIOMARINE ASA, NO
(74) Agent: SMART & BIGGAR

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(54) Title: METHODS FOR PREPARING CLA ISOMERS

(57) **Abrégé/Abstract:**

Novel compositions containing conjugated linoleic acid isomers can be used for controls in gas chromatography and for animal feeding studies. Purified isomers of conjugated linoleic acid can be treated to produce a preparation containing two isomers of CLA. The preparation can be used as it is or further processed to separate the two isomers. The isomers can be used in their free fatty acid form or converted to alkylesters or triglycerides. Furthermore, the isomers are useful as supplements for food products or feed.

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(71) Applicant (for all designated States except US): **CON-
LINCO, INC.** [US/US]; Suite 308, 211 W Holmes Street,
Detroit Lakes, MN 56502 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SAEBO, Asgeir**
[NO/NO]; Davane, N-6037 Eidsnes (NO). **SAEBO, Per,**
Christian [NO/NO]; Plassevegen 20, N-6100 Volda (NO).

(74) Agents: **JONES, J., Mitchell** et al.; Medlen & Carroll,
LLP, Suite 350, 101 Howard Street, San Francisco, CA
94105 (US).

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(54) Title: METHODS FOR PREPARING CLA ISOMERS

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METHODS FOR PREPARING CLA ISOMERS

FIELD OF THE INVENTION

The present invention relates to lipid biochemistry, and in particular to the preparation of various isomers of conjugated linoleic acid.

BACKGROUND OF THE INVENTION

In 1978, researchers at the University of Wisconsin discovered the identity of a substance contained in cooked beef that appeared to inhibit mutagenesis. The substance was found to be a mixture of positional isomers of linoleic acid (C18:2) having conjugated double bonds. The c9,t11 and t10,c12 isomers are present in greatest abundance, but it is uncertain which isomers are responsible for the biological activity observed. It has been noted from labelled uptake studies that the 9,11 isomer appears to be somewhat preferentially taken up and incorporated into the phospholipid fraction of animal tissues, and to a lesser extent the 10,12 isomer (Ha, *et al.*, Cancer Res., 50: 1097 [1990]).

The biological activity associated with conjugated linoleic acids (termed CLA) is diverse and complex. At present, very little is known about the mechanisms of action, although several preclinical and clinical studies in progress are likely to shed new light on the physiological and biochemical modes of action. The anticarcinogenic properties of CLA have been well documented. Administration of CLA inhibits rat mammary tumorigenesis, as demonstrated by Birt, *et al.*, Cancer Res., 52: 2035s [1992]. Ha, *et al.*, *supra*, reported similar results in a mouse forestomach neoplasia model. CLA has also been identified as a strong cytotoxic agent against target human melanoma, colorectal and breast cancer cells *in vitro*.

Although the mechanisms of CLA action are still obscure, there is evidence that some component(s) of the immune system may be involved, at least *in vivo*. U.S. Pat. No. 5,585,400 (Cook, *et al.*), discloses a method for attenuating allergic reactions in animals mediated by type I or IgE hypersensitivity by administering a diet containing CLA. CLA in concentrations of about 0.1 to 1.0 percent was also shown to be an effective adjuvant in preserving white blood cells. U.S. Pat. No. 5,674,901 (Cook, *et al.*), disclosed that oral or parenteral administration of CLA in either free acid or salt form resulted in elevation in

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CD-4 and CD-8 lymphocyte subpopulations associated with cell-mediated immunity. Adverse effects arising from pretreatment with exogenous tumor necrosis factor could be alleviated indirectly by elevation or maintenance of levels of CD-4 and CD-8 cells in animals to which CLA was administered. Finally, U.S. Pat. No. 5,430,066 (Cook, *et al.*), describes the effect of CLA in preventing weight loss and anorexia by immune stimulation.

Apart from potential therapeutic and pharmacologic applications of CLA as set forth above, there has been much excitement regarding the use of CLA as a dietary supplement. CLA has been found to exert a profound generalized effect on body composition, in particular redirecting the partitioning of fat and lean tissue mass. U.S. Patent No. 5,554,646 (Cook, *et al.*), discloses a method utilizing CLA as a dietary supplement in which pigs, mice, and humans were fed diets containing 0.5 percent CLA. In each species, a significant drop in fat content was observed with a concomitant increase in protein mass. It is interesting that in these animals, increasing the fatty acid content of the diet by addition of CLA resulted in no increase in body weight, but was associated with a redistribution of fat and lean within the body. Another dietary phenomenon of interest is the effect of CLA supplementation on feed conversion. U.S. Pat. No. 5,428,072 (Cook, *et al.*), provided data showing that incorporation of CLA into animal feed (birds and mammals) increased the efficiency of feed conversion leading to greater weight gain in the CLA supplemented birds and mammals. The potential beneficial effects of CLA supplementation for food animal growers is apparent.

Another important source of interest in CLA, and one which underscores its early commercial potential, is that it is naturally occurring in foods and feeds consumed by humans and animals alike. In particular, CLA is abundant in products from ruminants. For example, several studies have been conducted in which CLA has been surveyed in various dairy products. Aneja, *et al.*, (J. Dairy Sci., 43: 231 [1990]) observed that processing of milk into yogurt resulted in a concentration of CLA. (Shanta, *et al.*, Food Chem., 47: 257 [1993]) showed that a combined increase in processing temperature and addition of whey increased CLA concentration during preparation of processed cheese. In a separate study, Shanta, *et al.*, J. Food Sci., (60: 695 [1995]) reported that while processing and storage conditions did not appreciably reduce CLA concentrations, they did not observe any increases. In fact, several studies have indicated that seasonal or

interanimal variation can account for as much as three fold differences in CLA content of cows milk (See *e.g.*, Parodi, *et al.*, J. Dairy Sci., 60: 1550 [1977]). Also, dietary factors have been implicated in CLA content variation (Chin, *et al.*, J. Food Comp. Anal., 5: 185 [1992]). Because of this variation in CLA content in natural sources, ingestion of prescribed amounts of various foods will not guarantee that the individual or animal will receive the optimum doses to ensure achieving the desired nutritive effect.

In the development of a defined commercial source of CLA for both therapeutic and nutritional applications, a process for generating large amounts of defined material is needed. The problem with most CLA products made by conventional approaches is their heterogeneity, and substantial variation in isoform from batch to batch. A recent publication documents this variation and indicates the need for producers of CLA to appreciate the complex nature of their products (See Christie *et al.*, JAOCS, 74(11): 1231 [1997]).

Considerable attention has also been given to the fact that the ingestion of large amounts of hydrogenated oils and shortenings, instead of animal tallow, results in diets high in trans- fatty acid content. For example, Holman, *et al.*, (Proc. Nat'l. Acad. Sci., 88:4830 [1991]) showed that rats fed hydrogenated oils gave rise to an accumulation in rat liver of unusual polyunsaturated fatty acid isomers, which appeared to interfere with the normal metabolism of naturally occurring polyunsaturated fatty acids. Therefore, there exists a strong need for chemical and biological analysis of the various isomers of CLA.

SUMMARY OF THE INVENTION

The present invention relates to lipid biochemistry, and in particular to the preparation of various isomers of conjugated linoleic (octadecadienoic) acid. The present invention is not limited to the production of any particular octadecadienoic acid isomer. Indeed, a variety isomers may be prepared including, but not limited to, c9,t11 octadecadienoic acid, t8,c10 octadecadienoic acid, t10,c12 octadecadienoic acid, c11,t13 octadecadienoic acid, c7,t9 octadecadienoic acid, t6,c8 octadecadienoic acid, t11,c13 octadecadienoic acid, c12,t14 octadecadienoic acid, c6,t8 octadecadienoic acid, t5,c6 octadecadienoic acid, c5,t7 octadecadienoic acid, t4,c6 octadecadienoic acid, t3,c5 octadecadienoic acid, t12,c14 octadecadienoic acid, c13,t15 octadecadienoic acid, and c14,t16 octadecadienoic acid. The isomers can be provided as free fatty acids,

alkylesters, or acylglycerides. Accordingly, in some embodiments, the present invention provides a composition comprising greater than 25% of one of the preceding isomers. In still other embodiments, the present invention provides a composition comprising greater than 40% of one of the preceding isomers. In still other embodiments, the present invention provides a composition comprising greater than 50% of one of the preceding isomers. Again, the isomers can be provided as free fatty acids, alkylesters, or acylglycerides. In some other preferred embodiments, the present invention provides a composition comprising greater than 50% c11,t13 CLA. In some embodiments, the present invention provides a composition comprising greater than 60% c11,t13 CLA.

In particularly preferred embodiments, the present invention provides a mixture of acylglycerols comprising residues of isomers of conjugated linoleic acid, wherein the fatty acyl content of said acylglycerols is at least 25% of a conjugated linoleic acid isomer selected from the group consisting of t8,c10 octadecadienoic acid, c11,t13 octadecadienoic acid, c7,t9 octadecadienoic acid, t6,c8 octadecadienoic acid, t11,c13 octadecadienoic acid, c12,t14 octadecadienoic acid, c6,t8 octadecadienoic acid, t5,c6 octadecadienoic acid, c5,t7 octadecadienoic acid, t4,c6 octadecadienoic acid, t3,c5 octadecadienoic acid, t12,c14 octadecadienoic acid, c13,t15 octadecadienoic acid, and c14,t16 octadecadienoic acid. In other particularly preferred embodiments, the present invention provides a mixture of acylglycerols comprising residues of isomers of conjugated linoleic acid, wherein the fatty acyl content of said acylglycerols is at least 45% of a conjugated linoleic acid isomer selected from the group consisting of t8,c10 octadecadienoic acid, c11,t13 octadecadienoic acid, c7,t9 octadecadienoic acid, t6,c8 octadecadienoic acid, t11,c13 octadecadienoic acid, c12,t14 octadecadienoic acid, c6,t8 octadecadienoic acid, t5,c6 octadecadienoic acid, c5,t7 octadecadienoic acid, t4,c6 octadecadienoic acid, t3,c5 octadecadienoic acid, t12,c14 octadecadienoic acid, c13,t15 octadecadienoic acid, and c14,t16 octadecadienoic acid. In still further particularly preferred embodiments, the present invention provides a mixture of acylglycerols comprising residues of isomers of conjugated linoleic acid, wherein the fatty acyl content of said acylglycerols is at least 50% of a conjugated linoleic acid isomer selected from the group consisting of t8,c10 octadecadienoic acid, c11,t13 octadecadienoic acid, c7,t9 octadecadienoic acid, t6,c8 octadecadienoic acid, t11,c13 octadecadienoic acid, c12,t14 octadecadienoic acid, c6,t8 octadecadienoic acid, t5,c6 octadecadienoic acid, c5,t7

octadecadienoic acid, t4,c6 octadecadienoic acid, t3,c5 octadecadienoic acid, t12,c14 octadecadienoic acid, c13,t15 octadecadienoic acid, and c14,t16 octadecadienoic acid.

In other embodiments, the present invention provides a composition comprising isomers of conjugated linoleic acid, wherein the composition comprises or consists essentially of at least 25% of a first isomer of octadecadienoic acid and at least 25% of a sister isomer of said first isomer. The present invention is not limited to any one pair of sister isomers. Indeed, a variety of sister isomer pairs are contemplated, including, but not limited to, c9,t11 octadecadienoic acid and t8,c10 octadecadienoic acid, t10,c12 octadecadienoic acid and c11,t13 octadecadienoic acid, c7,t9 octadecadienoic acid and t6,c8 octadecadienoic acid, t11,c13 octadecadienoic acid and c12,t14 octadecadienoic acid, c6,t8 octadecadienoic acid and t5,c6 octadecadienoic acid, c5,t7 octadecadienoic acid and t4,c6 octadecadienoic acid, c4,t6 octadecadienoic acid and t3,c5 octadecadienoic acid, t12,c14 octadecadienoic acid and c13,t15 octadecadienoic acid, and t13,c15 octadecadienoic acid and c14,t16 octadecadienoic acid. The isomers can be provided as free fatty acids, alkylesters, or acylglycerides.

In still other embodiments, the present invention provides methods for preparing pairs of octadecadienoic acid isomers and/or individual octadecadienoic acid isomers comprising: a) providing a first partially purified octadecadienoic acid isomer; and b) treating the first octadecadienoic isomer under conditions such that the conjugated bond system migrates, thereby forming a mixture containing at least first and second isomers of octadecadienoic acid. In still further embodiments, the methods further comprise step c) separating the at least first and second isomers of octadecadienoic acid to provide a second purified octadecadienoic acid isomer. In other embodiments of the present invention, the conditions comprise heating the partially purified octadecadienoic acid isomer. In still further embodiments, the separating step is accomplished by gas liquid chromatography. In some embodiments, the first partially purified octadecadienoic acid isomer is one of c9,t11 and t8,c10 octadecadienoic acids and the second octadecadienoic acid isomer is the other of t8,c10 and c9,t11 octadecadienoic acids. In some embodiments, the first partially purified octadecadienoic acid isomer is one of t10,c12 and c11,t13 octadecadienoic acids and the second octadecadienoic acid isomer is the other of t10,c12 and c11,t13 octadecadienoic acids. In some embodiments, the first partially purified octadecadienoic acid isomer is one of c7,t9 and t6,c8 octadecadienoic acids and the second octadecadienoic acid isomer the other of c7,t9 and t6,c8

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octadecadienoic acids. In some embodiments, the first partially purified octadecadienoic acid isomer is one of t11,c13 and c12,t14 octadecadienoic acids and the second octadecadienoic acid isomer is the other of t11,c13 and c12,t14 octadecadienoic acids. In some embodiments, the first partially purified octadecadienoic acid isomer is one of c6,t8 and t5,c6 octadecadienoic acids and the second octadecadienoic acid isomer is the other of c6,t8 and t5,c6 octadecadienoic acids. In other embodiments, the first partially purified octadecadienoic acid isomer is one of c5,t7 and t4,c6 octadecadienoic acids and the second octadecadienoic acid isomer is the other of c5,t7 and t4,c6 octadecadienoic acids. In some embodiments, the first partially purified octadecadienoic acid isomer is one of t3,c5 and c4,t6 octadecadienoic acids and the second octadecadienoic acid isomer is the other of c4,t6 and t3,c5 octadecadienoic acids. In some embodiments, the first partially purified octadecadienoic acid isomer is one of t12,c14 and c13,t15 octadecadienoic acids and the second partially purified octadecadienoic acid isomer is the other of t12,c14 and c13,t15 octadecadienoic acids. In other embodiments, the first partially purified octadecadienoic acid isomer is one of c14,t16 and t13,c15 octadecadienoic acids and the second octadecadienoic acid isomer is the other of said c14,t16 and t13,c15 octadecadienoic acids.

In still further embodiments, the present invention provides compositions produced by the methods described above. In some embodiments, the second isomer is present in a concentration greater than 30% of the first isomer. In other embodiments, the second isomer is present in a concentration greater than 40% of the first isomer. In still further embodiments, the second isomer is present in a concentration between 20% and 80% of the first isomer.

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According to one aspect of the present invention, there is provided a method comprising: a) providing a first partially purified octadecadienoic acid isomer having a conjugated bond system; and b) heating said first partially purified octadecadienoic acid isomer to 200 to 240°C such that said conjugated bond system migrates, thereby forming a mixture comprising at least said first partially purified octadecadienoic acid isomer and a second octadecadienoic acid isomer.

According to another aspect of the present invention, there is provided a method comprising: a) providing a first partially purified octadecadienoic isomer having a conjugated bond system; b) heating said first partially purified octadecadienoic isomer to 200 to 240°C such that said conjugated bond system migrates, thereby forming a mixture comprising said first partially purified octadecadienoic acid isomer and a second octadecadienoic acid isomer; and c) separating said first partially purified octadecadienoic acid isomer and said second octadecadienoic acid isomer to provide a second partially purified octadecadienoic acid isomer.

DEFINITIONS

As used herein, "conjugated linoleic acid" or "CLA" refers to any conjugated linoleic acid or octadecadienoic free fatty acid. It is intended that this term encompass and indicate all positional and geometric isomers of linoleic acid with two conjugated carbon-carbon double bonds any place in the molecule. CLA differs from ordinary linoleic acid in that ordinary linoleic acid has double bonds at carbon atoms 9 and 12. Examples of CLA include cis- and trans isomers ("E/Z isomers") of the

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following positional isomers: 2,4-octadecadienoic acid, 4,6-octadecadienoic acid, 6,8-octadecadienoic acid, 7,9-octadecadienoic acid, 8,10-octadecadienoic acid,

9,11-octadecadienoic acid and 10,12 octadecadienoic acid, 11, 13 octadecadienoic acid. As used herein, "CLA" encompasses a single isomer, a selected mixture of two or more isomers, and a non-selected mixture of isomers obtained from natural sources, as well as synthetic and semisynthetic CLA.

As used herein, the term "isomerized conjugated linoleic acid" refers to CLA synthesized by chemical methods (*e.g.*, aqueous alkali isomerization, non-aqueous alkali isomerization, or alkali alcoholate isomerization).

As used herein, the term "conjugated linoleic acid moiety" refers to any compound or plurality of compounds containing conjugated linoleic acids or derivatives. Examples include, but are not limited to fatty acids, alkyl esters, and triglycerides of conjugated linoleic acid.

As used herein, it is intended that "triglycerides" of CLA contain CLA at any or all of three positions (*e.g.*, SN-1, SN-2, or SN-3 positions) on the glycerol backbone. Accordingly, a triglyceride containing CLA may contain any of the positional and geometric isomers of CLA. "Acylglycerol" as used herein refers to a glycerol molecule having one (monoacylglycerol), two (diacylglycerol), or three (triacylglycerol or triglyceride) fatty acyl residues at any one of the available positions on the glycerol molecule. Mixtures of triglycerides or acylglycerols can contain varying amounts of particular isomers of CLA depending on the concentration of the particular isomers in the CLA used to produce the triglyceride and, if enzymatic procedures are used to make the triglyceride or acylglycerol, the specificity of the lipase or lipases utilized. Accordingly, it is convenient to characterize the resulting triglyceride or acylglycerol mixture or composition in terms of the amount, on a percentage basis, of a particular CLA isomer contained within the entire mixture or composition. By way of non-limiting example, a triglyceride or acylglycerol composition containing CLA residues at the various positions on the glycerol backbone can be said to have a fatty acyl residue content of at least 25% of a particular conjugated linoleic acid isomer when that conjugated linoleic acid isomer occupies at least 25% of any of the SN-1, SN-2, and SN-3 positions of the triglycerides in the mixture. Viewed another way, if the fatty acyl residues are removed by saponification, the resulting mixture of salts of fatty acids will comprise at least 25% of the particular isomer.

As used herein, it is intended that "esters" of CLA include any and all positional and geometric isomers of CLA bound through an ester linkage to an alcohol or any other chemical group, including, but not limited to physiologically acceptable, naturally occurring alcohols (*e.g.*, methanol, ethanol, or propanol). Therefore, an ester of CLA or esterified CLA may contain any of the positional and geometric isomers of CLA.

It is intended that "non-naturally occurring isomers" of CLA include, but are not limited to c11,t13; t11,c13; t11,t13; c11,c13; c8,t10; t8,c10; t8,t10; c8,c10; and trans-trans isomers of octadecadienoic acid. However, this definition does not include t10,c12 and c9,t11 isomers of octadecadienoic acid. "Non-naturally occurring isomers" may also be referred to as "minor isomers" of CLA, as these isomers are generally produced in low amounts when CLA is synthesized by alkali isomerization.

As used herein, "low impurity" CLA refers to CLA compositions, including free fatty acids, alkylesters, and triglycerides, which contain less than 1% total 8,10 octadecadienoic acids, 11,13 octadecadienoic acids, or trans-trans octadecadienoic acids.

As used herein, "c" encompasses a chemical bond in the cis orientation, and "t" refers to a chemical bond in the trans orientation. If a positional isomer of CLA is designated without a "c" or a "t", then that designation includes all four possible isomers. For example, 10,12 octadecadienoic acid encompasses c10,t12; t10,c12; t10,t12; and c10,c12 octadecadienoic acid, while t10,c12 octadecadienoic acid or CLA refers to just the single isomer.

As used herein, the term "oil" refers to a free flowing liquid containing long chain fatty acids (*e.g.*, CLA), triglycerides, or other long chain hydrocarbon groups. The long chain fatty acids, include, but are not limited to the various isomers of CLA.

As used herein, the term "physiologically acceptable carrier" refers to any carrier or excipient commonly used with oily pharmaceuticals. Such carriers or excipients include, but are not limited to, oils, starches, and sugars (*e.g.*, sucrose and lactose).

As used herein, the term "oral delivery vehicle" refers to any means of delivering a pharmaceutical orally, including, but not limited to, capsules, pills, tablets and syrups.

As used herein, the term "food product" refers to any food or feed suitable for consumption by humans, non-ruminant animals, or ruminant animals. The "food product" may be a prepared and packaged food (*e.g.*, mayonnaise, salad dressing, bread, or cheese food) or an animal feed (*e.g.*, extruded and pelleted animal feed or coarse

mixed feed). "Prepared food product" means any pre-packaged food approved for human or animal consumption.

As used herein, the term "foodstuff" refers to any substance fit for human or animal consumption.

As used herein, the term "volatile organic compound" refers to any carbon-containing compound which exists partially or completely in a gaseous state at a given temperature. Volatile organic compounds may be formed from the oxidation of an organic compound (*e.g.*, CLA). Volatile organic compounds include, but are not limited to pentane, hexane, heptane, 2-butenal, ethanol, 3-methyl butanal, 4-methyl pentanone, hexanal, heptanal, 2-pentyl furan, and octanal.

As used herein, the term "metal oxidant chelator" refers to any antioxidant that chelates metals. Examples include, but are not limited to lecithin and citric acid esters.

As used herein, the term "partially purify" means any process that removes some of a contaminant from the component of interest, such as removing unwanted isomers from a conjugated linoleic acid preparation. The percent of a purified component is thereby increased in the sample (*i.e.*, the component of interest is concentrated).

As used herein, the term "sister isomers" refers to pairs of conjugated linoleic acid isomers that can be interconverted by some treatment (*e.g.*, heating). Examples of sister isomers are provided in Table 1, *infra*.

As used herein, the term "conjugated bond system" refers to the following arrangement of unsaturated double bonds in a carbohydrate chain: $-C=C-C=C-$ (*i.e.*, two double bonds separated by a single bond).

As used herein, the term "migrate," when used in reference to a conjugated bond system in a carbohydrate chains, refers to the rearrangement of unsaturated carbon-carbon double bonds within the carbohydrate chain so that the double bonds move up or down the chain.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to lipid biochemistry, and in particular to the preparation of various isomers of conjugated linoleic acid. The rearrangement of the double bonds of linoleic acid to conjugated positions has been shown to occur during treatment with catalysts such as nickel or alkali at high temperatures, and during auto oxidation. Theoretically, eight possible geometric isomers of 9,11 and 10,12

octadecadienoic acid (c9,c11; c9,t11; t9,c11; t9,t11; c10,c12; c10,t12; t10,c12 and t10,t12) would form from the isomerization of c9,c12-octadecadienoic acid. A general mechanism for the isomerization of linoleic acid was described by J.C. Cowan (JAOCS 72:492-99 [1950]). It is believed that the double bond is polarized by the result of a collision with an activating catalyst. The polarized carbon atom and its adjoining carbon are then free to rotate and the forces are such as to make the deficient carbon atom essentially planar. When the system then moves to relieve these forces set up as a result of the collision, both cis and trans isomers are formed. The formation of certain isomers of CLA is thermodynamically favored. This is due to the co-planar characteristics of the five carbon atoms around the conjugated double bond and a spatial conflict of the resonance radical. The relatively higher distribution of 9,11 and 10,12 isomers apparently results from the further stabilization of the c9,t11 or t10,c12 geometric isomers.

Advances in gas chromatography have enabled researchers to precisely determine the isomer composition of samples of CLA. These studies demonstrate that many more than 8 isomers are actually formed during conjugation. In Christie *et al.*, (JAOCS 74 (11):1231 [1997]), it was reported that the isomer distribution of a commercial sample of CLA was as follows: 8,10 (14%); 9,11 (30%); 10,12 (31%), and 11,13 (24%). In another study by Christie *et al.* in (Lipids 33(2):217-21 [1998]), the following CLA isomer composition of a commercial CLA preparation was reported: t11,t13 (.74%); t10,t12 (1.23%); t9,t11 (1.18%); t8,t10 (0.46%); c11,t13 and t11,c13 (21.7%) c10,t12 and t10,c12 (29.0%); c9,t11 and t9,c11 (29.5%); c8,t10 and t8,c10 (12.3%); c11,c13 (0.96%); c10,c12 (0.88%); c9,c11 (0.88%); and c8,c10 (0.20%). As can be seen from these studies, even though the formation of certain isomers are favored, other isomers of CLA can contribute greatly to the composition of alkali isomerized CLA preparations.

A. Migration of Conjugated Double Bonds

The present invention provides methods for producing compositions enriched for different isomers of CLA. In some embodiments of the invention, a partially purified or concentrated isomer of CLA is treated under conditions that cause migration of the double bond system. In preferred embodiments, the conditions comprise heating at least one isomer to about 200-240°C, preferably to about 220°C. In other embodiments, the conditions further comprise reacting the partially purified or concentrated isomer or

isomers under nitrogen in a sealed container. Referring to Table 1, the preparations of isomers in column 1 can be used to produce preparations containing a substantial amount of the corresponding isomer in column 2. After the initial conversion reaction, the preparation will contain both the starting isomer and the "sister" isomer. Likewise, the preparations of isomers in column 2 can be used to produce substantial amounts of the corresponding isomer in column 1. The preparations containing both isomers may be further treated to purify the sister isomer (*e.g.*, by gas chromatography). As will be understood by those skilled in the art, it is possible to start with more than one partially purified isomer, thereby producing a preparation containing four, six, eight or more isomers. In further embodiments, a purified preparation of the sister isomer may be prepared by methods known in the art (*i.e.*, gas-liquid chromatography) from the treated preparation containing the initial isomer and its sister isomer.

Table 1	
Column 1	Column 2
c9,t11	t8,c10
t10,c12	c11,t13
c7,t9	t6,c8
t11,c13	c12,t14
c6,t8	t5,c6
c5,t7	t4,c6
c4,t6	t3,c5
t12,c14	c13,t15
t13,c15	c14,t16

As demonstrated in the Examples, treatment of purified t10,c12 octadecadienoic acid resulted in the production of c11,t13 octadecadienoic acid. Likewise, concentrated or partially purified c11,t13 octadecadienoic acid can be used to produce t10,c12

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octadecadienoic acid. After the partially purified isomer is treated to produce a sister isomer, the resulting preparation preferably comprises about greater than 20% of the sister isomer as compared to the initial partially purified isomer. In other embodiments, the resulting preparation comprises about greater than 40% of the sister isomer as compared to the initial partially purified isomer. In still further embodiments, the resulting preparation comprises between about 20% and 80% of the sister isomer as compared to the initial partially purified isomer. In still other embodiments, the resulting preparation comprises or consists essentially of greater than about 20% of the sister isomer on a weight/weight basis (*e.g.*, the preparation contains about 20 grams of the sister isomer and about 80 grams of the initial partially purified isomer). In further embodiments, the resulting preparation comprises greater than about 40% of the sister isomer on a weight/weight basis. In still further embodiments, the preparation comprises between about 20% and 80% of the sister isomer on a weight/weight basis.

B. Uses of Purified Isomer and Sister Isomer Pairs

The purified isomers and sister isomer pairs described above have many uses. In some embodiments of the present invention, the purified isomers or sister isomer pairs find use as standards in gas chromatography and liquid chromatography. Purified or enriched sources of these isomers can be used as standards for the analysis of commercial CLA products which may have contained heretofore unidentified peaks representing unidentified isomers.

In other embodiments of the present invention, the preparation containing the purified isomers or sister isomer pairs are used in animal feeding studies and as nutritional supplements. It is contemplated that the purified isomers and sister isomer pairs can be used as controls in animal feeding studies so that the biological effects (*e.g.*, partitioning within organs and cells, effects on lipid biosynthesis, and metabolism) of the isomers can be studied. The isomers may be provided as free fatty acids, alkylesters (*e.g.*, methyl or ethyl esters of CLA), triglycerides, or combinations thereof. In some preferred embodiments, the isomers are provided orally. In other embodiments, the isomers may be formulated with suitable carriers such as starch, sucrose or lactose in tablets, pills, dragees, capsules, solutions, liquids, slurries, suspensions and emulsions. Preferably, the isomer formulations contain antioxidants, including, but not limited to ControxTM (Grunau (Henkel), Illertissen, DE), Covi-OXTM (Grunau (Henkel), Illertissen, DE),

lecithin, and oil soluble forms of vitamin C. The isomers may be provided in aqueous solution, oily solution, or in any of the other forms discussed above. The tablet or capsule of the present invention may be coated with an enteric coating which dissolves at a pH of about 6.0 to 7.0. A suitable enteric coating which dissolves in the small intestine but not in the stomach is cellulose acetate phthalate. In some embodiments, the isomers are provided as soft gelatin capsules. The CLA may also be provided by any of a number of other routes, including, but not limited to, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal means. Further details on techniques for formulation for and administration and administration may be found in the latest edition of *Remington's Pharmaceutical Sciences* (Maack Publishing Co., Easton, PA).

An effective amount of isomers may also be provided as a supplement in various food products, including animal feeds and nutritional drinks. For the purposes of this application, "food products containing CLA isomers" refers to any natural, processed, diet or non-diet food product to which exogenous CLA isomers have been added. Likewise, "feed products containing CLA" refers to any animal feed to which exogenous CLA isomers have been added. The CLA may be added in the form of free fatty acids, esters of conjugated linoleic acid, or as an oil containing partial or whole triglycerides of CLA. Therefore, CLA may be directly incorporated into various food products, including, but not limited to diet drinks, diet bars, supplements, prepared frozen meals, candy, snack products (e.g., chips), prepared meat products, milk, cheese, yogurt and any other fat or oil containing foods.

In further embodiments of the present invention, the isomer preparations can be further purified (e.g., by molecular distillation or adsorption) to remove volatile organic compounds. Therefore, it is contemplated in some embodiments of the present invention provide isomer preparations having less than 100 ppm volatile organic compounds, and preferably less than 5 ppm volatile organic compounds.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: M (molar); mM (millimolar); μ M (micromolar); kg (kilograms); g (grams); mg (milligrams); μ g (micrograms); ng (nanograms); L or l (liters); ml (milliliters); μ l (microliters); cm (centimeters); mm (millimeters); nm (nanometers); °C (degrees centigrade); KOH (potassium hydroxide); HCL (hydrochloric acid); and Hg (mercury).

Example 1

Preparation of CLA Isomers

This Example describes the production c11,t13 octadecadienoic acid from t10,c12 octadecadienoic acid. Fifty grams of KOH were dissolved in propylene glycol under moderate heating. One hundred grams of 98% linoleic acid were then added to the mixture, and the mixture heated to 150 °C and stirred for 3 hours. The mixture was then cooled and washed several times with hot water and then dried under vacuum at moderate heat. The resulting CLA mixture consisted of c9,t11 and t10,c12 octadecadienoic acid as well as traces of CLA isomers. The mixture was converted to methylester by reflux boiling in acidic methanol. Fifty grams of conjugated free fatty acids were dissolved in methanol containing 4.5% sulfuric acid and boiled under reflux conditions for 1 hour in a water bath. The mixture was cooled and the bottom layer discarded. Fresh methanol with 4.5% sulfuric acid was added and the mixture boiled for an additional hour under reflux conditions. After cooling, this methylester mixture was washed several times with water and then dried under vacuum at moderate heat. Ten grams of the methylester were dissolved in acetone and cooled overnight to -60 °C in a freezer. A solid precipitate was recovered by filtration and re-dissolved in acetone and again cooled to -60 °C overnight. The precipitate was dried under vacuum and shown by GLC analysis to contain 97% t10,c12 CLA. The analytical equipment consisted of a

Perkin Elmer GLC with auto-sampler. The column was a highly polar fused silica type.

The following program setting were used:

Injection: Splitless at 250 °C
 Detection: Flame Ionization Detector at 280 °C
 Carrier: Helium at psig.
 Oven program: 80 °C - 130 °C (45 °C/min), then 1 °C/min to 220 °C and
 220 °C throughout for 10 min.
 Column: WCOT FUSED SILICA 0.25mm X 100m, CP-SIL 88 for
 FAME, df = 0.2.

One gram of purified t10,c12 isomer was then covered with nitrogen in a sealed tube and heated for two hours at 220 °C. After cooling, the resulting methylesters were analyzed by GC as above. The relative content of t10,c12 in the mixture was reduced to 52.32% and the c11,t13 isomer was present at a level of 41.96% (See Table 2).

Table 2		
Conversion of t10,c12 isomer to c11,t13 isomer		
Isomer	% Before heating	% After heating
c11,t13	0	41.57
t10,c12	97.34	51.72
C11,c13	0	1.44
c10,c12	0	2.70
t11,t13	0	0.54
t10,t12	0.7	1.05

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Example 2**Preparation of CLA Isomers**

This Example describes the production t8,c10 octadecadienoic acid from c9,t11 octadecadienoic acid. Purified c9,t11 octadecadienoic acid may be obtained from commercial sources (Matreya, State College, PA) or by fermentation with rumen microorganisms (*See, e.g.*, U.S. Pat. No. 5,674,901).

The purified c9,t11 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) t8,c10 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

Example 3**Preparation of CLA Isomers**

This Example describes the production t6,c8 octadecadienoic acid from c7,t9 octadecadienoic acid. Purified c7,t9 octadecadienoic acid may be obtained by preparative scale gas chromatography. The purified c7,t9 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) t6,c8 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

Example 4**Preparation of CLA Isomers**

This Example describes the production c12,t14 octadecadienoic acid from t11,c13 octadecadienoic acid. Purified t11,c13 octadecadienoic acid may be obtained by preparative scale gas chromatography (*e.g.*, following the process described in Example 1). The purified t11,c13 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) c12,t14 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

Example 5

Preparation of CLA Isomers

This Example describes the production t5,c6 octadecadienoic acid from c6,t8 octadecadienoic acid. Purified c6,t8 octadecadienoic acid may be obtained by preparative scale gas chromatography. The purified c6,t8 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) t5,c6 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

Example 6

Preparation of CLA Isomers

This Example describes the production t4,c6 octadecadienoic acid from c5,t7 octadecadienoic acid. Purified c5,t7 octadecadienoic acid may be obtained by preparative scale gas chromatography. The purified c5,t7 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) t4,c6 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

Example 7

Preparation of CLA Isomers

This Example describes the production t3,c5 octadecadienoic acid from c4,t6 octadecadienoic acid. Purified c4,t6 octadecadienoic acid may be obtained by preparative scale gas chromatography. The purified c4,t6 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) t3,c5 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

Example 8

Preparation of CLA Isomers

This Example describes the production of c13,t15 octadecadienoic acid from t12,c14 octadecadienoic acid. Purified t12,c14 octadecadienoic acid may be obtained by preparative scale gas chromatography. The purified t12,c14 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) c13,t15 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

Example 9

Preparation of CLA Isomers

This Example describes the production of c14,t16 octadecadienoic acid from t13,c15 octadecadienoic acid. Purified t13,c15 octadecadienoic acid may be obtained by preparative scale gas chromatography. The purified t13,c15 octadecadienoic is converted to a high percentage (*e.g.*, 25% to 50%) c14,t16 octadecadienoic acid by placing the c9,t11 octadecadienoic acid in a sealed tube under nitrogen and heating to 220 °C for about 2 hours.

EXAMPLE 10

The Preparation of Triacylglycerols of CLA by Direct Esterification.

Immobilized *Candida antarctica* lipase (1.25 g) is added to a mixture of glycerol (1.22 g, 13.3 mmol) and the desired CLA isomer. The mixture is gently stirred on a magnetic stirrer hot plate at 65° C under continuous vacuum of 0.01-0.5 Torr. The volatile water produced during the progress of the reaction is continuously condensed into liquid nitrogen cooled traps. After 48 h the reaction is discontinued, n-hexane added and the enzyme separated off by filtration. The organic phase is treated with an alkaline aqueous solution of sodium carbonate to remove excessive free fatty acids (when required). The organic solvent (after drying over anhydrous magnesium sulfate when appropriate) is removed *in vacuo* on a rotary evaporator followed by high-vacuum treatment to produce the virtually pure product. When stoichiometric amounts of free fatty acids are used, titration by standardized sodium hydroxide is applied to determine the free fatty acid content of the crude reaction product.

EXAMPLE 11**Rations Containing CLA Isomers**

This Example provides typical animal rations containing the CLA free fatty acids, triglycerides, and esters of the present invention. The CLA isomer preparation may be in the form of free fatty acids, alkylesters, triglycerides, or combinations thereof. It is not intended that the present invention be limited to any particular formulation. Indeed, it is intended that CLA isomers in various concentrations will find use in a variety of rations.

A. PIG STARTER RATIONS**Table 3**

Ingredients	lbs.	kgs.
Corn, yellow (8.4% protein)	1067	484.7
Soy bean meal, solvent extracted, dehulled (47% protein)	570	259
CLA isomer prep.	5	2.3
Whey, dried (12.0% protein)	300	136
Dicalcium phosphate	24	11
Limestone	16	7
Iodized salt	5	2
Trace mineral premix	5	2
Vitamin premix	8	4
Totals	2000	908

**B. GROWER-FINISHER RATIONS FOR PIGS
(FROM 40-240 LBS[18-109KGS])**

Table 4

Ingredients	lbs.	kgs.
Corn, yellow (8.4% protein)	1566	
Soybean meal, solvent extracted (44% protein)	380	
CLA isomer prep.	5	
Dicalcium phosphate	21	
Limestone	15	
Iodized Salt	5	
Trace Mineral Premix	3	
Vitamin Premix	3	
Total	2000	

**C. PIG GROWER FINISHER RATIONS
(FOR PIGS 121-240LBS[55-109KGS])**

Table 5

Ingredients	lbs.	kgs.
Corn, yellow (8.4% protein)	1687	
Soybean meal, solvent extracted (44% protein)	265	
CLA isomer prep.	5	
Dicalcium phosphate	18	
Limestone	15	
Iodized salt	5	
Trace mineral premix	2	
Vitamin premix	3	
Total	2000	

1. COMPOSITION AND ANALYSIS OF PIG TRACE MINERAL REMIX

Table 6

Element	Source	Amount (lbs)
Copper (Co)	Copper Sulfate	1.500
Iodine (I)	Potassium Iodide	0.010
Iron (Fe)	Ferrous Sulfate	25.000
Manganese (Mn)	Manganese Sulfate	2.500
Selenium (Se)	Sodium Selemite)	0.025
Zinc (Zn)	Zinc Sulfate	25.000
	Carrier	45.965
Total		100.000

2. COMPOSITION OF PIG VITAMIN PREMIX

Table 7

Vitamins	Amount
Essential	
Vitamin A.....(million IU)	5.0
Vitamin D.....(million IU)	0.6
Vitamin E.....(thousand IU)	26.0
Niacin.....(g)	25.0
d-Pantothenic acid.....(g)	20.0
Riboflavin.....(g)	6.0
Vitamin B-12.....(mg)	25.0
Optional	
Biotin.....(g)	0.3
Menadione.....(g)	4.0
Carrier.....	to 10lbs
Total	10.0

D. 18% PROTEIN LAYER RATIONS FOR HENS**Table 8**

Ingredients	lbs.	kgs.
Ground yellow corn	1242	564.5
CLA isomer prep.	5	2.3
Alfalfa meal, 17%	25	11.3
Soybean meal, dehulled	451.6	205.3
Meat and bone meal (47%)	50	23.0
DL-methionine	1.0	.5
Dicalcium phosphate	7	3.1
Ground limestone	174	79.1
Iodized salt	7	3.1
Stabilized yellow grease	37	17.2
Mineral and vitamin supplements		
Calcium pantothenate (mg)	5,000	
Manganese (g)	52	
Selenium (mg)	90.8	
Zinc (g)	16	
Vitamin A (IU)	6,000,000	
Vitamin D ₃ (IU)	2,000,000	
Choline (mg)	274,000	
Niacin (mg)	12,000	
Riboflavin (mg)	2,000	
Vitamin B-12	6	
Total	2000	909.4

E. STARTER AND FINISHER RATIOS FOR BROILERS**Table 9**

Ingredients	Starter (up to 24 days)		Finisher (25 days to market)	
	lbs.	kgs.	lbs.	kgs.
Ground yellow corn	1,106	503	1235	561
CLA isomer prep.	5	2.3	5	2.3
Soybean meal, dehulled	605	275	420	191
Alfalfa meal, 17%	-	-	25	11
Corn gluten meal, 60%	50	23	75	34
Fish meal, herring, 65%	50	23	50	23
Meat and bone meal, 47%	50	23	50	23
Dicalcium phosphate	10	4	9	4
Ground limestone	16	7	14	6.3
DL-methionine	0.8	0.3	-	-
Stabilized yellow grease	101	45.7	110	49.4
Iodized salt	7	3	7	3
Mineral and vitamin supplement				
Calcium pantothenate (mg)	5,000		5,000	
Manganese (g)	75		75	
Organic arsenical supplement	0.1		0.1	
Selenium (mg)	90.8		90.8	
Zinc (g)	30		30	
Vitamin A (IU)	4,000,000		4,000,000	
Vitamin D (IU)	1,000,000		1,000,000	
Vitamin E (mg)	2,000		2,000	
Vitamin K (mg)	2,000		2,000	
Choline (mg)	503,000		672,000	
Niacin (mg)	20,000		20,000	
Riboflavin (mg)	3,000		3,000	

Ingredients	Starter (up to 24 days)		Finisher (25 days to market)	
Vitamin B-12 (mg)	12		12	
Total	2000.9	909.3	2000.1	909.5

F. GROWER/FINISHER TURKEY RATIOS**Table 10**

Ingredients	Grower (8-16 weeks)		Finisher (16 weeks-market)	
	lbs.	kgs.	lbs.	kgs.
Ground yellow corn	1194	595	1490	677.2
Wheat middlings	50	23	-	-
Alfalfa meal, 17%	25	11.3	25	11.3
Soybean meal, dehulled	570	259	335	152.3
Meat and bone meal, 47%	50	23	50	23
Dicalcium phosphate	32	14.5	23	10.5
Ground limestone	14	6	17	8
Stabilized yellow grease	45	20.7	45	20.7
CLA isomer prep.	5	2.3	5	2.3
Iodized Salt	10	4.5	10	4.5
Mineral and vitamin supplements				
Calcium pantothenate (mg)	4,500		4,500	
Manganese (g)	30		30	
Selenium (mg)	181.6		181.6	
Zinc (g)	30		30	
Vitamin (IU)	1,500,000		7,500,000	
Vitamin D (IU)	1,700,000		1,700,000	
Vitamin E (IU)	10,000		10,000	
Biotin (mg)	100		100	
Choline (mg)	388,000		417,000	

Ingredients	Grower (8-16 weeks)		Finisher (16 weeks-market)	
Niacin (mg)	46,000		48,000	
Riboflavin (mg)	5,000		5,000	
Vitamin B-12	6		6	
Total	2000	909.3	2000	909.3

G. DRY DOG FOOD FORMULA

Table 11

Ingredients	Formula 1, %	Formula 2, %
Meat and bone meal, 50% CP	8.0	15.0
Fish meal, 60% CP, low fat	5.0	3.0
Soybean meal, 44% CP	12.0	-
Soybean meal, 50% CP	-	19.0
Wheat germ meal, 25% CP	8.0	5.0
Skimmed milk, dried	4.0	2.75
Cereal grains, mixed	51.23	-
Corn, flaked	-	23.25
Wheat bran	4.0	-
Wheat, flaked	-	23.35
Animal fat	1.75	2.75
CLA isomer prep.	.25	.25
Steamed bone meal	2.0	-
Brewers yeast	2.0	5.0
Fermentation solubles, dehydrated	1.0	-
Salt and trace minerals	0.5	0.5
Vitamin mixture	0.25	0.25
Ferric oxide	0.02	-
Total	100.00	100.00

H. SEMI-MOIST DOG FOOD FORMULA**Table 12**

Ingredients	Formula 1, %	Formula 2, %
Soy flakes	30.9	33.5
Meat byproducts, 70% moisture	32.0	-
Meat and bone meal, dehydrated	-	7.3
Water	-	25.6
Sugar	21.0	21.0
Calcium and phosphorous supplement	3.3	-
Soybean hulls	3.1	3.1
Skimmed milk, dried	2.5	-
Propylene glycol	2.1	2.1
Sorbitol	2.0	2.0
Animal fat	.75	3.95
CLA isomer prep.	.25	.25
Emulsifiers	0.9	-
Potassium sorbate	0.35	0.35
Salt	0.6	0.6
Vitamins	0.25	0.25
Total	100.000	100.000

EXAMPLE 12**Production of Triacylglycerides**

CLA isomers are prepared as described above and distilled on a molecular distillation plant at 150°C and a pressure of 10^{-2} mbar. Next, 1000 kg of the distilled product is mixed with 97 kg of pure glycerol and 80 kg lipase. The reaction is allowed to proceed for 12 hours at 55°C under vacuum and with stirring. The triacylglyceride product is distilled on a molecular distillation apparatus to remove unreacted fatty acids.

EXAMPLE 13

Synthesis of c11, t13 CLA

This Example describes the production of a CLA sample containing approximately 60% of the c11,t13 isomer of CLA. Briefly, two portions of a 95% t10,c12 fatty acid ethyl ester mixture, 30 g each, were transferred to 70 ml test tubes. The test tubes were purged with nitrogen and sealed. The test tubes were preheated for one minute in a 100°C water bath and then placed directly into a 220°C oil bath. After two hours, one of the test tubes was removed from the oil bath and allowed to cool. The other test tube was removed after one more hour and then allowed to cool. The isomer profiles of the initial sample and two heated samples were determined by GC (Table 13).

Three samples were withdrawn from each of the two heated samples and diluted into 3.5, 4.5, or 5.5 ml acetone per gram fat in 70 ml test tubes (Table 14). The test tubes with acetone solution were then cooled to -60°C in a freezer. After 3 days, samples were withdrawn from the supernatant fractions. The isomer profile of the supernatant fractions were determined by GC (Table 15).

The heating of the initial sample (total CLA approximately 99%, oleic acid 0.9%) to 220°C for 2 and 3 hours caused a partial conversion of the t10,c12 isomer into the c11,t13 isomer. The heated samples contained 35-45% c11,t13 isomer (Table 13). The exact content of the isomer could not be determined due to incomplete separation under the selected GC conditions.

The crystallization of the sample heated for two hours differed from the crystallization of the sample heated for three hours by giving better (more solid) crystals. The sample heated for 3 hours gave slushy crystals. The analysis of the supernatant fractions revealed that the crystallization of the sample heated for two hours gave a better separation with a higher content of c11,13 in the supernatant fraction. The content of c11,t13 and t10,c12 CLA in these supernatant fractions was 56-60% and 27-30% respectively. The content of c11,t13 was slightly higher in the supernatant fractions of the samples with the lower fat/acetone ratio (Table 15).

Table 13 Fatty Acid Composition of Initial and Heated Samples*								
Sample	GC-no	c9,t11	c11,t13	t10,c12	c10,c12	c11,c13	t11,t13	t,t other
Initial	5723	2.8	nd	94.8	0.8	0.2	nd	0.7
220°C 2 h	5724	2.8	38	55	0.7	1.4	0.3	0.8
220°C 3 h	5725	2.6	46	46	1.1	1.9	0.4	1.0

* In the heated samples, the values given in the c9,t11 column represent c9,t11 and t8,c10 due to a similar conversion from c9,t11. The values given for the amounts of c10,c12 and c11,c13 are the values integrated by the GC. These values may deviate from the real content due to incomplete separation on the chromatograms.

Table 14 Dilution of the Initial Sample in Acetone Prior To Cooling*		
Dilution No.	Amount of fat (g)	Amount of acetone (ml)
1 (1:3.5)	7.0	24.5
2 (1:4.5)	5.5	24.8
3 (1:5.5)	4.5	24.8

* The two heated samples were diluted in equal amounts.

Table 15 Fatty Acid Profile of the Supernatant Fractions*								
Sample	GC-no	c9,t11	c11,t13	t10,c12	c10,c12	c11,c13	t11,t13	t,t other
2h, Dil. 1	5726	4.3	56	31	1.0	2.4	0.7	1.1
2h, Dil. 2	5727	4.8	60	28	1.2	2.4	0.6	1.1
2h, Dil. 3	5728	5.6	59	27	1.2	3.0	0.6	1.1
3h, Dil. 1	5729	2.8	41	50	1.2	2.0	0.5	1.1
3h, Dil. 2	5733	3.2	48	42	1.2	2.3	0.7	1.3
3h, Dil. 3	5734	3.7	56	33	1.3	2.6	0.6	1.1

* In the heated samples, the values given in the c9,t11 column represent c9,t11 and t8,c10 due to a similar conversion from c9,t11. The values given for the amounts of c10,c12 and c11,c13 are the values integrated by the GC. These values may deviate from the real content due to incomplete separation on the chromatograms.

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It is clear from above that the present invention provides methods for preparing novel compositions of CLA isomers. These CLA isomers may be used for various purposes, including but not limited to, gas chromatography controls and for controls in feeding studies, as well as nutritional supplements for humans and animals.

Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in food science, animal science, medicine, biochemistry, or related fields are intended to be within the scope of the following claims.

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CLAIMS:

1. A method comprising:

a) providing a first partially purified octadecadienoic acid isomer having a conjugated bond system;
5 and

b) heating said first partially purified octadecadienoic acid isomer to 200 to 240°C such that said conjugated bond system migrates, thereby forming a mixture comprising at least said first partially purified
10 octadecadienoic acid isomer and a second octadecadienoic acid isomer.

2. The method of claim 1, further comprising:

c) separating said first partially purified octadecadienoic acid isomer and said second octadecadienoic
15 acid isomer to provide a second partially purified octadecadienoic acid isomer.

3. The method of claim 2, wherein said separating step is accomplished by gas liquid chromatography.

4. The method of claim 1, wherein said
20 octadecadienoic acid isomer comprises an alkylester.

5. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of c9,t11 and t8,c10 octadecadienoic acids and said second octadecadienoic acid isomer is the other of t8,c10 and
25 c9,t11 octadecadienoic acids.

6. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of t10,c12 and c11,t13 octadecadienoic acids and said second

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octadecadienoic acid isomer is the other of t10,c12 and c11,t13 octadecadienoic acids.

7. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of
5 c7,t9 and t6,c8 octadecadienoic acids and said second octadecadienoic isomer is the other of c7,t9 and t6,c8 octadecadienoic acids.

8. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of
10 t11,c13 and c12,t14 octadecadienoic acids and said second octadecadienoic acid isomer is the other of t11,c13 and c12,t14 octadecadienoic acids.

9. The method of claim 1, wherein said first partially purified octadecadienoic isomer is one of c6,t8
15 and t5,c6 octadecadienoic acids and said second octadecadienoic isomer is the other of c6,t8 and t5,c6 octadecadienoic acids.

10. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of
20 c5,t7 and t4,c6 octadecadienoic acids and said second octadecadienoic isomer is the other of c5,t7 and t4,c6 octadecadienoic acids.

11. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of
25 t3,c5 and c4,t6 octadecadienoic acids and said second octadecadienoic acid isomer is the other of c4,t6 and t3,c5 octadecadienoic acids.

12. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of
30 t12,c14 and c13,t15 octadecadienoic acids and said second

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octadecadienoic acid isomer is the other of t12,c14 and c13,t15 octadecadienoic acids.

13. The method of claim 1, wherein said first partially purified octadecadienoic acid isomer is one of c14,t16 and t13,c15 octadecadienoic acids and said second octadecadienoic acid isomer is the other of said c14,t16 and t13,c15 octadecadienoic acids.

14. The method of claim 1, further comprising synthesizing an acylglycerol with said mixture comprising at least said first partially purified octadecadienoic acid isomer and a second octadecadienoic acid isomer.

15. The method of claim 14, wherein said acylglycerol is a triglyceride.

16. A method comprising:

15 a) providing a first partially purified octadecadienoic isomer having a conjugated bond system;

b) heating said first partially purified octadecadienoic isomer to 200 to 240°C such that said conjugated bond system migrates, thereby forming a mixture comprising said first partially purified octadecadienoic acid isomer and a second octadecadienoic acid isomer; and

20 c) separating said first partially purified octadecadienoic acid isomer and said second octadecadienoic acid isomer to provide a second partially purified octadecadienoic acid isomer.

17. The method of claim 16, wherein said separating step is accomplished by gas liquid chromatography.

18. A composition comprising isomers of conjugated linoleic acid, wherein said composition comprises at

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least 25% of a first isomer of octadecadienoic acid and at least 25% of a sister isomer of said first isomer.

19. The composition of claim 18, wherein said conjugated linoleic acid is or comprises an alkylester.

5 20. The composition of claim 18, wherein said conjugated linoleic acid isomers are contained in an acylglycerol form.

21. The composition of claim 18, wherein said first isomer is c9,t11 octadecadienoic acid and said sister isomer
10 is t8,c10 octadecadienoic acid.

22. The composition of claim 18, wherein said first isomer is t10,c12 octadecadienoic acid and said sister isomer is c11,t13 octadecadienoic acid.

23. The composition of claim 18, wherein said first
15 isomer is c7,t9 octadecadienoic acid and said sister isomer is t6,c8 octadecadienoic acid.

24. The composition of claim 18, wherein said first isomer is t11,c13 octadecadienoic acid and said sister isomer is c12,t14 octadecadienoic acid.

20 25. The composition of claim 18, wherein said first isomer is c6,t8 octadecadienoic acid and said sister isomer is t5,c6 octadecadienoic acid.

26. The composition of claim 18, wherein said first isomer is c5,t7 octadecadienoic acid and said sister isomer
25 is t4,c6 octadecadienoic acid.

27. The composition of claim 18, wherein said first isomer is c4,t6 octadecadienoic acid and said sister isomer is t3,c5 octadecadienoic acid.

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28. The composition of claim 18, wherein said first isomer is t12,c14 octadecadienoic acid and said sister isomer is c13,t15 octadecadienoic acid.

29. The composition of claim 18, wherein said first
5 isomer is t13,c15 octadecadienoic acid and said sister isomer is c14,t16 octadecadienoic acid.

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