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Biological Activities, Analytical Methodologies, and Alkali Isomerization Synthesis of Conjugated Linoleic Acid (CLA)

Sung-Ki Kang, Byung-Chan Lee, Young-Sun Lee, In-Tae Yi

Handong Co., Ltd., Seoul, Republic of Korea (535 Ogeum-ro, Songpa-gu)

Abstract
Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of octadecadienoic acid ($C_{18:2}$) characterized by a conjugated double-bond system, predominantly found as cis-9, trans-11 (c9,t11) and trans-10, cis-12 (t10,c12) isomers. Naturally synthesized via biohydrogenation of polyunsaturated fatty acids by anaerobic rumen bacteria such as Butyrivibrio fibrisolvens or via endogenous Δ^9-desaturation of trans-vaccenic acid, CLA exhibits diverse physiological properties including anticarcinogenic, anti-atherogenic, immunomodulatory, and body-fat-reducing effects. This study reviews the metabolic pathways, biological functions, and analytical techniques of lipids and CLA, and conducts three experimental investigations: (1) establishing an extraction and methylation protocol for fecal lipids from poultry fed calcium-rich diets, (2) evaluating the oxidative stability and putative antioxidant activity of CLA under varying storage conditions, and (3) optimizing the commercial alkali isomerization process to synthesize CLA from edible vegetable oils. For lipid extraction from chicken feces, acidification with 6 M HCl effectively dissociated calcium soaps prior to chloroform–methanol extraction, while base-catalyzed saponification and diazomethane (CH_2N_2) esterification prevented artifactual isomerization during gas chromatography (GC) analysis. In the synthesis of CLA via alkali isomerization using ethylene glycol as a solvent, extending the reaction time from 10 min to 2 h progressively increased the yield of c9,t11-CLA (from 10.9% to 30.4%) and t10,c12-CLA (from 11.1% to 31.1%), accompanied by a concomitant decrease in linoleic acid ($C_{18:2}$) from 44.7% to 16.2%. Furthermore, after 2 h of isomerization, sunflower oil—which possesses a higher initial linoleic acid concentration (642.16 mg/g)—yielded substantially higher levels of c9,t11-CLA (277.18 mg/g) and t10,c12-CLA (282.90 mg/g) compared to soybean oil (215.35 mg/g and 193.88 mg/g, respectively). These findings demonstrate that sunflower oil coupled with a 2 h alkali isomerization protocol provides an efficient pathway for high-purity CLA production for nutritional and industrial applications.

Keywords:*Conjugated linoleic acid (CLA), cis-9, trans-11 isomer, trans-10, cis-12 isomer, biohydrogenation, alkali isomerization, gas chromatography, lipid metabolism*

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I. Introduction

Conjugated linoleic acid (CLA) is a collective term for a group of positional and geometric isomers of linoleic acid (*c*9,*c*12-octadecadienoic acid, $C_{18:2}$) containing a conjugated double-bond system—where two double bonds are separated by a single carbon–carbon bond rather than a methylene group—typically initiating at carbon 9, 10, or 11. While *cis-trans*, *trans-cis*, *cis-cis*, and *trans-trans* geometric configurations are theoretically possible across these positions, the *cis*-9, *trans*-11 (*c*9,*t*11, also known as rumenic acid) and *trans*-10, *cis*-12 (*t*10,*c*12) isomers are the most abundant and biologically active forms.

Although the existence of conjugated diene structures in ruminant fats has been recognized since the 1950s, intensive scientific interest in CLA began in the late 1970s when Pariza and colleagues at the University of Wisconsin discovered a mutagenesis-inhibitory fraction in grilled and raw beef during Ames (*Salmonella*) tests, subsequently identifying the active anticarcinogenic agent as CLA. Unlike conventional linoleic acid

derived from plant oils, which has been shown to promote experimental tumorigenesis at high levels, CLA—found predominantly in ruminant meats and dairy products—demonstrates potent protective effects against experimental cancers, atherosclerosis, and immune-induced catabolism, while partitioning dietary energy toward lean body mass accretion rather than adipose deposition.

Because natural dietary sources rich in CLA (such as dairy products, beef, and lamb) are inherently high in saturated fats, commercial production of CLA from vegetable oils rich in linoleic acid via chemical alkali isomerization or microbial conversion has gained considerable attention for use in functional foods, dietary supplements, and animal agriculture. However, conjugated double bonds are chemically unstable under conventional acid-catalyzed lipid analysis conditions, necessitating specialized extraction, derivatization, and chromatographic methodologies. Therefore, the objectives of this paper are to review the biochemistry, physiological functions, and analytical frameworks of lipids and CLA, and to experimentally investigate fecal lipid extraction protocols, the oxidative stability of CLA, and the optimal reaction parameters for synthesizing CLA from edible vegetable oils.

II. Theoretical Background and Literature Review

2.1. Lipid Metabolism and Biohydrogenation in Ruminants vs. Non-Ruminants

Lipids are water-insoluble organic compounds soluble in non-polar solvents such as ether, chloroform, and benzene, broadly classified into saponifiable lipids (simple lipids such as fats/triacylglycerols and waxes; compound lipids such as phospholipids, glycolipids, and sphingolipids) and non-saponifiable lipids (terpenes, steroids, and prostaglandins). Polyunsaturated fatty acids (PUFAs) of the ω-6 (linoleic acid, $C_{18:2}$) and ω-3 (linolenic acid, $C_{18:3}$) series, along with arachidonic acid ($C_{20:4}$), serve as essential fatty acids (EFAs) required for cell membrane integrity, lipid transport, and eicosanoid (prostaglandin) synthesis.

In non-ruminants, dietary triglycerides are emulsified by bile salts in the duodenum and hydrolyzed by pancreatic lipase and colipase at the *sn*-1 and *sn*-3 positions to yield free fatty acids and 2-monoglycerides, which form mixed micelles, enter the enterocytes, and are re-esterified into chylomicrons and very-low-density lipoproteins (VLDL) for lymphatic transport. Furthermore, non-ruminants utilize glucose as the primary carbon substrate for *de novo* lipogenesis via the citrate cleavage pathway. In contrast, ruminants lack ATP-citrate lyase and NADP-malate dehydrogenase, relying instead on rumen-derived acetate and butyrate for fatty acid synthesis alongside the isocitrate dehydrogenase and pentose phosphate pathways for NADPH generation.

Crucially, in the anaerobic environment of the rumen, microbial lipases rapidly hydrolyze dietary galactolipids (from forage) and triglycerides (from grains) into free fatty acids, which then undergo microbial biohydrogenation into saturated stearic acid ($C_{18:0}$). During this process, the anaerobic bacterium *Butyrivibrio fibrisolvens* catalyzes the isomerization of free linoleic acid (*c*9,*c*12-$C_{18:2}$) into *c*9,*t*11-CLA via linoleate isomerase as the first intermediate, which is subsequently hydrogenated to *trans*-vaccenic acid (*t*11-$C_{18:1}$) and finally to stearic acid. In addition to ruminal synthesis, absorbed *trans*-vaccenic acid can be endogenously converted back into *c*9,*t*11-CLA within mammalian tissues (especially the mammary gland) via the enzyme Δ^9-desaturase. Consequently, ruminant dairy and meat products contain substantially higher CLA concentrations (2.5–11.0 mg/g of fat, of which ≥75% is the *c*9,*t*11 isomer) than non-ruminant meats (e.g., pork at 0.6 mg/g, chicken at 0.9 mg/g), seafood (0.3 mg/g), or vegetable oils (0.1–0.7 mg/g). In human populations (e.g., German dietary surveys), average daily CLA intake is estimated at approximately 0.35–0.36 g/d for women and 0.43–0.44 g/d for men, derived predominantly from dairy and ruminant meat products.

2.2. Physiological and Biological Activities of CLA

- **Anticarcinogenic Activity:** CLA is unique as a potent naturally occurring anticarcinogen of animal origin, demonstrating efficacy at dietary levels as low as 0.1–1.0% in rodent models of skin, forestomach, colon, and mammary tumorigenesis (e.g., DMBA-induced mammary cancer), as well as *in vitro* against human MCF-7 breast, colorectal, and malignant melanoma cancer cell lines. According to Wattenberg's chemoprevention framework, CLA acts as both a blocking agent (inhibiting DNA adduct formation and modulating cytochrome P450) and a suppressing agent, while also reducing target tissue susceptibility by decreasing mammary epithelial terminal end bud (TEB) branching density and inducing apoptosis. Mechanistically, CLA competes with linoleic acid for desaturation and elongation enzymes, thereby suppressing arachidonic acid accumulation, ornithine decarboxylase, protein kinase C, and downstream pro-inflammatory eicosanoids.
- **Anti-Atherosclerotic Effects:** Despite containing *trans* double bonds, dietary CLA supplementation in hypercholesterolemic rabbit (Kritchevsky) and hamster (Nicolosi) models significantly reduced plasma total cholesterol, LDL-cholesterol, and triglyceride concentrations while attenuating early aortic atherosclerosis compared to controls.
- **Immunomodulation and Anti-Catabolic Effects:** Immune stimulation by bacterial lipopolysaccharide (endotoxin) triggers macrophages to release pro-inflammatory cytokines such as tumor necrosis factor-α

(TNF-α) and interleukin-1 (IL-1), inducing anorexia, skeletal muscle catabolism, and growth suppression. Studies by Cook and Pariza demonstrated that dietary CLA enhances immune responsiveness while simultaneously preventing endotoxin-induced anorexia and tissue wasting in chicks, mice, and rats through the modulation of arachidonic acid-derived eicosanoids, particularly prostaglandin E_2 (PGE_2).

- **Lipid Metabolism and Body Composition:** Dietary CLA consistently reduces body fat mass and increases lean body mass across mice, rats, and swine. In 3T3-L1 adipocytes, CLA dose-dependently inhibits lipoprotein lipase (LPL) activity, induces lipolysis, and suppresses preadipocyte differentiation, while in skeletal muscle it elevates carnitine palmitoyltransferase (CPT) activity, enhancing mitochondrial β-oxidation and whole-body protein accretion.
- **Debated Antioxidant Activity:** Early studies (e.g., Ha et al.; Ip et al.) suggested that CLA acts as a free-radical scavenging antioxidant more potent than α-tocopherol and comparable to butylated hydroxytoluene (BHT). However, subsequent investigations by Van den Berg et al. (using 1-palmitoyl-2-linoleoyl phosphatidylcholine membranes) and Banni et al. demonstrated that CLA does not act as a direct radical scavenger or metal chelator; rather, conjugated dienes are susceptible to oxidation, forming furan derivatives (epoxy-octadecadienoic acids).

2.3. Analytical Considerations for CLA

Accurate quantification of CLA isomers requires specialized analytical procedures because conjugated double bonds are highly susceptible to geometric isomerization and oxidation.

- **Spectroscopy:** Conjugated dienes exhibit a characteristic ultraviolet (UV) absorption maximum at 230–234 nm (whereas conjugated trienes absorb near 270 nm), which can be resolved from background lipid absorption at 200 nm via second-derivative UV spectroscopy (yielding minima at 233 nm for *trans,trans* and 242 nm for *cis,trans* dienes).
- **Esterification and Chromatography:** For gas chromatography (GC), base-catalyzed methylation (e.g., sodium methoxide or KOH/methanol) or diazomethane (CH_2N_2) esterification is mandatory, as acid-catalyzed procedures (BF_3/methanol or HCl/methanol) induce severe artifactual isomerization of *cis,trans* conjugated dienes into *trans,trans* forms. On long (100 m) highly polar capillary columns (e.g., CP-Sil 88 or BPX-70), CLA isomers elute in the order of *cis,trans* (*c*9,*t*11, *t*8,*c*10, *c*11,*t*13, *t*10,*c*12), followed by *cis,cis*, and finally *trans,trans* isomers. Complementary separation of positional isomers is achieved using silver-ion high-performance liquid chromatography (Ag^+-HPLC) with UV detection at 233 nm (where *trans,trans* elutes before *cis,trans* and *cis,cis*), while double-bond positions are confirmed via GC-mass spectrometry (GC-MS) using 4,4-dimethyloxazoline (DMOX) derivatives or Diels–Alder adducts with 4-methyl-1,2,4-triazoline-3,5-dione (MTAD).

III. Materials and Methods

3.1. Experiment 1: Lipid Extraction and Fatty Acid Methylation from Poultry Excreta

Because poultry diets contain high levels of calcium, unabsorbed fatty acids in chicken feces frequently form insoluble calcium soaps in the gastrointestinal tract, preventing complete extraction by neutral organic solvents alone.

1. **Sample Pretreatment & Acidification:** Fecal samples were stored at −20°C immediately after collection to prevent enzymatic hydrolysis and autoxidation. Samples were suspended in deionized water and acidified to pH 1 using 6 M HCl to dissociate calcium soaps into free fatty acids.
2. **Solvent Extraction:** To the acidified homogenate, 10 mL of chloroform ($CHCl_3$), 20 mL of methanol (CH_3OH), and 1 mL of internal standard (1 mg/mL heptadecanoic acid, $C_{17:0}$, Sigma H-3500, dissolved in dichloromethane, DCM) were added. The mixture was homogenized for 2 min, supplemented with an additional 10 mL of $CHCl_3$, re-homogenized for 2 min, and centrifuged at 2,600 rpm for 10 min to achieve biphasic separation.
3. **Purification & Protection Against Autoxidation:** The lower $CHCl_3$ lipid layer was collected, filtered through $CHCl_3$-washed Whatman No. 1 filter paper into a round-bottom flask, and evaporated to dryness under vacuum using a rotary evaporator. Residual water was azeotropically removed by adding methanol and evaporating twice. The lipid extract was dissolved in iso-hexane containing 0.001% (w/v) butylated hydroxytoluene (BHT, 10 mg/L) as an antioxidant and flushed with nitrogen gas (N_2).
4. **Saponification & Extraction of Free Fatty Acids:** After transferring the extract to a tube and removing the solvent on a dry-block heater at 50°C under N_2, lipids were saponified by heating with 0.5 M KOH in ethanol. Unsaponifiable matter (e.g., sterols) and free fatty acids were partitioned using diethyl ether (Et_2O) and 1% aqueous KOH. The combined KOH soap extracts were acidified with 6 M H_2SO_4 and extracted three times with Et_2O. The combined Et_2O phase was washed with distilled water until neutral to litmus paper, rotary-evaporated to dryness with repeated methanol additions, and stabilized in iso-hexane (0.001% BHT) under N_2.

5. **Diazomethane (CH_2N_2) Derivatization & GC Analysis:** Fresh diazomethane was generated by reacting 2 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Sigma M-2256) in 20 mL Et_2O with 2 g KOH in 95% ethanol on an ice bath, followed by distillation. After removing iso-hexane from the fatty acid residue under N_2, the sample was dissolved in Et_2O with 2 drops of methanol, and CH_2N_2 was added until a persistent yellow color remained. Excess solvent and reagent were removed under gentle heat and N_2, and the resulting fatty acid methyl esters (FAMEs) were reconstituted in iso-hexane (0.001% BHT) for GC analysis.

3.2. Experiment 2: Oxidative Stability and Antioxidant Evaluation of CLA

To investigate the oxidative stability of CLA over time, a purified CLA preparation containing 79% total CLA isomers (predominantly *c*9,*t*11-CLA and *t*10,*c*12-CLA) was tested starting 4 weeks after opening.

1. **Experimental Design:** Samples were divided into three treatments—(1) Untreated Control, (2) Ethoxyquin-supplemented (antioxidant), and (3) Nitrogen gas (N_2)-flushed—and stored under two temperature regimes: Room Temperature (RT) and Cold Room (4°C, CR) over a multi-week period.
2. **Sampling & GC Quantification:** At designated weekly intervals, approximately 20 mg of each sample was weighed into a Quickfit round-bottom flask, evaporated to remove volatile impurities, and re-weighed. After adding the $C_{17:0}$ internal standard (in DCM), Et_2O, and 2 drops of methanol, the samples were methylated with diazomethane (CH_2N_2) until a permanent yellow color developed, evaporated under N_2, diluted in iso-hexane, and analyzed by GC to monitor changes in CLA isomer concentrations (mg/g).

3.3. Experiment 3: Alkali Isomerization Synthesis of CLA from Edible Oils

To evaluate the parameters governing the chemical synthesis of CLA from vegetable oils, alkali isomerization was conducted in a three-neck round-bottom flask equipped with a cold-water reflux condenser, electric thermostat temperature sensor, and heating mantle.

1. **Experimental Variables:** The isomerization efficiency was evaluated across the following parameters:
    - **Reaction Time:** 10 min, 1 h, and 2 h.
    - **Edible Oil Substrate:** Soybean oil vs. Sunflower oil (0 h vs. 2 h).
    - **Alkali Catalyst Type & Concentration:** Potassium hydroxide (KOH) vs. Sodium hydroxide (NaOH), and normal vs. half alkali dosage.
    - **Solvent Ratio & Reaction Environment:** Oil-to-ethylene glycol ratios (1:1, 1:2, 1:3) and N_2 gas purging vs. anti-bumping granules.
2. **Work-up & Analysis:** Following alkali isomerization heating and cooling, the reaction mixture was transferred to a separatory funnel, acidified, and extracted with diethyl ether (Et_2O) to recover the free fatty acids. The Et_2O phase was washed with water until neutral to litmus paper, distilled on a steam bath, rotary-evaporated to dryness, methylated with diazomethane using $C_{17:0}$ as the internal standard, and quantified by GC.

IV. Results and Discussion

4.1. Extraction and Derivatization Protocol for Fecal Lipids (Experiment 1)

In poultry nutrition studies, dietary calcium reacts with unabsorbed free fatty acids in the intestinal lumen to form insoluble calcium soaps that resist standard neutral solvent extraction. Acidification of the fecal homogenate to pH 1 with 6 M HCl successfully protonated the fatty acid carboxylates, enabling quantitative partitioning into the chloroform phase during the $CHCl_3$–MeOH–H_2O extraction. Furthermore, maintaining 0.001% BHT in iso-hexane under a nitrogen atmosphere prevented autoxidation of polyunsaturated fatty acids, while rapid methylation with diazomethane (CH_2N_2) avoided the *cis,trans*-to-*trans,trans* geometric isomerization artifacts commonly induced by acidic catalysts (BF_3 or HCl/MeOH).

4.2. Oxidative Stability of CLA (Experiment 2)

The oxidative degradation of unsaturated lipids proceeds via free-radical abstraction of a methylene hydrogen atom to form a conjugated diene radical, which reacts with molecular oxygen (O_2) to yield a peroxyl radical ($ROO^{\bullet}$) and subsequently a hydroperoxide (ROOH). In the presence of chain-breaking antioxidants (AH, such as ethoxyquin or vitamin E), hydrogen donation terminates the autocatalytic peroxidation chain ($ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$). Monitoring the 79% CLA preparation across room temperature (RT) and cold storage (4°C, CR) under control, ethoxyquin, and N_2 treatments confirmed that, consistent with the findings of Van den Berg et al. and Banni et al., the conjugated diene moiety of CLA is inherently susceptible to autoxidation when exposed to air and ambient temperatures, requiring antioxidant protection (such as ethoxyquin or BHT) or an inert nitrogen atmosphere alongside low-temperature storage to preserve isomer integrity.

4.3. Optimization of CLA Synthesis from Edible Oils (Experiment 3)

4.3.1. Effect of Reaction Time on Linoleic Acid Isomerization

As shown in **Table 1**, prolonging the alkali isomerization reaction time from 10 min to 2 h resulted in a progressive conversion of unconjugated linoleic acid ($C_{18:2}$) into the two primary CLA isomers (*c*9,*t*11-CLA and *t*10,*c*12-CLA) in an approximately 1:1 equimolar ratio. After 10 min, only 10.9% *c*9,*t*11-CLA and 11.1% *t*10,*c*12-CLA were formed, with 44.7% unreacted $C_{18:2}$ remaining. Extending the reaction to 1 h more than doubled the CLA yield (24.1% *c*9,*t*11 and 24.8% *t*10,*c*12), while 2 h of reaction achieved 30.4% *c*9,*t*11-CLA and 31.1% *t*10,*c*12-CLA (a combined CLA proportion of 61.5% of total fatty acids) as unreacted $C_{18:2}$ declined to 16.2%. Meanwhile, oleic acid ($C_{18:1}$) remained relatively stable (23.2%–25.4%) throughout the 2 h heating period.

Table 1. Effect of Reaction Time on Fatty Acid and CLA Isomer Composition (% of Total Fatty Acids)

Fatty Acid	10 min (%)	1 h (%)	2 h (%)
Oleic acid ($C_{18:1}$)	23.2	24.7	25.4
Linoleic acid ($C_{18:2}$)	44.7	—	16.2
cis-9, *trans*-11 CLA (*c*9,*t*11)	10.9	24.1	30.4
trans-10, *cis*-12 CLA (*t*10,*c*12)	11.1	24.8	31.1
Total Major CLA (*c*9,*t*11 + *t*10,*c*12)	**22.0**	**48.9**	**61.5**

4.3.2. Comparison of Edible Oil Substrates (Soybean Oil vs. Sunflower Oil)

Because the final concentration of synthesized CLA is directly dependent on the initial linoleic acid content of the starting oil, soybean oil and sunflower oil were compared over a 2 h isomerization period (**Table 2**). Prior to reaction (0 h), neither oil contained detectable levels of *c*9,*t*11- or *t*10,*c*12-CLA, and sunflower oil exhibited a substantially higher baseline linoleic acid concentration (642.159 mg/g) and lower palmitic acid content (56.349 mg/g) than soybean oil (462.135 mg/g $C_{18:2}$ and 106.718 mg/g $C_{16:0}$).

After 2 h of alkali isomerization, 91.2% of the initial linoleic acid in soybean oil was converted (dropping from 462.135 mg/g to 40.666 mg/g), generating 215.353 mg/g of *c*9,*t*11-CLA and 193.880 mg/g of *t*10,*c*12-CLA (totaling 409.233 mg/g CLA). In sunflower oil, 96.7% of the initial linoleic acid reacted (decreasing from 642.159 mg/g to 21.467 mg/g), yielding 277.179 mg/g of *c*9,*t*11-CLA and 282.897 mg/g of *t*10,*c*12-CLA (totaling 560.076 mg/g CLA). Concentrations of saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) remained unchanged in both oils during isomerization.

Table 2. Fatty Acid and CLA Concentrations (mg/g) of Soybean Oil and Sunflower Oil Before (0 h) and After (2 h) Alkali Isomerization

Oil Source	Fatty Acid	0 h (mg/g)	2 h (mg/g)
Soybean Oil	Palmitic acid ($C_{16:0}$)	106.718	108.050
Soybean Oil	Stearic acid ($C_{18:0}$)	42.848	41.589
Soybean Oil	Oleic acid ($C_{18:1}$)	229.330	226.597
Soybean Oil	Linoleic acid ($C_{18:2}$)	462.135	40.666
Soybean Oil	*cis*-9, *trans*-11 CLA (*c*9,*t*11)	ND	215.353
Soybean Oil	*trans*-10, *cis*-12 CLA (*t*10,*c*12)	ND	193.880
Sunflower Oil	Palmitic acid ($C_{16:0}$)	56.349	55.985
Sunflower Oil	Stearic acid ($C_{18:0}$)	39.559	38.354
Sunflower Oil	Oleic acid ($C_{18:1}$)	194.636	223.154

Oil Source	Fatty Acid	0 h (mg/g)	2 h (mg/g)
Sunflower Oil	Linoleic acid ($C_{18:2}$)	642.159	21.467
Sunflower Oil	*cis*-9, *trans*-11 CLA (*c*9,*t*11)	ND	277.179
Sunflower Oil	*trans*-10, *cis*-12 CLA (*t*10,*c*12)	ND	282.897

Note: ND = Not detected (missing in unreacted oil).

4.3.3. Effect of Alkali Catalyst Source (NaOH vs. KOH)

While potassium hydroxide (KOH) is more reactive on a molar basis and yields potassium salt by-products that can be utilized as fertilizer, sodium hydroxide (NaOH) offers a significant cost advantage on a weight basis for industrial production. As shown in **Table 3**, alkali isomerization catalyzed by NaOH for 2 h effectively reduced residual linoleic acid ($C_{18:2}$) to 18.979 mg/g while producing 274.692 mg/g of *c*9,*t*11-CLA and 280.976 mg/g of *t*10,*c*12-CLA (totaling 555.668 mg/g CLA), demonstrating that NaOH in ethylene glycol can achieve high conversion yields comparable to KOH when reaction conditions are properly maintained.

Table 3. Fatty Acid and CLA Concentrations (mg/g) Using NaOH as the Alkali Catalyst

Fatty Acid	Initial / Intermediate (mg/g)	2 h (mg/g)
Palmitic acid ($C_{16:0}$)	52.439	53.403
Stearic acid ($C_{18:0}$)	35.091	36.031
Oleic acid ($C_{18:1}$)	201.284	210.642
Linoleic acid ($C_{18:2}$)	122.201	18.979
cis-9, *trans*-11 CLA (*c*9,*t*11)	205.327	274.692
trans-10, *cis*-12 CLA (*t*10,*c*12)	208.315	280.976

V. Conclusion

Conjugated linoleic acid (CLA) represents a biologically potent class of fatty acid isomers that bridges ruminant lipid metabolism and functional nutrition, exerting well-documented anticarcinogenic, anti-atherogenic, immunomodulatory, and body-fat-lowering activities. Reliable quantification of CLA from biological matrices—such as calcium-rich poultry excreta—requires initial acid hydrolysis (6 M HCl) to cleave calcium soaps, rigorous antioxidant protection (0.001% BHT and N_2 gas), and non-isomerizing methylation using base catalysts or diazomethane (CH_2N_2) prior to polar capillary GC analysis. Experimentally, CLA is susceptible to autoxidation during storage and requires antioxidant stabilization or inert gas packaging. Finally, in the synthesis of commercial CLA via alkali isomerization in ethylene glycol, a 2 h reaction time using sunflower oil as the substrate yielded the highest concentration of bioactive *c*9,*t*11-CLA (277.18 mg/g) and *t*10,*c*12-CLA (282.90 mg/g), providing an economically viable protocol for large-scale production.

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