J Biochem Tech (2018) Special Issue (2): 1-8
ISSN: 0974-2328

Different Conditions for Alkaline Isomerization and the Production of Conjugated Linoleic Acid (CLA) from Linoleic Acid

Mohamad-Reza Shayanmehr*, Amir-Hossein Elhamirad and Mohamad Armin

Received: 04 December 2017 / Received in revised form: 11 May 2018, Accepted: 15 May 2018, Published online: 05 September 2018

Abstract

Linoleic acid isomerization was performed in this study and the production of conjugated Linoleic acid was tested. Treatment groups comprised four variable temperatures (80, 110, 140 and 170 °C), four durations (0.25, 0.5, 1, 2, 3.5, 5 and 6.5 hours), four solvents (water, ethanol, propylene and glycol), and four alkalis (sodium hydroxide, potassium hydroxide, sodium methoxide and potassium methoxide) .The relevant tests were aimed at identifying the best temperature, treatment period, solvent and alkali for the production of conjugated linoleic acid, which was 140 °C and 30 min. Results showed that the excessive increase in temperature caused the conversion of beneficial isomers) C9-T11 and T10-C12) to other isomers of conjugated linoleic acid and other unusual products. The excessive increase in temperature reduced the desirable product, but longer duration of time did not cause this problem. It was concluded that there is approximately a 50/50 ratio between the two isomers under optimum conditions.

Keywords: Linoleic acid, Conjugated linoleic acid, Isomerization, Solvent, Alkali.

Introduction

In recent years, the demand for the production of safe and healthy foods has increased significantly. Fats are one of the main components of food and there are many concerns about the use of fats due to the association of lipids with cardiovascular disease, diabetes and obesity (O'Keefe and et al, 2088). In contrast, another group of lipids exhibits beneficial physiological effects (Akoh, 2005). One of the healthier or pragmatic lipids is conjugated linoleic acid (CLA). Conjugated linoleic acid represents a group of linoleic acid isomers (cis-9, cis-12 octadecadienoic acid) that have been explored in recent years due to their physiological beneficial effects. These fatty acids have attracted much attention as biological functional lipids. The unique activities of CLA have been studied intensively, and it is expected that conjugated linoleic acid is a substance that can be used for pharmaceutical and dietary supplementation.

Today, conjugated linoleic acid is produced as a dietary supplement by the chemical isomerization of linoleic acid. Recent studies have shown that each of the isomers can do a different effect on cell metabolism and function. So far, cis9-trans11 and -trans10 cis12 isomers are more important because of important biological activities (Sæbø, 2003; Pariza and et al, 2001). Appropriate isomers have to be selected when considering the use of conjugated linoleic acid for medical and nutritional purposes. Conjugated linoleic acid supplements for human consumption have been available since 1995. These products contain between 60 and 80% of conjugated linoleic acid in the form of free fatty acids. The history of using conjugated linoleic acid for industrial purposes goes back to the past 100 years (Sæbø, 2003).

Moreover, CLA isomers, either in their free or esterified forms, have not only been associated with diverse health and physiological effects, but also are interesting renewable compounds in the production of industrial products such as paints, glues, and polymers, due to their very reactive conjugated double bond system (Philippaerts and et al, 2013).

Also, a variety of positive health effects have been attributed to CLAs. CLAs has been reported to have diverse health benefits and biological properties, including being reducing arterial dilation (Kritchevsky and et al, 2002), ant carcinogenic, antiatherogenic, antidiabetic, ant obesity, antioxidative, immunomodulatory, antibacterial, cholesterol lowering, and growth promoting (Pariza and et al,

Mohamad-Reza Shayanmehr*, Amir-Hossein Elhamirad
Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran

Mohamad Armin
Department of Agriculture, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran

***Email:** MRShayanmehr2010@gmail.com

2001; Onakpoya and et al, 2012; Rainer & Heiss, 2004). Studies in different animals and in humans have shown that CLA regulates the composition of corporal fat and proteins, it produces anti-atherosclerotic and modulates inflammatory responses (Dilzer & Park, 2012; Viladomiu and et al, 2016).

The commercial production of CLA is accomplished by chemical and microbial synthesis methods. The established and industrial approach to the production of CLA is isomerization under alkaline conditions, which converts linoleic acids and their alkyl esters with alkali bases or potassium alkoxides into CLAs (Guo and et al, 2003; Philippaerts and et al, 2011a; 2011b). 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 (Philippaerts and et al, 2013; Villeneuve and et al, 2055). However, the alkali isomerization of LA is the most specific and least expensive process to produce bulk synthetic CLA isomers (Rocha-Uribe & Hernandez, 2004a; 2004b; Pakdeechanuan and et al, 2010). The isomers produced by each of these methods are different.

In contrast to the heterogeneous catalysis, microbiological methods possess a high specificity Results and discussion toward the production of bioactive CLA isomers, mainly toward the cis9, trans11-CLA isomer (Khaskheli and et al, 2013). Unfortunately, the low conversion of LA to CLA and the long reaction times employed by the microbiological methods make them uncompetitive against alkali isomerization (Khaskheli and et al, 2013, Salamon and et al, 2012).

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 (Lundberg, 1958). 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 (Salamon and et al, 2012; Yang & Liu, 2004). Silva-Ramırez *et al*. (Silva Ramírez and et al, 2017) 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.

This study considered linoleic acid sunflower oil and its direct use for the production of CLA. The effects of solvents and different alkali were studied on alkaline CLA isomerization in sunflower oil.

Materials and Methods

The treatments

In this study, four variables were used, i.e. temperature (80, 110, 140, 170 °C), time (0.25, 0.5, 1, 2, 3.5, 5, 6.5 hours), solvent (water, ethanol, propylene glycol), and alkali (sodium hydroxide, potassium hydroxide, sodium methoxide, potassium methoxide).

Sunflower oil was purchased from Nina Corporation (company), which contained about 55% linoleic acid. Solvents, alkali and boron trifluoride (BF3) in methanol were purchased from Merck Company.

Production of conjugated linoleic acid with linoleic acid isomerization was performed as described by Chin *et al.* (Chin and et al, 1992). Accordingly, 1.2 gram of the desired alkali were dissolved in 5 ml of the selected solvent under nitrogen gas blast conditions for 20 minutes at 80,110,140,170 ° C. Then 2.5 ml of oil was added to the mixture. The temperature and time required to perform isomerization reaction were kept constant. The mixture was cooled after the reaction at room temperature was neutralized with 40 milliliters of hydrochloride acid (HCl). The pH was reduced to less than 4 (about 3) .

According to Reaney *et al.* (Reaney and et al,2002) and Guo *et al.* (Guo and et al, 2003), strong acids such as sulfuric acid or hydrochloride acid can lead to a chemical reaction with CLA. To reduce hydrolyzing soaps pH to lower than 4 we used of Phosphoric acid.

Then distilled water was added and mixed for 5 minutes. The extraction procedure was performed 3 times with 20 ml Hexane. To remove hexane, a mixture of sodium chloride or calcium chloride 5% was rinsed with distilled water or with a mixture of methanol 30% in distilled water. Finally, hexane was removed by vacuum rotary evaporation and the purification and dewatering step was performed by sodium sulfate (Chin and et al, 1992).

Analysis of fatty methyl esters

The fatty acid methyl esters according to the ISO standard were analyzed by the gas chromatography machine technologies 6890 N Agilent manufactured in China under the license of the USA equipped with FID detector and HP-5 capillary column (30 M, 0.320 MM,

0.25 μM) under the following conditions: The injector temperature was 250 °C and the aeration temperature was 215-160 °C. The air pressure and nitrogen were set to be 6 bars.

Methylation of oil samples

About 10 drops of oil were poured into test tubes. Then, 7 ml of hexane solvent were added to gas chromatography and then 2 ml of methanol potassium 2 molar were added to the test tubes. The tubes were sealed and well stirred. Then, the test tubes were placed in a serum-bromine temperature of 55-50 °C for 15 minutes, and the binomial temperature was regularly controlled by the thermometer to remain constant at the desired temperature every 5 minutes. The contents of the test tubes were stirred. After 15 minutes, the test tube remained stationary for 5 minutes in Bin-Marie at 55-50 °C. Then, the test tubes were relocated without shaking and the supernatant was used to inject gas chromatography.

Results and Discussion

Evaluation of the effect of different solvents

In the first step, each of the 3 solvents reacted with 4 selected alkali at 80 °C for 5 hours. The conversion of linoleic acid to the conjugated linoleic acid in each of the treatments was investigated. The effect of alkali and solvent type on the amount of LA during isomerization was documented (Fig. 1A). Results showed that the alkali and solvent used for linoleic acid isomerization had a statistically significant effect on LA ($p<0.05$). The interaction between solvent and alkali was statistically significant ($p<0.05$). In the water solvent, the lowest and highest levels of LA were related to alkali CH3OK and NaOH, and there was no significant difference between the two other alkalies ($p<0.05$). In the ethanol solvent, the highest amount of LA was related to CH3OK alkali, and the KOH and CH3ONa solvents had the lowest amount. There was no significant difference in LA of the propylene glycol solvent ($p<0.05$). The results of Fig. 1B showed that the alkali and solvent used for linoleic acid isomerization had a statistically significant effect on C9, T11 ($p<0.05$). The interaction of solvent and alkali was statistically significant ($p<0.05$). In the water solvent, the highest C9, T11 was related to KOH alkali and the CH3ONa alkali had the lowest. In ethanol solvent, the highest amounts of C9, T11 were KOH alkali, and the lowest was for alkali NaOH and CH3OK. In propylene glycol solution, the highest C9, T11 was NaOH alkali, while the two alkalies CH3ONa and CH3OK had the lowest value. The results of Fig. 1C showed that the alkali and solvent used for linoleic acid isomerization had a statistically significant effect on T10, C12 ($p<0.05$). The interaction of solvent and alkali was statistically significant ($p<0.05$). As seen in the water solvent, the highest amounts of T10, C12 were NaOH and KOH alkali, and there was no significant difference between this two alkali ($p<0.05$). No significant difference was found between the two other alkalies ($p<0.05$). In the ethanol solvent, the smallest and highest amounts of T10, C12 isomers were respectively NaOH and KOH alkali. In the propylene glycol solvent, the two alkalies KOH and CH3ONa had the highest T10, C12 isomers, and the least amount occurred by NaOH and CH3OK. The results of Fig. 1D showed that the alkali and solvent used for linoleic acid isomerization had a statistically significant effect on the total amount of linoleic acid isomers ($p<0.05$). The interaction between solvent and alkali was statistically significant ($p<0.05$). The effect of alkali and solvent type on the total amount of linoleic acid isomers during isomerization appears in Fig. 1D. In the water solvent, the highest total isomers were related to KOH alkali, and the CH3ONa and CH3OK alkali had the lowest amount. There was no significant difference between this two alkali ($p<0.05$). In ethanol, the highest total isomers were related to KOH alkali and the lowest was by NaOH and CH3OK. In the propylene glycol solvent, the highest total isomers were related to NaOH followed by KOH and CH3OK which caused the lowest.Fig. 1D shows that the use of this temperature and time in treatments related to water solvents causes the isomerization and conjugated linoleic acid production to be very low, and almost none of the CLA isomers were produced in these conditions. Reany *et al*. (2002) invented the use of water as a reaction medium and vegetable oil containing more than 60% linoleic acid to produce CLA. They found that the reaction in water produced several isomers, but the isomer ratio was controlled by variables and reaction kinetics. They showed that at 170 °C, the reaction value accelerated with increasing the temperature. The temperature used in their study (170 °C) was much higher than the temperature used in this study (80 °C), which indicates the effect of temperature on chemical reactions. Sæbø (Sæbø, 2003) avoided the formation of isomers T8, C10, and C11, T13 by using KOH and NaOH catalysts in the water solvent. They concluded that isomerization is not possible without producing very little amounts of these isomers. Ma *et al*. (Ma and et al, 1999) stated that water use should also be carried out in a pressure vessel in a closed and continuous operation. Fig. 1D shows that treatments using ethanol solvents were not successful and the amount of conjugated linoleic acid was very low at 5 °C and 80 °C. Ma *et al*. (Ma and et al, 1999) used alcohol to produce CLA and stated that the use of this material requires special equipment that is safe to ignite. Also, the molecular weight of alcohols has the difficulty of being recycled and the remaining alcohol in the oil makes it unacceptable for use. In experiments of using propylene glycol as the reaction medium, the production of conjugated linoleic acid was higher than other solvents, and the results from Fig. 1D shows that the propylene glycol solvent provides a more suitable environment for isomerization and the production of conjugated linoleic acid. Iwata *et al*. (Iwata and et al, 1999) showed that with the use of propylene glycol as a solvent, a higher conversion rate of linoleic acid to CLA was observed compared to that of ethylene glycol, while the CLA products obtained by this method had a much lower coloration than other conventional methods and more solubility than other solvents. The use of water for the isomerization reaction requires high temperatures and the use of special equipment, such as a pressure vessel and a closed and

continuous operation. Also, when using ethanol, the problem of alcohol recycling becomes an important matter, and the remaining alcohol makes the product unusable. The production of conjugated linoleic acid is close to zero under the conditions provided by the two solvents of water and ethanol. Therefore, these two solvents were not used further in the experiments.

Propylene glycol is the most common solvent used in the current CLA preparation by alkali isomerization of LA; nevertheless, it is also the most expensive solvent used for this purpose (Pakdeechanuan and et al, 2010). Generally, the use of propylene glycol in comparison to ethylene glycol leads to the production of more trans isomers and increases the production of CLA c9-t11 and t10-c12 isomers (Silva-Ramírez and et al, 2017) .At the end of this step, this substance was selected as the preferred solvent because of the higher concentrations of conjugated linoleic acid isomers in treatments using propylene glycol solution, as Rocha-Uribe and Hernandez (Rocha-Uribe & Hernandez, 2004a; 2004b) and Silva-Ramırez *et al*. (Silva-Ramírez and et al, 2017) stated in their study. Also, according to a report by Iwata *et al*. (Iwata and et al, 1999), if propylene glycol is used for the isomerization reaction to produce conjugated linoleic acid, the resulting product would contain less color and, because propylene glycol is not toxic, the product can be used in food.

Evaluation of the effect of different temperature levels

In the case of propylene glycol solvent, the effects of different temperature levels were investigated for each of the alkalies. The effect of the temperature factor of 110, 140 and 170 °C of the propylene glycol solvent was investigated by increasing the temperature to 30 °C. The effect of alkali and time on LA is shown in Fig. 2A and shows that the alkali type and temperature used for linoleic acid isomerization has a statistically significant effect on LA ($p<0.05$). The interaction between alkali and temperature was statistically significant ($p<0.05$). As observed in the figure, in all alkali samples, with increasing temperature, LA significantly decreased ($p<0.05$), so that the highest amount of linoleic acid related to KOH, CH3ONa and CH3OK alkalis at 110 °C and KOH alkali at 170 °C had the lowest LA content. The effect of alkali and time on the amount of C9, T11 is shown in Fig. 2B and shows that the alkali type and temperature used for linoleic acid isomerization has a statistically significant effect on C9, T11 ($p<0.05$). The interaction between alkali and temperature was statistically significant ($p<0.05$). As shown in Fig. 2B, in CH3ONa and CH3OK alkali, increasing the temperature causes the C9, T11 levels to increase significantly ($p<0.05$), while in NaOH and KOH alkali, increasing the temperature from 110 to 140 °C led to a different outcome. The isomer increased significantly and then declined again with increasing temperatures from 140 to 170 °C. The highest C9, T11 isomer was KOH alkaline at 140 °C and alkaline CH3OK at 170 °C. Alkaline CH3OK had the lowest C9, T111 at 110 °C. The effect of alkali and time on the amount of T10, C12 is shown in Fig. 2C and shows that the alkali type and temperature used for linoleic acid isomerization had a statistically significant effect on T10, C12 ($p<0.05$). The interaction of alkali and temperature was statistically significant ($p<0.05$). As seen in the figure, the CH3ONa and CH3OK alkali cause the T10,C12 levels to increase significantly with increasing temperature ($p<0.05$), while in NaOH and KOH alkali the increase in temperature from 110 to 140 °C caused this isomer to increase significantly and then decrease again with increasing temperatures from 140 to 170 °C. The highest T10, C12 isomer was KOH alkaline at 140 °C and alkaline CH3OK at 170 ° C. The CH3OK alkaline at 110 °C had the lowest T10, C12. The effect of alkali type and time on the amount of linoleic acid isomers is shown in Fig. 2D and shows that the alkali type and temperature used for linoleic acid isomerization had a significant effect on the total amount of linoleic acid isomers ($p<0.05$). The interaction between alkali and temperature was statistically significant ($p<0.05$). As shown in the figure, the use of CH3ONa and CH3OK alkali caused the temperature to increase significantly ($p<0.05$), while in alkali NaOH and KOH, the increase in temperature from 110 to 140 °C caused the amount of linoleic acid isomers to increase significantly, and then declined again with increasing temperatures from 140 to 170 °C. The highest total amount of linoleic acid isomers was related to KOH alkaline at 140 °C and alkaline CH3OK at 170 °C. The alkaline CH3OK at 110 °C had the least amount of linoleic acid isomers. Fig. 2A shows that using a temperature of 110 °C led to the amount of isomerization reaction to being only about 6% for both of conjugated linoleic acid isomers and 90% of the linoleic acid content, which indicates that the conversion efficiency is very low. Fig. 2A shows that applying the temperature of 140 °C brings about better conditions for an isomerization reaction, and isomerization efficiency in this temperature is about 90%. In Fig. 2A, the use of a temperature of 170 °C is observed and the effect of applying high temperatures was demonstrated. Despite the conversion factor being 100 percent, the amount of CLA isomer production was reduced. Examination of Fig. 2A and D show that the temperature of isomerization increased as well as the production of conjugated linoleic acid production, but due to the excessive increase in temperature, the production of the desired isomers (Cis9, Trans11, and Trans10, Cis12) decreased. This was because these major CLA isomers after production were converted again to other isomers that were not very useful and known as impurities, which reduced the amount of final products produced. At 140 °C, the isomerization and production of conjugated linoleic acid were almost complete and the production of isomers nearly approached its maximum production and the production of undesirable isomers was observed at a minimum. In previous studies, Yang and Lio Yang & Liu, 2004, Sæbø (Sæbø, A. (2003) and Yang *et al*.(Yang and et al, 2002) proved the use of higher temperatures (160 °C) to have a negative effect on the product, and despite increasing the conversion efficiency, the amount of trans-trans isomers increased, thereby making it undesirable. They found that both the isomers T,T 18:2 increased with increasing temperature, while the production of isomers C,T 18:2 and C,C 18:2 decreased. They showed that at low temperatures, the isomers C,C were more abundant and at high temperatures the T,T isomers were produced more. In their research, they showed that alkaline isomerization at 160 °C would be up to 90% efficient, and by reducing the temperature to 120 °C, the efficiency dropped by 20%.

Silva-Ramırez *et al.* (Silva-Ramírez and et al, 2017) declared the most suitable temperature for an isomerization reaction of 160 ° C with a 91% efficiency. 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 rise of the temperature increases the conjugated trans-trans isomers has been previously reported by Michaud (Michaud and et al, 2003) and explained as the higher thermodynamic stability. After the formation of the two primary isomers, additional isomers could be produced in function of temperature and the time of reaction.

By examining the above results, the temperature of 140 °C was chosen as the most suitable temperature for the isomerization of linoleic acid and the production of conjugated linoleic acid by the production of pure isomers was managed in order to determine the best time and other variables used from this temperature. Also, by analyzing the observations and results from the previous steps, it was found that potassium hydroxide is the most suitable alkali and has the best performance for conjugated linoleic acid production, which is consistent with previous studies. Yang and Lio (Yang & Liu, 2004) showed that the use of potassium hydroxide alkali increases the conversion efficiency. Accordingly, potassium hydroxide was used as the alkali for catalytic function during the experiments.

Study the effect of different time levels

At this stage, by causing a variation in the time spent in the experiments, different levels including 15 and 30 minutes and 1, 2, 3.5, 5, 6.5 hours in 7 different treatments of time were used so as to determine the appropriate duration for the isomerization and the conversion of linoleic acid to conjugated linoleic acid . The effect of isomerization time on LA is shown in Fig. 3A. It showed that the isomerization time had a significant effect on LA ($p<0.05$). As seen in the figure, the LA gradually decreased with increasing time, and this decrease was statistically significant by up to 3.5 hours ($p<0.05$). The effect of isomerization time on C9, T11 was shown in Fig. 3B and showed that the isomerization time had a statistically significant effect on C9, T11 ($p<0.05$). As shown in Fig. 3B, by increasing the duration from 0.25 to 2 hours, the C9 and T11 levels increased significantly ($p<0.05$), but the increase in time from 2 to 6.5 hours caused no significant difference in the amount of this isomer ($p<0.05$). The effect of isomerization time on T10, C12 is shown in Fig. 3C and shows that the isomerization time had a statistically significant effect on T10, C12 ($p<0.05$). As shown in Fig. 3C, with increasing the duration of time, the amount of T10, C12 increased initially and then decreased. The maximum amount of this isomer was related to the time of 2 hours and the minimum was obtained at 0.25 hours. The effect of isomerization time on the total amount of linoleic acid isomers is shown in Fig. 3D and shows that the isomerization time had a statistically significant effect on the total amount of linoleic acid isomers ($p<0.05$). As shown in Fig. 3D, with increasing time, the total amount of linoleic acid isomers increased initially (for up to 2 hours) and then decreased. The maximum amount of linoleic acid isomers was related to the duration of 2 hours, and the minimum was obtained by 0.25 hours. There was no significant difference between the total amount of linoleic acid isomers in 3-5, 5 and 6.5 hours ($p<0.05$). Fig. 3A and 3D showed that the increase in time for isomerization was successful during the first 2 hours of isomerization, by which time the reaction was almost complete. By the time the reaction had performed for 6.5 hours, the amount of the isomerization reaction was almost constant. The conversion of conjugated linoleic acid isomers was at its peak at 2 hours and reached about 50% of each of the CLA isomers. Using the time of more than 2 hours, the desired isomers of the conjugated linoleic acid were almost constant and, with an increase in time up to 6.5 hours, the amount of these isomers did not change, which indicates that the increase in time has had little effect on isomerization of conjugated linoleic acid isomers. No reduction was observed in the main desired isomers and the production of undesirable CLA isomers, or if this increase was time-consuming, the effect was negligible. Rocha-uribe and Hernandez (Rocha-Uribe & Hernandez, 2004a; 2004b) which used a temperature of 180 ° C for 2.15 hours) reached the maximum production of clause isomers in these conditions. Iwata *et al*. (Iwata and et al, 1999) showed that when methyl linolenate was heated to 200 °C for 7 hours in a potassium hydroxide and ethylene glycol solution, about 80% of the conjugation was performed, it is a counteraction to methyl linoleate in this condition. In contrast to other studies that have run reactions from 2 to 3 hours, due to economic results in time and cost, a duration of 30 minutes with about 65% conversion of LA to CLA was the most appropriate time to perform isomerization and proceeding the experiments were selected to reduce the duration of treatments. Under these conditions, the highest proportion of convenient and cost-effective time can be used for the production of C9 + T10 isomers. Moreno *et al.* (Moreno and et al,2012) stated their research, microwave irradiation decreased the reaction times in the chemoenzymatic conversion of LA and in dehydration of ricinoleic acid.

Conclusion

In this study, the best solvent was identified as propylene glycol dissolved in water and ethanol. In the case of using propylene glycol for production of conjugated linoleic acid, the product would contain less color. Since propylene glycol is not toxic, the product can be used for food production. Furthermore, it requires less equipment and facilities than other solvents. Potassium hydroxide was also selected as the most suitable alkali with the highest conversion factor for catalytic activity due to its higher solubility than sodium hydroxide (for catalytic converter) and better catalytic performance.

The increase in temperature causes an increase in reactive velocity and, as a result, isomerization is carried out more rapidly, but the excessive increase in temperature leads to a reaction that produces undesirable isomers. Nonetheless, 140 °C was identified as the best temperature with over 90% conversion factor when converting the LA to CLA. Furthermore, the increase in time has the same effect and increases the probability of producing inappropriate isomers over long durations. Economic reasons pertaining to time and cost make the appropriate duration for the isomerization reaction to be 30 minutes, along with the production of conjugated linoleic acid, by which the highest ratio of C9+T10 isomer production was observed along with the most economical conversion

References

Akoh, C. C. (2005). *Handbook of functional lipids*. CRC press.

Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L., & Pariza, M. W. (1992). Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *Journal of food composition and analysis*, *5*(3), 185-197.

Dilzer, A., & Park, Y. (2012). Implication of conjugated linoleic acid (CLA) in human health. *Critical reviews in food science and nutrition*, *52*(6), 488-513.

Guo Wei, Zhang Genwang, & Sun Yan. (2003) Preparation of conjugated linoleic acid and identification of its isomers. *Chinese Journal of Chemical Engineering: English, 11(2), 130-135*

Iwata, T., Kamegai, T., Sato, Y., Watanabe, K., & Kasai, M. (1999). *US Pat. 5,986,116* . Washington, DC: US Patent and Trademark Office.

Khaskheli, A. A., Talpur, F. N., Demir, A. S., Cebeci, A., & Jawaid, S. (2013). A highly selective whole cell biocatalysis method for the production of two major bioactive conjugated linoleic acid isomers. *Biocatalysis and Agricultural Biotechnology*, *2*(4), 328-332.

Kritchevsky, D., Tepper, S. A., Wright, S., & Czarnecki, S. K. (2002). Influence of graded levels of conjugated linoleic acid (CLA) on experimental atherosclerosis in rabbits. *Nutrition Research*, *22*(11), 1275-1279.

Lundberg, W. O. (1958). Alkali-isomerization reactions of unsaturated fatty acids. *The American journal of clinical nutrition*, *6*(6), 592-593.

Ma, D. W., Wierzbicki, A. A., Field, C. J., & Clandinin, M. T. (1999). Conjugated linoleic acid in Canadian dairy and beef products. *Journal of Agricultural and Food Chemistry*, *47*(5), 1956-1960.

Michaud, A. L., Yurawecz, M. P., Delmonte, P., Corl, B. A., Bauman, D. E., & Brenna, J. T. (2003). Identification and characterization of conjugated fatty acid methyl esters of mixed double bond geometry by acetonitrile chemical ionization tandem mass spectrometry. *Analytical chemistry*, *75*(18), 4925-4930.

Moreno, M., Gomez, M. V., Cebrian, C., Prieto, P., de la Hoz, A., & Moreno, A. (2012). Sustainable and efficient methodology for CLA synthesis and identification. *Green Chemistry*, *14*(9), 2584-2594.

O'Keefe, SF, Akoh, CC, & Min, DB (2008). Food Lipids: Chemistry, Nutrition, and Biotechnology.

Onakpoya, I. J., Posadzki, P. P., Watson, L. K., Davies, L. A., & Ernst, E. (2012). The efficacy of long-term conjugated linoleic acid (CLA) supplementation on body composition in overweight and obese individuals: a systematic review and meta-analysis of randomized clinical trials. *European journal of nutrition*, *51*(2), 127-134.

Pakdeechanuan, P. S., Salaemae, A., & Odpiban, S. (2010). Effect of reducing propylene glycol content on conjugated linoleic acid production from sunflower oil. *Warasan Witthayasat Mokho*.

Pariza, M. W., Park, Y., & Cook, M. E. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in lipid research*, *40*(4), 283-298.

Philippaerts, A., Aelst, J. V., & Sels, B. (2013). Conjugated linoleic acids and conjugated vegetable oils: From nutraceutical to bio-polymer. *European Journal of Lipid Science and Technology*, *115*(7), 717-720.

*P*hilippaerts, A., Goossens, S., Jacobs, P. A., & Sels, B. F. (2011). Catalytic production of conjugated fatty acids and oils. *ChemSusChem*, *4*(6), 684-702.

Philippaerts, A., Goossens, S., Vermandel, W., Tromp, M., Turner, S., Geboers, J., ... & Sels, B. F. (2011). Design of Ru–Zeolites for Hydrogen-Free Production of Conjugated Linoleic Acids. *ChemSusChem*, *4*(6), 757-767.

Rainer, L., & Heiss, C. J. (2004). Conjugated linoleic acid: health implications and effects on body composition. *Journal of the American Dietetic Association*, *104*(6), 963-968.

Reaney, M. J., Liu, Y. D., & Westcott, N. D. (2002). *U.S. Patent No. 6,420,577*. Washington, DC: U.S. Patent and Trademark Office.

Rocha Uribe, A., & Hernández, E. (2004). Síntesis de ácido linoleico conjugado por isomerización alcalina usando propilenglicol como solvente. *Revista mexicana de ingeniería química*, *3*(2).

Rocha-Uribe, A., & Hernandez, E. (2004). Solvent-free enzymatic synthesis of structured lipids containing CLA from coconut oil and tricaprylin. *Journal of the American Oil Chemists' Society*, *81*(7), 685-689.

Sæbø, A. (2003). Commercial synthesis of conjugated linoleate. *Advances in conjugated linoleic acid research*, *2*, 71-81.

Salamon, R. V., Varga-Visi, É., András, C. D., Kiss, Z. C., & Csapo, J. (2012). Synthetic methods for obtaining conjugated linoleic acids (CLA) by catalysis. *Acta Univ. Sapientiae, Alimentaria*, *5*, 32-51.

Silva-Ramírez, A. S., Rocha-Uribe, A., González-Chávez, M. M., & González, C. (2017). Synthesis of conjugated linoleic acid by microwave-assisted alkali isomerization using propylene glycol as solvent. *European Journal of Lipid Science and Technology*, *119*(4), 1600079.

Viladomiu, M., Hontecillas, R., & Bassaganya-Riera, J. (2016). Modulation of inflammation and immunity by dietary conjugated linoleic acid. *European journal of pharmacology*, *785*, 87-95..

Villeneuve, P., Lago, R., Barouh, N., Barea, B., Piombo, G., Dupré, J. Y., ... & Pina, M. (2005). Production of conjugated linoleic acid isomers by dehydration and isomerization of castor bean oil. *Journal of the American Oil Chemists' Society*, *82*(4), 261-269.

Yang, L., Huang, Y., Wang, H. Q., & Chen, Z. Y. (2002). Production of conjugated linoleic acids through KOH-catalyzed dehydration of ricinoleic acid. *Chemistry and Physics of lipids*, *119*(1), 23-31.

Yang, T. S., & Liu, T. T. (2004). Optimization of production of conjugated linoleic acid from soybean oil. *Journal of agricultural and food chemistry*, *52*(16), 5079-5084.

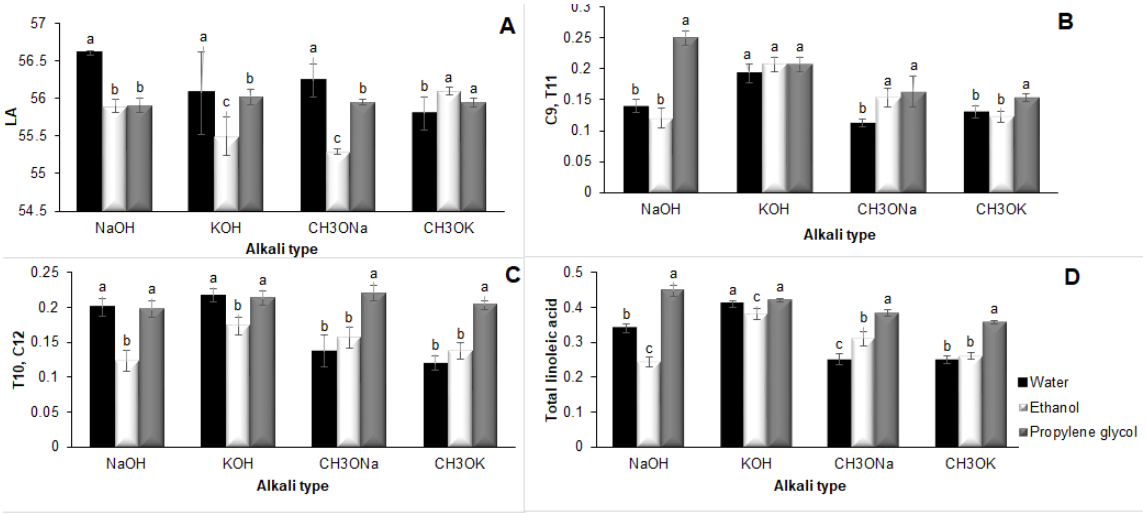

Fig. 1. Effect of solvent and alkali types on LA (A), C9, T11 (B), T10, C12 (C) and total linoleic acid (D).

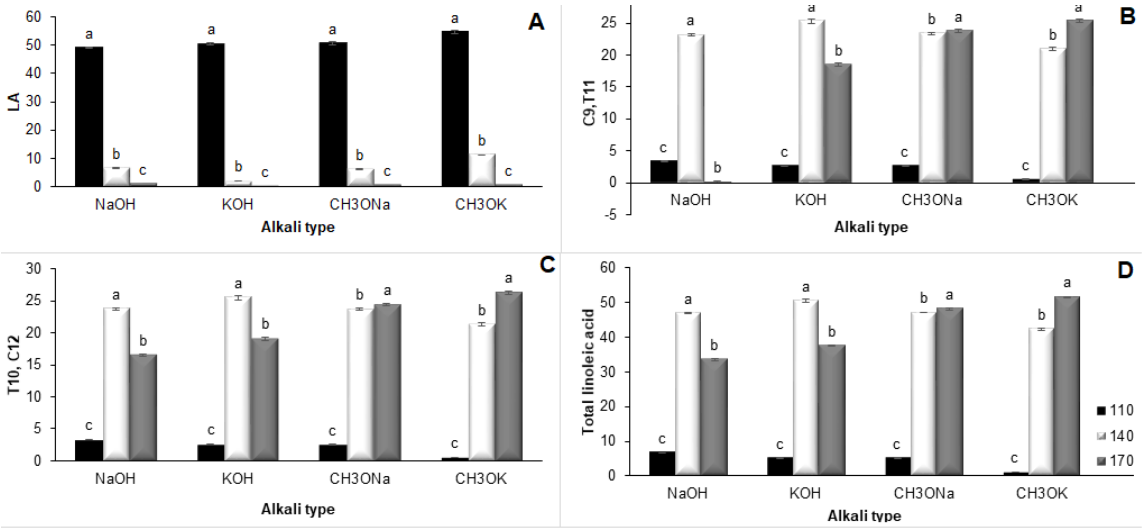

Fig. 2. Effect of alkali type and temperature on LA (A), C9, T11 (B), T10, and C12 (C) and total linoleic acid.

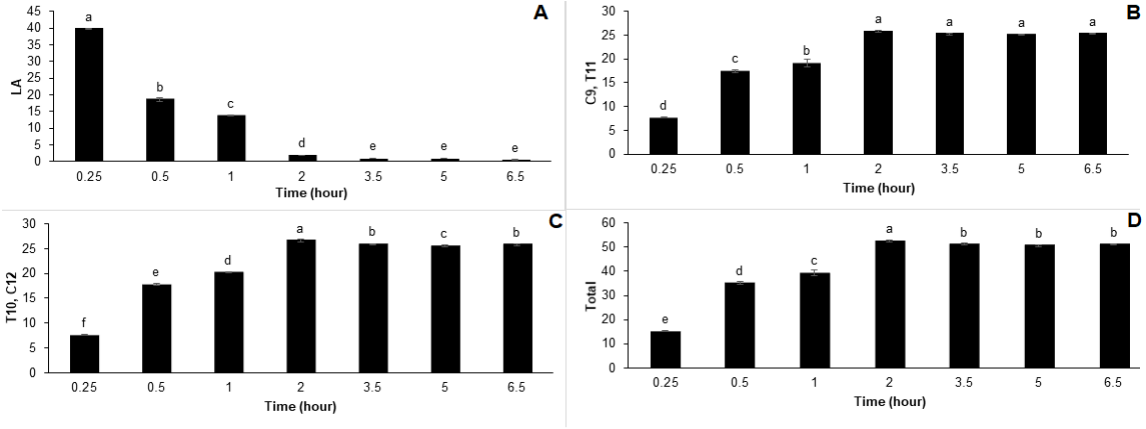

Fig. 3. Effect of different times on LA (A), C9, T11 (B), T10, and C12 (C) and total linoleic acid.