

Research Article

Synthesis of conjugated linoleic acid by microwave-assisted alkali isomerization using propylene glycol as solvent

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Conjugated linoleic acid (CLA) is a group of isomers of linoleic acid (LA) with a conjugated double-bond system that occurs in trace amounts in natural oils. As result of the beneficial properties on the health, bioactive isomers of CLA (*cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA) have been industrially produced by alkali isomerization of LA. A large amount of studies regarding the CLA production might be found; however, new approaches as the use of microwave irradiation have been recently suggested. Here, we develop an efficient and sustainable method to selectively produce bioactive CLA by microwave-assisted isomerization of LA using propylene glycol as solvent. The investigated reaction variables were solvent/LA mass ratio (1:1–6:1), catalyst/LA mass ratio (0.25:1–0.6:1), temperature (160–180°C), catalyst type (KOH or NaOH), and reaction time. The results showed that: (i) KOH increased the production of conjugated *trans-trans* isomers; (ii) a considerable reduction of propylene glycol on the reaction was possible using NaOH; and (iii) the reaction time was drastically reduced in comparison to conventional heating. Thus, the optimum conditions for the *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA production (91.21% in equimolar ratio) were: NaOH at a catalyst/LA mass ratio of 0.5:1 and solvent/LA mass ratio of 1:1 at 160°C during 4 min.

Practical applications: CLA has been subject of growing interest in nutrition and the food industry due to its wide range of biological activities including anti-carcinogenic and anti-atherogenic properties. Here, the chemical synthesis of CLA by microwave-assisted alkali isomerization was studied in order to lower the production costs by decreasing both, the propylene glycol content (the most expensive solvent used for this kind of reaction) and the reaction time. In comparison to conventional heating, reaction time was drastically reduced by employing microwave heating while the selective synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA was achieved at low solvent conditions when the reaction conditions were optimized. In this sense, the methodology developed in this work could be used in order to obtain more efficiently and sustainably the bioactive forms of CLA, which in turn could be used in the manufacture of functional foods or dietary supplements.

Keywords: Alkali isomerization / Conjugated linoleic acid / Microwave irradiation / Propylene glycol / Synthesis

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Abbreviations: CG, gas chromatography; CLA, conjugated linoleic acid; FAME, fatty acid methyl ester; FCC, food chemical codex; FDA, food and drug administration; FFA, free fatty acids; FID, flame ionization detection; GRAS, general regarded as safe; IR, infrared spectroscopy; LA, linoleic acid

1 Introduction

Conjugated linoleic acid (CLA) is a generic term to designate a group of positional and geometrical isomers of linoleic acid (LA, *cis*9,*cis*12-18:2) with conjugated double bonds (i.e., $-C=C-C=C-$) [1, 2]. As the discovery of CLA, 28 isomers out of a total of 56 isomers theoretically possible have been identified, including 6,8; 7,9; 8,10; 9,11; 10,12; 11,13; and 12,14 carbon positions in all the possible geometric configurations *cis-cis*, *cis-trans*, *trans-cis*, and *trans-trans* [3, 4].

However, only *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA isomers appear to be biologically active forms (Fig. 1) [5]. Studies in different animals and in humans have shown that CLA regulates the composition of corporal fat and proteins, it produces anti-atherosclerotic and anti-cancer effects and modulates inflammatory responses [6–9].

*Cis*9,*trans*11-CLA also called rumenic acid, is the main isomer found in dairy and meat products [10]. In ruminants, this isomer is naturally produced as an intermediary in the biohydrogenation of LA by *Butyrivibrio fibrisolvens* or through the desaturation of vaccenic acid (*trans*11–18:1) by δ -9 desaturase activity in the liver and intestinal tract of ruminant and non-ruminant species including the man [11]. Although the total content of CLA in the diet varies widely, dietary intake of this fatty acid from natural sources is not enough to provide the beneficial effects since it occurs at low concentrations [1, 12]. Accordingly, several synthesis processes for obtaining bioactive CLA have been developed.

Synthetic methods as the dehydration of castor oil or the conjugation of double bonds of LA by basic catalysis are currently used to obtain commercial CLA [13–15]. However, the alkali isomerization of LA is the most specific and least expensive process to produce bulk synthetic CLA isomers [16–18]. In alkali isomerization, the positions of protons are changed along the hydrocarbon chain of LA to conjugated positions of the double bonds, that is, without methylene carbons between them [1]. For this purpose, vegetable oils with a high LA proportion are heated at 180°C with a strong base as sodium or potassium hydroxide and ethylene glycol for a given period of time [1, 19–21]. However, the main problem in the alkali isomerization process is the difficulty to separate the homogeneous catalysts from the CLA products. In view of this, heterogeneous catalysts have been used in the CLA production because they can be regenerated more than once; nevertheless, the selectivity toward *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA synthesis has been negatively affected favoring the *trans*,*trans*-CLA production [22, 23]. In contrast to the heterogeneous catalysis, microbiological methods possess a high specificity

toward the production of bioactive CLA isomers, mainly toward the *cis*9,*trans*11-CLA isomer [24]. Unfortunately, the low conversion of LA to CLA and the long reaction times employed by the microbiological methods make them uncompetitive against alkali isomerization [19, 24].

Microwave irradiation has emerged as an alternative heating system in the organic synthesis including the preparation of CLA to considerably reduce the reaction time [25–27]. In order to selectively synthesize the *cis*9,*trans*11-CLA isomer, a chemoenzymatic method was designed in two steps [25]. First of all, the enzymatic conversion of LA into 10,hydroxy-12-octadecenoic acid by incubation of *Lactobacillus plantarum* was done, followed by its microwave-assisted dehydration to CLA. Although the selective synthesis of *cis*9,*trans*11-CLA was achieved in half the time used in biotransformation, the conversion was low at 19.2% [24, 25].

Ricinoleic acid (18:1, OH), the main fatty acid in castor oil, has been widely used in the synthesis of CLA [15, 28]. The catalytic dehydration of castor oil leads to a non-specific formation of a new double bond in the fatty acid chain, so the LA can also be synthesized [15, 29]. Microwave irradiation has been used in the dehydration of ricinoleic acid to decrease the reaction time in CLA production from hours to minutes, reaching conversions up to 43.1% of CLA and 49.2% of LA [26, 27]. In spite of that microwave irradiation decreased the reaction times in the chemoenzymatic conversion of LA and in dehydration of ricinoleic acid, the selective synthesis toward bioactive CLA isomers continues depleted. In view of this, the alkali isomerization remains as the most effective process for the selective synthesis of bioactive isomers of CLA.

Recently, microwave irradiation has been used as an alternative method of heating in the production of CLA by alkali isomerization [27]. However, high concentrations of ethylene glycol and KOH were necessary to yield the complete conversion of LA to CLA. Ethylene glycol is a highly toxic solvent which cannot be accepted in the synthesis of CLA since traces of solvent may remain in the final

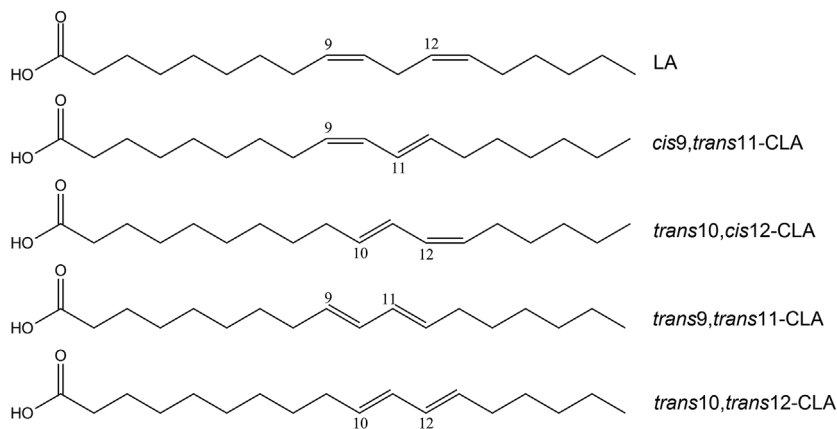


Figure 1. Geometric structures of LA, *cis*9,*trans*11-CLA, *trans*10,*cis*12-CLA, *trans*9,*trans*11-CLA, and *trans*10,*trans*12-CLA.

product; only GRAS (General Regarded as Safe) solvents are accepted [30].

To avoid the possible presence of toxic traces of ethylene glycol in the CLA, in the present study we have evaluated the replacement of ethylene glycol by propylene glycol FCC (Food Chemical Codex, food grade) in the microwave-assisted alkali isomerization of LA, as we did previously for the conventional heating [16, 17]. The type and concentration of catalyst, the concentration of solvent, the temperature, and the reaction time for the selective synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA were investigated in a microwave-assisted base catalysis using propylene glycol as solvent.

2 Materials and methods

2.1 Conjugated linoleic acid synthesis

LA ($\geq 99\%$, Cat. No. L1376, Sigma-Aldrich Inc., St. Louis, USA) was isomerized under inert nitrogen atmosphere to CLA by basic catalysis assisted by microwave irradiation in a batch mode, in accordance with Moreno et al. [27]. The microwave-heated reactions were carried out on a single-mode CEM Discover Instrument (Model No. 908005; Serial No. DU8774, Matthews, NC) at 210 W and 250 psi, in a 10 mL microwave reaction tube. Temperature and reaction time were set according to experimental design. The catalyst concentration, that is, KOH (purity 88%) and NaOH (purity 98.5%) ranging from 0.25:1 to 0.6:1 w/w of catalyst/LA, was evaluated on CLA synthesis keeping constant the proportion of propylene glycol FCC ($>99.5\%$, Cat. No. W294004, Sigma-Aldrich Inc.) at 12:1 w/w solvent/LA, the temperature at 160°C, and the reaction time at 10 min. It is important to note that the called “reaction time” is the hold time at the set temperature, but it should be considered that the total time of reaction includes the heating (40 s) and the cooling time (between 1.5 and 2 min). Subsequently, the effect of the catalyst (KOH or NaOH), the concentration of propylene glycol (1:1, 3:1, and 6:1 w/w solvent/LA), and the reaction temperature (160 or 180°C) were evaluated on selective synthesis of bioactive isomers at a constant proportion of catalyst (0.5:1 w/w catalyst/LA) during 10 min of reaction. Once that the best reaction conditions for the synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA were obtained, the optimal reaction time was determined by a kinetics at intervals of 2 min on a 0–10 min scale considering zero-time when the reaction temperature was reached. For this purpose, 200 mg of LA were reacted with 100 mg of NaOH and 200 mg of propylene glycol at 160°C under microwave irradiation. In the same way, we used corn oil as starting material to produce bioactive CLA from mixtures of fatty acids. In this regard, we reacted 269.7 mg of corn oil (56.18% of LA, 151.5 mg), 75.8 mg of NaOH, and 151.5 mg of propylene glycol in a reaction tube at the optimal reaction conditions for the selective synthesis of *cis*9,*trans*11-CLA and

*trans*10,*cis*12-CLA. The total mass in the reaction tube was kept constant, at 500 mg, for all experiments, in order to avoid differences in the response due to differences in the mass amount. The reaction mixtures were acidified to pH = 2 with 6 N HCl after being homogenized with 5 mL of methanol. The lipid fraction was separated by liquid–liquid extraction using 5 mL of hexane as organic solvent. Then the organic phase was washed twice with 5 mL of 30% v/v methanol/water and twice with 5 mL of distilled water. Finally, it was dried over anhydrous MgSO_4 and the solvent was removed under a nitrogen gas stream. Fatty acids were stored at -20°C after being purged with nitrogen gas [16, 17]. CLA production was monitored by a quantitative analysis of LA, *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA.

2.2 Fatty acid analysis by gas chromatography

The fatty acid analysis was performed by gas chromatography with flame ionization detector (GC-FID). Two milligram of free fatty acids (FFA) obtained from alkali isomerization were methylated with 250 μL of HCl/MeOH (5:95, v/v) solution at 70°C for 2 min [16, 17, 31]. The reaction was stopped by the addition with 250 μL of saturated NaCl solution and the resulting fatty acid methyl esters (FAME) were extracted with 500 μL of hexane. FAME contained in 1 mL of solution were identified and quantified using a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with a flame ionization detector set to 300°C. Stabilwax capillary column (60 m \times 0.25 mm internal diameter, 0.25 μm stationary phase film, Cat. No. 10626, Restek Corp., USA) was used in the chromatographic separation. The oven temperature was programmed from 150 to 200°C at a rate of 10°C min $^{-1}$ and kept for 1 min, then it was raised to 250°C at 3°C min $^{-1}$ and held for 1 min. Hydrogen at a flow rate of 2 mL/min was used as carrier gas. The injector temperature was adjusted to 250°C (split flow 50:50). FAME were identified by comparing their retention times with known standards of LA, *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA (Cat. No. L1376, 16413, and 92321, Sigma-Aldrich Inc.). Heptadecanoic acid (C17:0) was used as internal standard for quantitative analysis (Cat. No. H3500, Sigma-Aldrich Inc.). The presence of conjugated *trans-trans* isomers was confirmed by infrared spectroscopy as is described below.

2.3 Geometrical elucidation

The geometrical isomerism of LA, CLA obtained under optimum reaction conditions (0.5:1 w/w of NaOH/LA, 1:1 w/w of propylene glycol/LA, 160°C and 4 min of reaction) and CLA produced at 0.5:1 w/w of KOH/LA, 1:1 w/w of propylene glycol/LA, 180°C and 10 min was confirmed by infrared spectroscopy (IR). The IR spectra of the samples were obtained from Nicolet Nexus 470 FT-IR Spectrometer (Thermo Nicolet, USA) using KBr-pellets method.

2.4 Statistics

Data were collected from three independent experiments. One-way analysis of variance (ANOVA) followed by a *post-hoc* Tukey test was used to detect significant differences at $p < 0.05$ among treatments, after the normal distribution was corroborated by Kolmogorov–Smirnov's test. Statistical analysis was performed using the Statistica 7.0 software package (StatSoft, Tulsa, OK), whereas Graph Pad Prism V 5.01 (Graph-Pad Software Inc.) was used for data plotting.

3 Results and discussion

In this work, we have implemented the microwave irradiation heating in alkali isomerization of LA to selectively synthesize the *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA (Fig. 1) using propylene glycol as solvent. First of all, we have reproduced the reaction conditions reported by Moreno et al. [27] for the microwave-assisted synthesis of CLA: 0.25 g of LA (0.87 mmol), 0.78 g of KOH (12 mmol), and 2.5 mL of ethylene glycol, that is, 2.75:1 w/w KOH/LA and 11.1:1 w/w ethylene glycol/LA at 160°C and 15 min. Although the results showed a maximum conversion of LA to CLA of $98.96 \pm 0.39\%$, we obtained only $47.11 \pm 0.24\%$ of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA isomers in equimolar ratio (Fig. S1A). Our results suggest that these reaction conditions generate a high amount of CLA without constituting a selective process for the synthesis of the isomers of biological interest. By another hand, the replacing of ethylene glycol by propylene glycol resulted in an increase of conjugated *trans-trans* isomers and a decrease in the production of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA (Fig. S1B). As the synthesis of bioactive isomers of CLA was not favored under these reaction conditions, in the present study, we evaluated the effect of the type and concentration of catalyst, the concentration of solvent, temperature, and reaction time on the selective synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA using propylene glycol as solvent.

3.1 Effect of proportion of catalyst

The effect of catalyst concentration, KOH and NaOH (ratios from 0.25:1 to 0.6:1 w/w catalyst/LA), was determined by homogeneous catalysis in a microwave irradiation system using propylene glycol as solvent (12:1 w/w solvent/LA), 160°C and 10 min. Fig. 2 shown the effect of KOH on conversion of LA to CLA and the synthesis of bioactive isomers *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA while the effect of NaOH is shown in Fig. 3. The results indicate that increasing concentrations of both KOH or NaOH catalysts significantly affect the synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA, reaching the maximum generation of

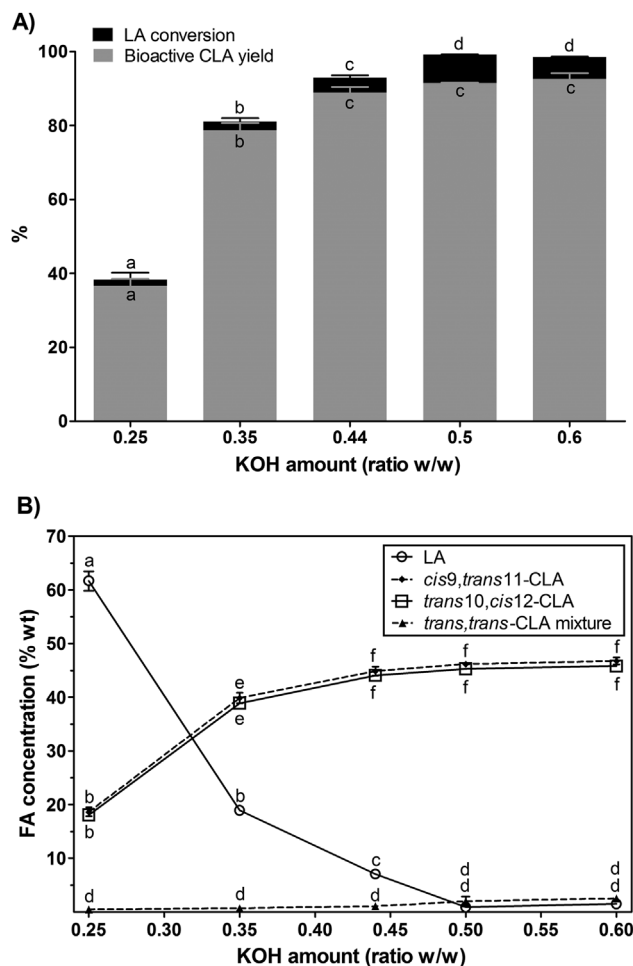


Figure 2. KOH effect on the synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA. (A) Conversion of LA to CLA and yield of the bioactive isomers using KOH as catalyst. (B) Production of *cis*9,*trans*11-CLA, *trans*10,*cis*12-CLA, and *trans,trans*-CLA mixture using KOH as catalyst. KOH at ratios ranging from 0.25:1 to 0.6:1 w/w catalyst/LA was used in the microwave-assisted alkali isomerization of LA in propylene glycol FCC (12:1 w/w solvent/LA) at 160°C and 10 min of reaction. The total mass in the reaction tube was kept constant for all experiments. Different letters indicate significant differences ($p < 0.05$). Each value represents the mean \pm SD.

bioactive isomers at a ratio of 0.44:1 w/w catalyst/LA. However, the complete conversion of LA to CLA was achieved at a ratio of 0.5:1 w/w catalyst/LA, so this ratio was considered as the optimum concentration for both catalysts. Our results are in agreement with previous reports about the alkali isomerization of LA by conventional heating, where the amount of catalyst needed to generate the complete conversion of LA into CLA is low [18]. By contrast, in this work we have decreased the amount of catalyst to produce CLA by microwave-assisted heating in almost twofold compared to the previous study [27].

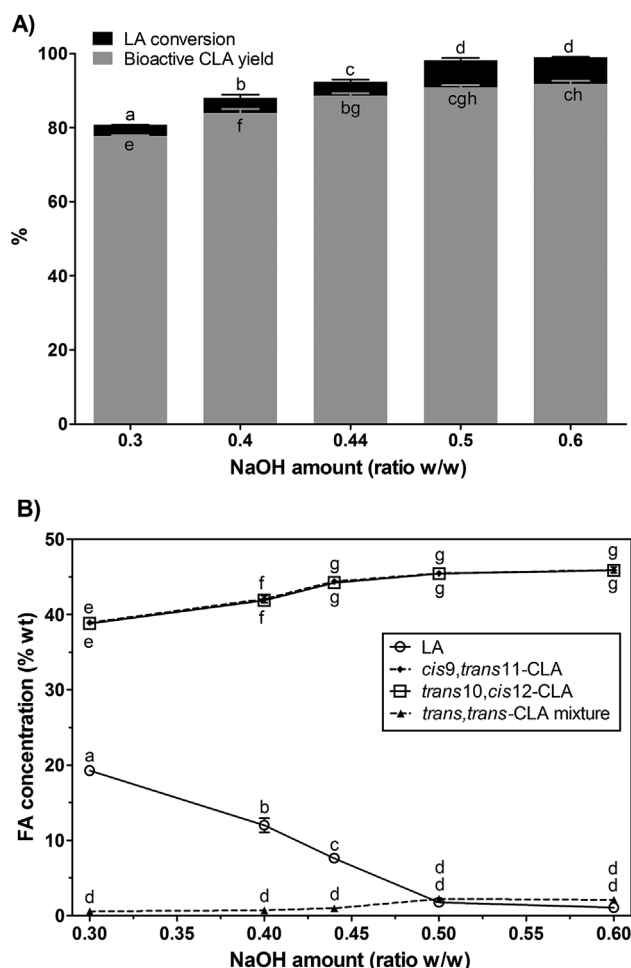


Figure 3. NaOH effect on the synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA. (A) Conversion of LA to CLA and yield of the bioactive isomers using NaOH as catalyst. (B) Production of *cis*9,*trans*11-CLA, *trans*10,*cis*12-CLA, and *trans,trans*-CLA mixture using NaOH as catalyst. NaOH at ratios ranging from 0.3:1 to 0.6:1 w/w catalyst/LA was used in the microwave-assisted alkali isomerization of LA in propylene glycol FCC (12:1 w/w solvent/LA) at 160°C and 10 min of reaction. The total mass in the reaction tube was kept constant for all experiments. Different letters indicate significant differences ($p < 0.05$). Each value represents the mean \pm SD.

3.2 Effect of catalyst type, temperature, and proportion of solvent

Although both KOH and NaOH at the same mass ratio (0.5:1 w/w catalyst/LA) produced selectively the isomers of interest (around 90% of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA in equimolar ratio), a high proportion of propylene glycol (12:1 w/w solvent/LA) was necessary to achieve such selectivity (Figs. 2 and 3 and Table 1). Propylene glycol is the most commonly solvent used in the current CLA preparation by alkali isomerization of LA; nevertheless, it is also the most

expensive solvent used for this purpose [18]. In order to decrease the proportion of solvent in the reaction mixture, the effect of the catalyst (KOH and NaOH) and the reaction temperature (160 and 180°C) were evaluated on the selective synthesis of the bioactive isomers of CLA at low proportions of propylene glycol (1:1, 3:1, and 6:1 w/w solvent/LA), using a reaction time of 10 min.

In spite of high yields of total CLA were obtained in all treatments (around 99% of conversion of LA to CLA), the profile of CLA isomers was affected by the reaction conditions (Table 1). NaOH as catalyst and ratios of 1:1, 3:1, or 6:1 w/w of solvent/LA at 160°C generated about 90% of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA isomers in equimolar proportion; whereas KOH favors such selectivity only when the isomerization was carried out at a ratio of 6:1 w/w of solvent/LA and 160°C. In this regard, we have decreased the proportion of solvent in almost tenfold by replacing KOH by NaOH [27]. These results indicate that the selection of catalyst is crucial in selective production of bioactive CLA when the amount of solvent is reduced.

The reduction of propylene glycol on alkali isomerization of CLA was previously studied under conventional heating [18]. The authors showed that the reduction of propylene glycol content at ratios of 1:1 and 1:2 v/v of solvent/oil (sunflower oil with 57.57% of LA), that is, 1.74:1 and 0.87:1 v/v of solvent/LA respectively, resulted in a 97.52 and 90.11% conversion of LA to CLA (*cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA in equimolar ratio) when the reaction was carried out at 180°C, 80 min, stirring speed of 300 rpm, and 1:2 catalyst(NaOH)/oil. Compared with our data at 1:1 w/w propylene glycol/LA, 0.5:1 w/w NaOH/LA, 160°C and 10 min (which produced $44.39 \pm 0.55\%$ of *cis*9,*trans*11-CLA and $44.67 \pm 1.35\%$ of *trans*10,*cis*12-CLA), the conversion of LA to CLA was similar. However, the reaction time was significantly reduced, which resulted to be the main advantage of microwave irradiation against conventional heating.

On the other hand, our results (Fig. 1) at 10 min of reaction time indicated that KOH catalyst was less selective than the catalyst based on sodium because KOH promoted the formation of conjugated *trans-trans* isomers when the lower ratio of solvent/LA was used. Also, it was established that the selective synthesis of bioactive isomers was favored at the lowest temperature evaluated of 160°C, while an increase at 180°C decreases their production. This singularity where a raise of the temperature increases the conjugated *trans-trans* isomers has been previously reported and explained as the higher thermodynamic stability [1, 32]. After the formation of the two primary isomers, additional isomers could be produced in function of temperature and the time of reaction [32]. Temperatures below 160°C were discarded from the study, since 140°C did not produce the complete conversion of LA to CLA at 10 min ($67.92 \pm 0.23\%$ conversion) or even at 15 min of reaction ($76.68 \pm 0.31\%$ of conversion). Accordingly, in the CLA synthesis it is

Table 1. Effect of temperature, catalyst type and proportion of propylene glycol as solvent on the production of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA, during 10 min of reaction time and 0.5:1 w/w of catalyst/LA

Catalyst	T (°C)	Solvent/LA (ratio w/w)	Fatty acid (% wt)			
			LA	<i>cis</i> 9, <i>trans</i> 11-CLA	<i>trans</i> 10, <i>cis</i> 12-CLA	<i>trans,trans</i> -CLA mixture
KOH	160	1:1	0.757 ^a ± 0.031	36.753 ^a ± 0.061	37.315 ^a ± 0.124	16.172 ^a ± 0.281
		3:1	0.679 ^a ± 0.038	42.590 ^b ± 0.353	42.851 ^b ± 0.442	07.016 ^b ± 0.597
		6:1	0.838 ^a ± 0.064	45.672 ^c ± 0.325	45.618 ^c ± 0.372	2.486 ^c ± 0.448
		12:1	0.832 ^{ab} ± 0.033	46.164 ^c ± 0.282	45.311 ^c ± 0.221	1.928 ^c ± 0.923
	180	1:1	0.915 ^{ab} ± 0.078	14.666 ^d ± 0.943	14.900 ^d ± 0.388	44.358 ^d ± 1.019
		3:1	1.108 ^{ab} ± 0.041	16.474 ^d ± 1.114	16.197 ^d ± 1.923	41.884 ^d ± 2.343
		6:1	1.239 ^{ab} ± 0.344	29.338 ^c ± 0.676	29.608 ^c ± 0.722	24.395 ^c ± 1.133
		12:1	1.090 ^{ab} ± 0.293	44.393 ^{bc} ± 0.554	44.672 ^{bc} ± 1.352	03.707 ^{bc} ± 1.387
NaOH	160	3:1	1.021 ^{ab} ± 0.393	44.235 ^{bc} ± 0.818	44.866 ^{bc} ± 0.715	03.903 ^{bc} ± 1.456
		6:1	1.303 ^{ab} ± 0.418	46.056 ^c ± 0.295	45.951 ^c ± 0.276	01.804 ^c ± 0.320
		12:1	1.739 ^b ± 0.523	45.463 ^c ± 0.274	45.415 ^c ± 0.315	2.190 ^c ± 0.056
	180	1:1	0.857 ^a ± 0.043	31.730 ^f ± 0.269	31.520 ^e ± 0.508	21.204 ^c ± 0.756
		3:1	1.064 ^{ab} ± 0.043	35.121 ^a ± 1.319	34.376 ^f ± 0.111	16.878 ^a ± 0.647
		6:1	0.780 ^a ± 0.046	39.953 ^g ± 0.616	39.252 ^a ± 0.201	10.139 ^b ± 0.866

The total mass in the reaction tube was kept constant for all experiments.

Different letters indicate significant differences for the same column ($p < 0.05$). Each value represents the mean ± SD.

necessary to maximize the content of bioactive isomers and minimize the formation of undesirable isomers through the optimization of reaction conditions.

3.3 Effect of time

NaOH as catalyst at a ratio of 0.5:1 w/w catalyst/LA, 160°C and a mass ratio of 1:1 of solvent/LA were selected to set the optimum time of reaction. A kinetics at intervals of 2 min on a scale of 0–10 min determined that the minimum time required for maximum production of the bioactive isomers of CLA was 4 min (Fig. 4). Under these conditions, we obtained 176 mg of fatty acids and a conversion of $97.37 \pm 0.84\%$ of LA to CLA, out of which $91.21 \pm 0.77\%$ consisted of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA isomers in equimolar ratio and 6.16% of others CLA isomers. It is noteworthy that at 0 min of reaction the concentration of LA does not correspond to 100%, this is due to isomerization reactions were carried out during the temperature rise and the time 0 corresponds to the time when 160°C were achieved. Similarly, alkali isomerization reactions took place during the cooling step. Therefore, the total time reaction was about 6 min.

Additionally, we have carried out the alkali isomerization of LA in corn oil by microwave irradiation at the optimal reaction conditions for the selective synthesis of bioactive CLA (Fig. S2). In this approach, the only significant change was the conversion of LA ($56.18 \pm 0.35\%$ of LA in corn oil) to CLA in a $98.77 \pm 0.15\%$ (out of which $45.62 \pm 0.26\%$ corresponded to *cis*9,*trans*11-CLA and the $45.19 \pm 0.20\%$ to *trans*10,*cis*12-CLA), whereas saturated and monounsaturated fatty acids

were not affected by the reaction conditions similarly to previous reports [16, 17].

We previously reported the selective production of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA by alkali isomerization of LA in safflower oil (74.53% of LA) using propylene glycol as solvent (5.8:1 w/w solvent/oil or 7.78:1 w/w solvent/LA) and NaOH as catalyst (0.44:1 w/w catalyst/oil or 0.59 w/w

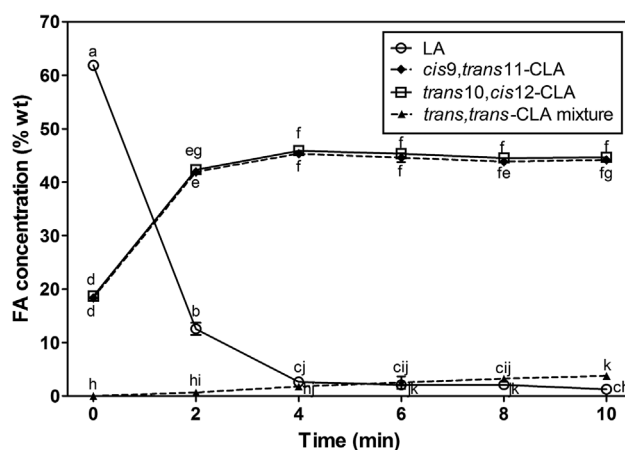


Figure 4. Effect of reaction time on the synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA. The microwave-assisted alkali isomerization of LA was performed in propylene glycol FCC (1:1 w/w solvent/LA) using NaOH (0.5:1 w/w catalyst/LA) at 160°C and different times of reaction (0–10 min). The total mass in the reaction tube was kept constant for all experiments. Different letters indicate significant differences ($p < 0.05$). Each value represents the mean ± SD.

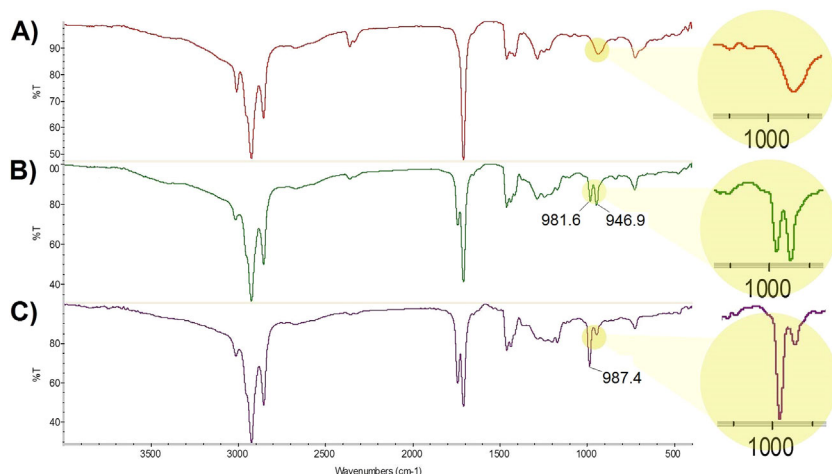


Figure 5. Geometrical isomerism of LA (A), 50:50 of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA (B), and 25:25:50 of *cis*9,*trans*11-CLA, *trans*10,*cis*12-CLA, and *trans,trans*-CLA mixture (C). CLA in B was produced by microwave-assisted alkali isomerization using 0.5:1 w/w of NaOH/LA and 1:1 w/w of propylene glycol/LA at 160°C and 4 min of reaction. CLA in C was produced at 0.5:1 w/w of KOH/LA, 1:1 w/w of propylene glycol/LA, 180°C and 10 min by microwave-assisted alkali isomerization. Infrared spectra showed the presence of *cis-cis* isomers in A (no bands between 900 and 1000 cm⁻¹), *cis-trans* and *trans-cis* isomers in B (two bands at 946.9 and 981.6 cm⁻¹ for both), and *trans-trans* isomers in C (one band at 987.4 cm⁻¹).

catalyst/LA) in a conventional heating system at 180°C for 2.15 h [16, 17]. Compared with our previous work, here we have significantly reduced the amount of solvent in almost sevenfold as well as the reaction time from 129 to 4 min by replacing conventional heating by microwave radiation. This reduction of reaction time could be explained by the ability of propylene glycol to absorb microwave energy and convert it into heat, since propylene glycol (as ethylene glycol) is considered as a solvent with a high capacity of absorb microwave energy [33]. According to Lidström et al. [34], in microwave heating the change of electrical field that interacts with the molecular dipoles and/or charged ion causes a rapid rotation of such entities, and heat is generated due to friction of this motion.

3.4 Geometrical elucidation

Infrared spectroscopy differentiates CLA isomers by the geometry of their double bonds [4]. The geometrical isomerism of CLA obtained by microwave-assisted alkali isomerization to the optimal reaction conditions was analyzed by IR and it was compared to its control, the LA. Unsaturated fatty acids of *cis-cis* isomerism as the LA showed a lack of bands in the region between 900 and 1000 cm⁻¹ of the infrared spectrum corresponding to the C–H out-of-plane deformation vibrations in H–C=C–H groups (Fig. 5A). In contrast, *cis-trans* and *trans-cis* conjugated dienes as the bioactive CLA isomers had a characteristic doublet near 945 and 985 cm⁻¹ (Fig. 5B) [4, 35, 36]. On the other hand, a sharp absorption band near to 990 cm⁻¹ (corresponding to conjugated *trans-trans* isomers) was observed for the CLA obtained at 0.5:1 w/w of KOH/LA, 1:1 w/w of propylene glycol/LA, 180°C and 10 min of reaction, suggesting that this CLA mostly consisted of conjugated *trans-trans* isomers (Fig. 5C). *Trans* fats are defined by the Food and Drug Administration (FDA) as unsaturated fatty acids that contain one or more non-conjugated double bonds in *trans* configuration and a band in 966 cm⁻¹ of the IR spectra, excluding from this definition to

trans fatty acids with conjugated double bonds as CLA [8, 37]. Although CLA is not considered as *trans* fat, the toxic effects of *trans-trans* isomers of CLA have not been widely elucidated to date; therefore, the selective synthesis of bioactive CLA remains necessary [38–40].

4 Conclusions

In the present study, we evaluated the effect of the type and concentration of catalyst, the concentration of solvent, temperature, and reaction time on the selective synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA using propylene glycol as solvent in a microwave-assisted alkali isomerization. The results indicated that alkali isomerization assisted by microwave irradiation at mass ratios of 0.5:1 of NaOH/LA and 1:1 of propylene glycol/LA, 160°C and 4 min of reaction might be an alternative in the selective production of bioactive isomers for the industry. Although the commercial production and dietary supplementation of individual isomers of CLA is desirable, it has been limited due to the high costs of purification. Therefore, the alkali isomerization of LA from sources rich in this fatty acid remains as the most effective method for the synthesis of *cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA in equimolar ratio. In the present study, we have showed that the selective synthesis of bioactive CLA is produced at the optimal reaction conditions even when oils rich in LA are used as raw material. In summary, the process described here is a safe (use of propylene glycol), fast, and selective method to produce bioactive CLA.

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